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ANALYSIS OF THE GENETIC BACKGROUND OF THYROID CANCER AND THE OUTCOMES OF PREOPERATIVE ASSESSMENT

PhD thesis

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LIST OF ABBREVIATIONS

ACR-TI-RADS: American College of Radiology Thyroid Imaging Reporting and Data System

AER: Absolute Excess Risk

AML: Acute myeloid leukemia

ATA: American Thyroid Association

BC: Breast cancer

BCa 95% CI: Bias-corrected and accelerated 95% bootstrap confidence interval

CI: Confidence Interval

CLS: Cowden-like syndromes

CS: Cowden syndrome

EDC: Endocrine disrupting chemical

ER: Estrogen Receptor

ER α : Estrogen Receptor Alpha

ETA: European Thyroid Association

EU-TIRADS: European Thyroid Imaging and Reporting Data System

FFPE block: Formalin-Fixed Paraffin-Embedded block

FNAB: Fine needle aspiration biopsy

GBq: Gigabecquerel

hCG: Human chorionic gonadotropin

HR: Hazard Ratio

IGV: Integrative genomics viewer

I-131: Iodine-131

ISP: Ion sphere particles

LN: Lymph node

lncRNS: long non-coding RNA

MPN: Myeloproliferative neoplasia

NHANES: National Health and Nutrition Examination Survey

NIS: Sodium/Iodide symporter

OR: Odds Ratio

RAI: Radioactive Iodine

RR: Relative Risk

SD: Standard deviation

SEER: Surveillance, Epidemiology, and End Results

SERM: Selective estrogen receptor modulator

SIR: Standardized Incidence Ratio

SMR: Standardized Mortality Ratio

SNP: Single-nucleotide polymorphism

SPM: Second Primary Malignancy

TC: Thyroid cancer

TG: Thyroglobulin

TPO: thyreoperoxidase

TRH: Thyrotropin-releasing hormone

T2DM: Type 2 Diabetes Mellitus

US: Ultrasound

1. INTRODUCTION

I. Synchronous/metachronous malignancies associated with thyroid cancer

Incidence of thyroid cancer has tripled in the last three decades all over the world, except for Africa possibly due to the insufficient detection. Thyroid cancer accounts for 1,0-1,5% of all new cancers found each year in USA (1, 2). According to IARC data in 2022, thyroid cancer has an age-standardized incidence rate of 13,6/100.000 in women and 4,2/100.000 in men in North America and Europe. Thus, for today, it is the most common endocrine malignancy and the fifth most frequent overall malignancy in women in the western region.

The synchronous or metachronous association of differentiated thyroid cancers with several other tumor types has long been recognized (3). The significance of which is that mortality, and thus prognosis, in survivors of malignant disease is significantly influenced by the development of second primary tumors.

A second primary tumor/malignancy (SPM) is a malignancy that develops in a survivor of a histologically or clonally distinct malignancy, with an incidence of 2-17% (4). Based on the relative timing of the two tumors, we can speak of synchronous (<6 months) or asynchronous/metachronous primary tumors (>6 months). The incidence of SPM compared to the age-adjusted general population can be expressed by SIR (standardised incidence ratio): observed incidence/expected incidence.

According to Hou et al. published in 2023 (5), the incidence of second primary malignancies after a first primary thyroid cancer was 15% during a median follow-up of 19,9 years. After thyroid cancer, the most common second primary malignant tumors in men were prostate, lung/bronchus, renal/renal pelvis cancer, followed by cutaneous melanoma, bladder cancer, nodal non-Hodgkin lymphoma, pancreatic cancer, multiple myeloma, rectal carcinoma. Women with thyroid cancer were followed most frequently by breast, lung/bronchus, uterine cancer, then by skin melanoma, renal/renal pelvis cancer, nodal non-Hodgkin lymphoma, ovarian, pancreatic carcinoma and multiple myeloma, in order of frequency. The 10-year survival of thyroid cancer without a second primary cancer was 93,7%, and 69,4% for SPM, showing that the presence of a second primary tumor significantly reduced survival. Their results showed that age over 55 years (HR= 3,102; 95% CI 2,949-3,263), male sex (HR= 1,363; 95% CI 1,292-1,438) and

multifocal thyroid tumor (HR= 1,135; 95% CI 1,076-1,197) were independent risk factors for a second primary tumor after differentiated thyroid cancer. A higher TNM stage III or IV was a protective factor (HR= 0,823; 95% CI 0,755-0,897). Compared to Caucasian race, the risk of SPM was the same in black ethnicity, but lower in Asian/ Pacific Islander (HR= 0,823; 95% CI 0,755-0,897) and other races (HR= 0,454; 95% CI 0,337-0,613). The different risk between races may be caused by different genetic backgrounds, different thyroid cancer treatment, different medical care, economic and lifestyle factors. The protective nature of the higher stage III/IV may be due to shorter survival, that is, insufficient time for the second primary tumor to develop (survival bias).

Subramanian et al's significant paper was published in 2007 in *Thyroid* (6). Their results showed that the overall SPM SIR for second primary malignancies in thyroid carcinomas was 1,2 (95% CI 1,17-1,24), which means that any second malignancy after thyroid cancer is 20% more common than in those without a history of thyroid cancer. After thyroid cancer, significantly higher incidence occurred in salivary gland (SIR= 2,97; 95% CI 1,78-4,15), gastric (SIR= 1,21; 95% CI 1,02-1,40), colon (SIR= 1,17; 95% CI 1,05-1,28), colorectal (SIR= 1,27; 95% CI 1,13-1,41), female breast (SIR= 1,25; 95% CI 1,17-1,32), prostate (SIR= 1,38; 95% CI 1,25-1,51), kidney (SIR= 2,30; 95% CI 1,99-2,62), brain/central nervous system (SIR= 1,45; 95% CI 1,12-1,78), adrenal (SIR= 6,54; 95% CI 2,73-10,35), bone/joint (SIR= 3,04; 95% CI 1,42-4,67) malignancies, and soft tissue sarcoma (SIR= 2,18; 95% CI 1,46-2,83), non-Hodgkin's lymphoma (SIR= 1,22; 95% CI 1,03-1,42), multiple myeloma (SIR=1,33; 95% CI 1,00-1,66) and leukemia (SIR= 1,74; 95% CI 1,46-2,02). However, there was a significantly lower incidence of second primary lung cancer (SIR= 0,84; 95% CI 0,75-0,92) and cervical cancer (SIR= 0,76; 95% CI 0,57-0,95).

Information on the rates of second primary tumors after thyroid cancer can be obtained from two large "pan-cancer" studies, i.e. comprehensive cancer registry analyses. One was published by Wang and colleagues (4) in 2024 based on 206 studies and analysis of the Surveillance, Epidemiology, and End Results (SEER) and National Health and Nutrition Examination Survey (NHANES) databases. Out of 9,617,643 patients with a first primary tumor, 677,430 patients developed a second primary tumor, with 3 significant associations in the pattern. Among these, one aspect of particular relevance to our study was that second primary breast cancer and colorectal cancer developed more frequently after first primary thyroid cancer (19% and 8%), fallopian tube cancer (28%

and 17%), ovarian carcinoma (24% and 15%), uterine carcinoma (26% and 14%) or vulvar and vaginal (22% and 11%) malignancies - i.e. after thyroid and gynecological tumors. Although no SIR value was reported, it is worth noting that after primary thyroid cancer, 19% develop a second primary breast cancer; 9% lung cancer; 8% colorectal carcinoma; 6% lymphoproliferative neoplasia and gastric carcinoma; 5% prostate carcinoma; 4% renal or uterine cancer; 2% bladder, pancreas, ovarian and liver cancers, melanoma and head and neck malignancies; 1% brain and central nervous system, gallbladder and biliary tract malignancies. However, there were no malignancies of the skin, esophagus, small intestine, vulva and vagina, ureter, bone, testis, eye and penis, after thyroid cancer.

The other large pan-cancer study was carried out by Ruan and colleagues (7) in 2023 using the SEER database, supplemented by a two-sample Mendelian randomisation study using UK Biobank data to search for causality. The Mendelian randomisation methodology uses genetic diversity to investigate possible causal associations between specific risk factors or clinical outcomes. In total, 264 significant SIRs were found in 22 solid tumors or hematologic malignancies. Notably, second primary thyroid cancer, small bowel cancer and endocrine malignancies were strongly and significantly associated with most first primary cancers. Second primary thyroid cancer was more common after first primary renal, oropharyngeal, gastric, pancreatic, male urothelial, lung, esophageal, liver, colorectal, ovarian, female breast, cervical, prostate and bladder malignancies, as well as lymphoma, primary melanoma, multiple myeloma and leukemia. On the other hand, first primary thyroid cancer was more common after second primary bone and soft tissue, kidney, male breast, female breast, small bowel, endocrine, oropharyngeal, prostate and brain malignancies, and also cutaneous melanoma, non-Hodgkin lymphoma, myeloma and leukemia. After thyroid cancer, however, esophageal and cervical malignancies were less common.

According to the results of the study (7), there was often no concordance between SIR values in SEER and Mendelian randomization results. For example, in thyroid cancer, no correlation with Mendelian randomization was found for any tumor. This may mean that there is no causal relationship between these tumors, but it seems more likely to indicate a detection bias due to increased diagnostic screening. It should be noted that the results of the Mendelian randomisation test are influenced by several factors, such as follow-up

time, so a causal relationship is likely with this method in the case of a positive correlation but cannot be excluded in the absence of a positive correlation.

Allen et al has provided data about the risk of second primary thyroid cancer after female (8) and also male (9) breast cancer. According to his results, the risk of second primary thyroid cancer was the highest among women with breast cancer (SIR= 1,89; 95% CI 1,49-2,38). Moreover, the risk of second primary thyroid cancer after male breast cancer (SIR= 5,58; 95% CI: 1,04-30,05) was also elevated.

The increased co-occurrence of breast cancer and differentiated thyroid cancer compared to chance alone has been found repeatedly in recent decades (4-15). A number of hypotheses have been suggested to explain these findings (12, 13, 15), implying the multifactorial origin of this association (Figure 1). These include the earlier detection of indolent thyroid cancers due to increasingly sensitive diagnostics, carcinogenic treatment (radiotherapy, chemotherapy, radioiodine therapy) given to breast cancer, radiation exposure due to increased diagnostic activity (e.g. mammography), hormonal factors, lifestyle factors (smoking, alcohol, obesity, diabetes, sedentary lifestyle) and rare genetic syndromes, as well as overall genetic susceptibility.

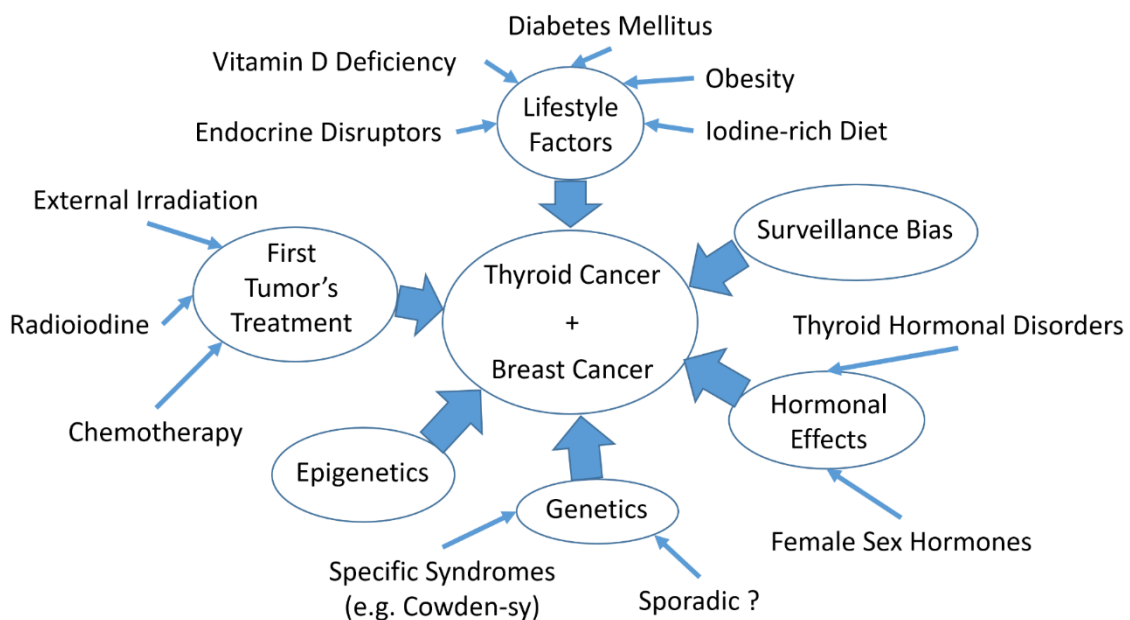


Figure 1. Potential factors associated with the co-occurrence of thyroid and breast cancer

A shared genetic background of these cancers (thyroid and breast cancer) is also a potential explanation for the observed association. Tan and colleagues (16) investigated

the causal association between thyroid cancer and breast cancer in a two-sample Mendelian randomization study. Their results found that, based on GWAS data from 342,499 individuals, a higher genetic predisposition to ER (estrogen receptor) positive breast cancer was also associated with a higher risk of thyroid cancer (OR=1,135; 95% CI: 1,006-1,279). However, this relationship was not observed for triple negative breast cancer.

PTEN hamartoma tumor syndrome consists of Cowden/Cowden-like syndrome, Bannayan-Riley-Ruvalcaba syndrome and Proteus-like syndrome, that are characterized by the presence of hamartomas and an increased risk of, among others, both thyroid and breast cancer. These conditions are mostly monogenic disorders with an autosomal dominant inheritance. Mutations in the PTEN, SDHB, SDHD, MTHFR, PARP4, MANCR and VEGF genes, and hypermethylation of KLLN have all been implicated (15, 17-20).

Much less is known about the genetic background of sporadic cases of metachronous or synchronous breast and thyroid cancer (20). In a 1994 publication (21), an increased risk of thyroid cancer was described in first-degree relatives of breast cancer patients (Family Relative Risk = 1,69; 95% CI 1,3-2,2), implicating a potential germline mutation underlying the association. No further genetic studies are available on the sporadic co-occurrence of thyroid and breast cancer.

II. MicroRNA expression profiling in papillary thyroid cancer

In cytological studies of thyroid nodules, Bethesda III and IV occur in approximately 20-30% of cases. These are considered indeterminate readings, carrying a risk of malignancy of 10-30% and 25-40%, respectively, therefore, further testing is necessary for clinical decision-making (22). A readily available minimal invasive diagnostic option in both categories is molecular testing, which can provide valuable information, taking into account cytological, radiological, and clinical factors (22, 23). In addition to DNA-based molecular testing, microRNA-based diagnostics also show promising results in indeterminate cytological readings (24), as well as in determining the prognosis of already diagnosed thyroid cancer (25).

MicroRNAs (miRNAs) form a special group of RNAs, being small and non-coding nucleic acids with a fine-tuning regulatory role in gene expression (26). Briefly, miRNAs

are around 18–25 nucleotides in length and take effect by binding to their complementary sequences on messenger RNA (mRNA) transcripts, which could result either in target degradation or translational repression and, by that, gene silencing (27) (Figure 2). miRNAs are quite stable molecules, making them accessible from a variety of biological samples such as fresh tissues, fine-needle aspiration biopsy (FNAB) specimens (28, 29), blood samples (30, 31), or even from formalin-fixed paraffin-embedded (FFPE) tissues (32). miRNAs represent a long-hidden and complex layer of gene regulation that is a crucial component in many physiological and pathological processes including cardiovascular diseases (e.g., cardiac fibrosis and cardiac hypertrophy) (33, 34), microbiome homeostasis (35), diabetes mellitus (36, 37), calcium and bone metabolism (38, 39), as well as in the pharmacodynamics of certain drugs (40). miRNAs influence many cellular processes such as differentiation, proliferation, apoptosis, and metastasis development (41-44). In addition, research on the role of miRNAs in anti-cancer drug sensitivity in thyroid cancer has also been published (31).

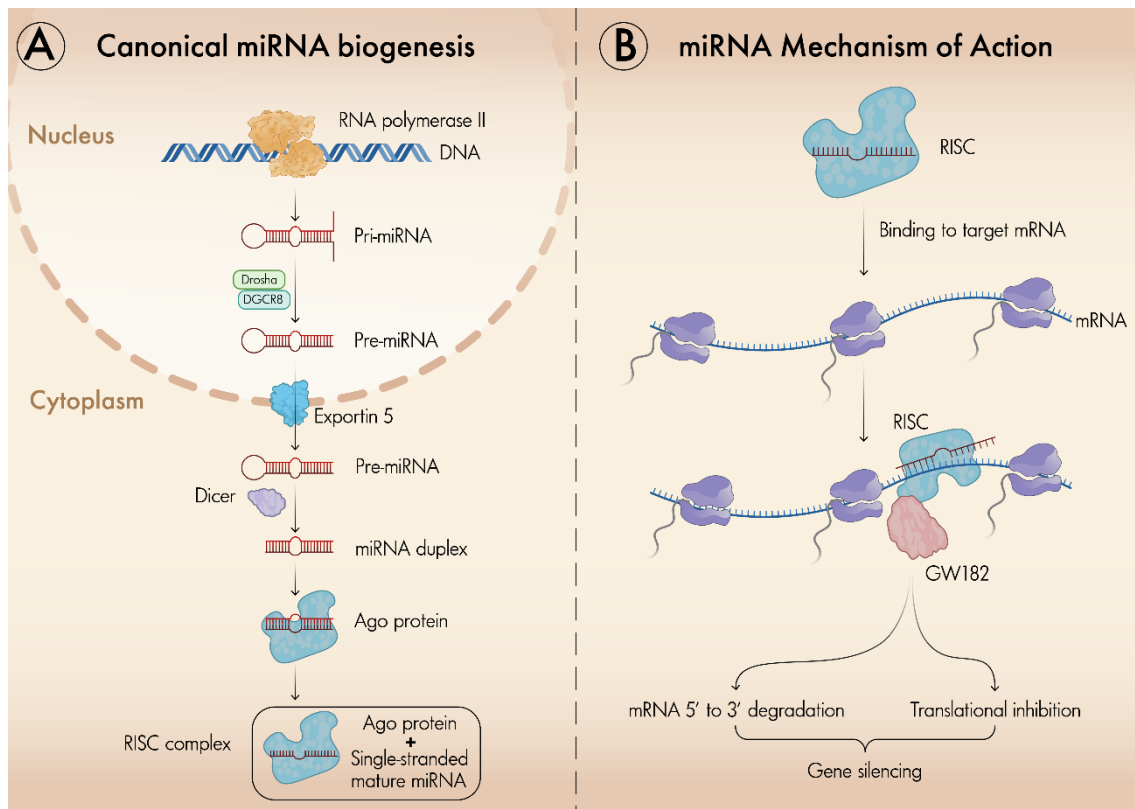


Figure 2. Canonical miRNA biogenesis and miRNAs' mechanism of action. A: pri-miRNA is transcribed from DNA by RNA-polymerase II. Then pri-miRNA is cropped by the Drosha-DGCR8 complex to produce the pre-miRNA. Pre-miRNA is transferred from the nucleus to the cytoplasm by Exportin 5. In the cytoplasm, Dicer cleaves the hairpin region of miRNA. The resulting double-stranded (duplex) miRNA binds to Ago protein (Ago1-4), after which one strand is eliminated, forming the RNA-induced silencing complex (RISC) containing the mature single-stranded miRNA and the Ago protein. B: The miRNA in RISC binds to the complementary region of the mRNA target, resulting in translational inhibition with/without mRNA degradation, mediated by GW182 protein. (45, 46) (Illustrated with Adobe Illustrator)

In oncology, miRNAs are receiving more and more attention due to their duality acting both as oncogenes and tumor suppressors (47). Emerging data suggest that different molecular backgrounds, including aberrant miRNA expressions, are a common feature of various cancers (48, 49), including thyroid malignancies and their most common form, PTC, in particular (50). PTC is seemingly well known by many clinicians and pathologists, although it is still slowly turning into a more heterogeneous category, which can be further divided not only into clinicopathological subtypes but molecular ones as well (51). Admittedly, some papers have already been published investigating the role of

miRNAs in PTC pathogenesis (52-54); however, establishing the exact depth of the correlation between specific miRNA expressions and the development of the disease lacks original molecular studies with sufficiently large control case numbers even to this day. Also, these studies provide markedly smaller coverage of miRNA types. For instance, even The Cancer Genome Atlas (TCGA) project involved PTCs with only 59 matched controls for miRNA analysis and evaluated 1046 different miRNAs while lacking clinicopathological aspects (52). miRNAs' association with the pathogenesis of other conventional thyroid cancer types such as follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), or anaplastic thyroid carcinoma (ATC) has also been noticed (55). In cases when differentiated thyroid carcinoma (DTC) (including PTC and FTC) was diagnosed, tumor development might be traced back to a DICER1 RNase IIIb hotspot mutation generating an unbalanced expression ratio of 5p:3p miRNAs (56).

PTC, characterized by its unique molecular signature, often exhibits alterations in miRNA expression (44, 48, 57). The link between these miRNA deviations and disease development is making specific miRNAs perfect candidates for being diagnostic or prognostic markers. Indeed, miR-21, miR-127, miR-136, miR-146b, miR-221, and miR-222 are frequently upregulated in PTC and are associated with a more aggressive course of the disease and an overall poorer prognosis (31, 44, 49, 52, 55, 58-65). Also, *BRAF*-targeting miR-486-5p, miR-9-5p, and miR-708-3p in PTC were previously described as downregulated and – in case of miR-486-5p – associated with lymph node metastasis development as well as more advanced tumor stage and recurrence risk (53). Based on previous findings, the 5p/3p expression ratio of specific miRNAs might also play a role in the onset of lymph node metastases (54). Upregulation of miR-181a in thyroid cancer has also been identified previously (44). Moreover, expression alterations of miRNAs such as miR-204, miR-146b, miR-221, and miR-222 have been described in association with important cancer features like extrathyroidal invasion and/or metastasis development (42, 44, 64, 65). Alternatively, a decrease in tumor-suppressing miRNA levels, such as let-7, miR-125b, and miR-204-5p (52, 66), has also been noted, propagating the malignant conversion of thyroid cells (67). Furthermore, miR-136, miR-21, and miR-127 showed significant correlation with the risk of persistent disease and potential relapse as well as with the risk categories defined by the American Thyroid Association's (ATA) risk stratification system (68, 69).

Highlighting the importance of miRNAs in papillary thyroid cancer, a large-scale, comprehensive research aiming miRNA expression profiling was warranted.

III. Preoperative assessment of thyroid cancer

Despite the increasing incidence of differentiated thyroid cancer, the mortality rate of the disease persists (2). Overdiagnosis by the general use of novel ultrasound (US) techniques is one of the main reasons for the „TC epidemic” (70-72). However, novel data shows a 1,1% per year increment of thyroid cancer mortality, moreover, a 2,9% per year increment of advanced stage papillary thyroid cancer (PTC) and 1,7% per year increment of all PTC between 1994-2013, suggesting a genuine increase of thyroid cancer incidence, which can not be explained by overdiagnosis alone (72). In another study (73), the 10-year survival was 99,9% and 97,8% for TNM stage I-II PTC, and 100,0% and 94,4% for follicular thyroid cancer (FTC). For stage IVb cancer, PTC had 52,3% and FTC had 15,2% mortality. Nevertheless, the mostly indolent behavior of differentiated thyroid cancer (68) requires the most conservative, cost-efficient, yet well-founded and free of complication management.

The most controversial issue is the first therapeutic step, the surgical approach of the 1–4 cm low risk DTCs in resectable cases. Papillary (83,6%) and follicular (10,8%) thyroid cancer are considered well differentiated, and these consists of the majority of thyroid cancers (beside medullary, anaplastic and others) (72). Of all thyroid cancers, 28.6% are under 1 cm and 9.6% are over 4 cm (13% of unknown size). Half of thyroid cancers (49%) are in the 1-4 cm size range (72). For low risk, well differentiated thyroid cancers of 1-4 cm, two main approaches exist: initial lobectomy or total thyroidectomy. Primary lobectomy alone may lead to a potentially lower surgical complication rate and less frequent need for thyroxine (T4) substitution. The 2015 guidelines of the American Thyroid Association (ATA) (68, 74), follow this approach and advocate for lobectomy in low risk DTCs between 1 to 4 cm in diameter. At the time of our research, the 2015 ATA guideline was in effect, therefore we used that as a basis, and this is what we refer to in the text as „ATA”. The most recent, 2025 ATA guideline introduce a similarly lenient approach (75), thus, the results of our findings do not lose their validity. The final risk stratification, however, is based on the postoperative pathologic assessment (e.g. lymph node metastasis, angioinvasion, extrathyroidal invasion, multifocal disease, etc.). The

initial diagnostics and the factors needed to be considered for the initial management of differentiated thyroid cancer is depicted on Figure 3.

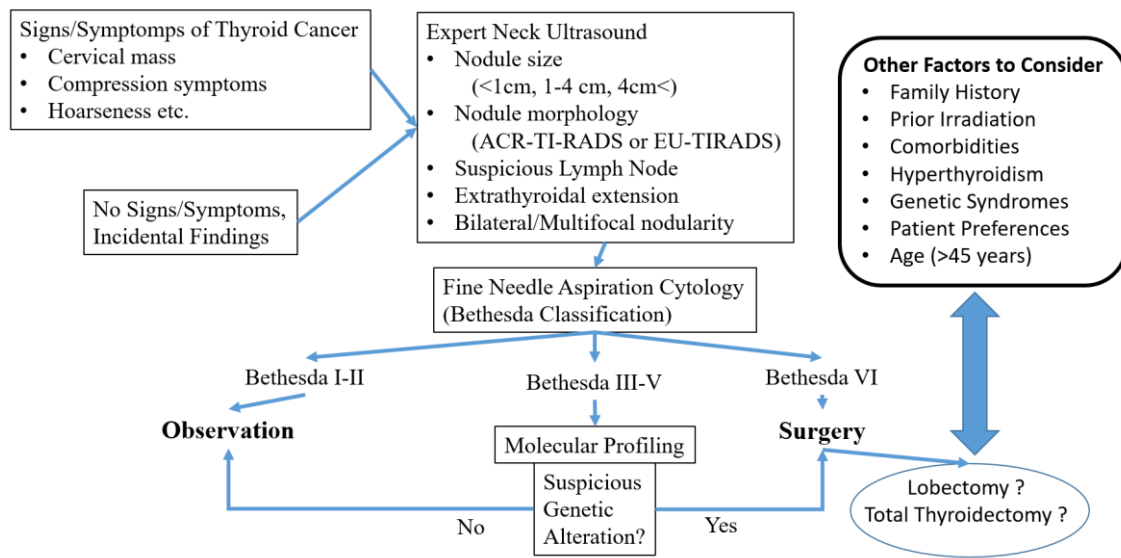


Figure 3. The initial diagnostics and the factors needed to be considered for the initial management of differentiated thyroid cancer. ACR-TI-RADS: American College of Radiology Thyroid Imaging Reporting and Data EU-TIRADS: European Thyroid Imaging and Reporting Data System.

After the final histology result, contralateral lobectomy is needed in certain cases. These microscopic features are less well predictable by prior clinical investigation (76). Undiscovered contralateral foci are also needed to be considered. Thus, the greatest concern about this approach is the high rate of completion thyroidectomy (77-79), with all its disadvantages which include a second general anesthesia and hospital admission, surgical difficulties raised by scar tissue, but also psychological concerns. Furthermore, without the total/near total removal of thyroid tissue, the adjuvant I-131 ablation and the serum thyroglobulin (Se TG) follow-up could not be achieved. Thus, the European Thyroid Association (ETA) guidelines (80) suggest a lower threshold (1 cm or above in tumor diameter) for total thyroidectomy taking into account the above detailed considerations.

It should be noted that the need for thyroxine supplementation is not avoidable in all cases after thyroid lobectomy alone. According to ATA recommendation (68), mild TSH suppression (TSH 0,5-2,0 mU/L) is recommended after hemithyroidectomy. Based on two studies, 76% (81) and 80% (82) of patients required levothyroxine substitution to

meet these criteria. With logistic regression, only preoperative THS value more than 2mU/L served as a predictive factor for postoperative thyroxin supplementation (OR= 1,7; 95% CI 1,059-3,403). In a large meta-analysis (83) the overall incidence of hypothyroidism showed 29% (95% CI 25-34%), and the transient hypothyroidism occurred in 34% of patients (95% CI 21-47%). Moreover, female sex, age, right sided lobectomy, the preoperative presence of anti-TPO and anti-Thyreoglobulin antibodies and Hashimoto's thyroiditis were all independent risk factors for thyroxin supplementation, beside preoperative high (≥ 2 mU/L) TSH level.

To assess the levothyroxine treatment dose after total/near total thyroidectomy, our research group has conducted a study in 2024 (84). Among the 44 patients treated with total thyroidectomy, based on the univariate analysis, only age ($F = 6.61$, $\beta = -0.011$, $p = 0.011$) affected LT4 dose (mcg/kgBW) significantly meaning that thyroxin requirement decreases with higher ages. Interestingly, the mean levothyroxine dose to achieve stable euthyroidism was $1,27 \pm 0,38$ mcg/kgBW (microgram/bodyweight kilogram), which is significantly lower, than it is suggested by the current guidelines (1,6-1,8 mcg/kgBW) (85).

In another study by our research group an investigation was carried out to establish the factors associated with quality of life in patients with well-controlled hypothyroidism. In our final 157 patients with $11,2 \pm 8,2$ years disease duration (70,7% Hashimoto's and 29,3% iatrogenic hypothyroidism) and with a TSH value of $1,8 \pm 0,9$ mIU/L, thyroid specific markers such as FT3, FT4, reverse-T3, TSH, anti-TPO antibody positivity, sex-hormone-binding globulin, the sum activity of deiodinase activity, neither was associated with thyroid-dependent quality of life. However, based on our multivariable model ($r^2 = 0,31$), psychological factors as well as somatosensory amplification ($p = 0,002$), depression ($p < 0,001$), also body mass index ($p = 0,021$) were predictors of thyroid-dependent quality of life, emphasised somatosensory amplification as a strong predictor (86).

Altogether, the choice of the primary surgical approach is of great importance and has significant implications. A comparison of the 2015 ATA guidelines, ETA guidelines, and real-life practice using a real patient data set would be warranted.

Based on the above, three major challenges can be identified:

1. We have observed an increased co-occurrence of thyroid cancer and breast cancer, and we aim to investigate the underlying genetic background of this association.
2. In cases classified as Bethesda category III-IV on cytology, which account for approximately 20-30% of cases, clinical decision-making remains uncertain. While DNA-based molecular testing from cytological samples is available, microRNA expression profiling may provide additional diagnostic value.
3. Once a diagnosis of thyroid cancer has been established, the optimal surgical approach remains controversial. Reoperation rate is relatively high, and current clinical guidelines differ in their recommendations regarding the extent of initial surgery.

2. OBJECTIVES

1. We investigated the potential for a shared genetic background of thyroid and breast cancers (20). The recognition and elucidation of such genetic risk factors could help in the identification of the other tumor, selection of the initial surgical approach (lobectomy/total thyroidectomy), follow-up, and potential preventive treatment of high-risk individuals with a first primary malignancy.
2. To increase the diagnostic accuracy in distinguishing between malignant and benign thyroid nodules in cases with indeterminate cytology, in a study we aimed to reveal differences in miRNA expression patterns between cancerous and non-cancerous thyroid tissue samples related to patients previously diagnosed with PTC (51). A better understanding of miRNA expression profiles of thyroid cancer may also provide insights into disease progression risk, histological findings, offering a more reliable target regarding choosing the extension of the first surgical approach, as well as potential therapeutic targets in the future (63, 65).
3. In a retrospective cohort study, our aim was to investigate the reliability of preoperative assessment of established malignant thyroid nodules, and to compare the differences in outcomes that are potentially achievable with adherence to ATA or ETA guidelines, using real-life patient data from our institution (87).

3. METHODS

I. Investigation of the shared genetic background of breast and thyroid cancer

I.1 Patients and setting

We analyzed the genetic polymorphisms in 112 hereditary cancer risk genes in a case-control study of patients with a history of either breast or both breast and thyroid cancer. (20)

Patients were selected from a pool of $n = 274$ thyroid cancer patients who had received I-131 treatment at our institution between January 2014 and October 2018. Twenty-one of these individuals received treatment for breast cancer before or after the diagnosis of a thyroid cancer. Two patients could not have been reached for consent, resulting in a final sample of 19. Given that the risk of thyroid cancer development after breast cancer is larger than vice versa, the control group consisted of 15 subjects who received treatment for breast cancer at our institution at least 12 years before the present study. Patients with a personal or a family history of thyroid cancer were excluded from this group. These criteria were specified to minimize contamination of the control group with patients with an increased genetic risk for thyroid cancer development. No other inclusion or exclusion criteria were set. (20)

We collected the following clinical characteristics from hospital and outpatient records: age at cancer diagnosis, tumor size, histology, staging and grading, presence or absence of lymph node and distant metastases, details of surgical-, radiation- chemotherapeutic-, hormonal and I-131 treatment. Cancer diagnosis for all cases was based on histological data using the WHO classification of breast and endocrine tumors (4th and 3/4th edition respectively). Staging was based on the TNM system (7th edition). Informed consent was obtained from all subjects before any study related procedures were performed. The study was approved by Semmelweis University Regional and Institutional Committee of Science and Research Ethics. (20)

I.2 Genetic analysis

We have aimed to cover all the well-established hereditary cancer risk loci. The final list of the 112 investigated genes was compiled based on literature data (88-90) and is shown in Table 1.

Table 1. List of cancer risk genes assessed. BC associated genes are underlined (20)

ACD	CDKN1B	FANCA	JAGN1	NFIX	PTEN	TERC
AIP	CDKN2A	FANCB	KIF1B	NHP2	RAD50	TERT
APC	<u>CHEK2</u>	FANCC	KIT	NOP10	RAD51	TINF2
<u>ATM</u>	DDB2	FANCD2	MAX	NSD1	RAD51C	TMEM127
ATR	DICER1	FANCE	MDH2	NTHL1	RAD51D	TP53
AXIN2	DIS3L2	FANCF	MEN1	<u>PALB2</u>	RB1	TSC1
BAP1	DKC1	FANCG	MET	PARN	RECQL	TSC2
BARD1	ELANE	FANCI	MITF	PDGFRA	RECQL4	UBE2T
BLM	EPAS1	FANCL	MLH1	PMS1	RET	VHL
BMPR1A	EPCAM	FANCM	MNX1	PMS2	RTEL1	VPS45
<u>BRCA1</u>	ERCC1	FH	MSH2	POLD1	SCG5	WAS
<u>BRCA2</u>	ERCC2	FLCN	MSH6	POLE	SLX4	WRN
BRIP1	ERCC3	G6PC3	MSR1	POLH	SMAD4	WT1
BUB1	ERCC4	GFI1	MUTYH	POT1	SMARCA4	XPA
<u>CDH1</u>	ERCC5	GREM1	NBN	PRKAR1A	STK11	XPC
CDK4	ERCC6	HOXB13	NF2	PTCH1	SUFU	XRCC2

Exome amplicon library was prepared using the Ion AmpliSeq Library Kit Plus combined with the Ion AmpliSeq Exome RDY kit (ThermoFisher, MA, USA). Briefly, 100 ng of genomic DNA was added to dried down, ultra-high multiplexed primer pairs (12 pools) in a 96-well plate and amplified with the following PCR conditions: at 99 °C for 2 min; at 99 °C for 15 s and at 60 °C for 16 min (10 cycles) and holding at 10 °C. Primers were

partially digested using a FuPa reagent, and then sequencing adapters and barcodes were ligated to the amplicons. The library was purified using the Agencourt AMPure XP Reagent (Beckmann Coulter, CA, USA). The concentration of the final library was determined by Ion Library TaqMan Quantitation Kit (ThermoFisher, MA, USA) on an ABI 7500 qPCR instrument with absolute quantification method. (20)

Template preparation was performed with Ion 540 OT2 Kit (ThermoFisher, MA, USA) on semi-automated Ion OneTouch 2 instrument using emPCR method. After breaking the emulsion, the non-templated beads were removed from the solution during the semi-automated enrichment process on Ion OneTouch ES (ThermoFisher, MA, USA) machine. Later adding the sequencing primer and polymerase, the fully prepared Ion Sphere Particles (ISPs) were loaded into an Ion 540 chip, and the sequencing runs were performed using the Ion S5 Sequencing kit (ThermoFisher, MA, USA) with 500 flows. (20)

Sequence data from the Ion Torrent run were analyzed using the platform-specific pipeline software Torrent Suite v5.10 for base calling, trim adapter and primer sequences, filtering out poor quality reads, and de-multiplex the reads according to the barcode sequences. Briefly, TMAP algorithm was used to align the reads to the hg19 human reference genome, and then, the variant caller plug-in was executed to search for germline variants in the targeted regions. Integrative genomics viewer (IGV) was used for visualization of the mapped reads. Variants were annotated using the Ion Reporter (ThermoFisher, MA, USA) software. Variant interpretation was restricted to the selected genes with known connection to oncology diseases. Variants were stratified by gene association with breast cancer and by variant association with cancer (no/potential/known) based on available data from the ClinVar database at the time of writing (91). Synonymous mutations were not excluded from the analysis. (20)

I.3 Statistical analysis

Descriptive data are given as means $\pm$ standard deviation (SD) for continuous data, and frequencies (percentages) for categorical variables. Between-group differences in clinical parameters were assessed using 2-sample t tests for continuous variables, and χ^2 tests or Fisher exact tests for categorical variables. (20)

When comparing the number of different variants across groups, the assumptions of normality and homogeneity of variances were assessed using the Shapiro–Wilk test and

Levene's test respectively. Given that in some cases these assumptions were not met, Welch's t-test was used to compare the number of variants with known, potential or no association with breast cancer and with any cancer. We also used the bootstrap procedure to get robust confidence intervals and *p*-values. To increase statistical power, we lumped together variants with potential and known associations using both weighted and unweighted scores. To generate the weighted scores, we gave double weight for variants with known association compared to those variants with potential association. To correct for multiple testing, we applied the Benjamini-Hochberg procedure targeting a false discovery rate < 15%. A corrected two-tailed *p*-value less than 0.05 was considered statistically significant. Effect sizes were computed as adjusted standardized mean differences (Hedge's *g*). Analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp. Released 2015 Armonk, New York, U.S.A.). (20)

II. MicroRNA profiling in papillary thyroid cancer

We conducted a comprehensive molecular investigation to detect specific miRNAs associated with PTC based on their expression differences between tumor sections and adjacent non-tumor tissues. We evaluated the miRNA profiles of thyroid tissue samples (one cancerous and one healthy sample per case) related to patients previously diagnosed with PTC and analyzed the sequencing data of the samples in the context of 2656 types of human miRNAs, doubling the control-matched sample size and more than doubling the examined miRNA cluster size compared to the TCGA study (50). We matched the miRNA profile with the clinicopathological variables of the patients, aiming to find a more accurate preoperative diagnostic approach. (51)

II.1 Study population, sample collection, and histopathological processing

Anonymized PTC tissue samples from patients who had consecutively undergone thyroid surgery and had previously been diagnosed and/or treated in our institution (Department of Medicine and Oncology, Semmelweis University, Hungary) were selected using clinical and histological databases. Then, the related clinicopathological data of the anonymized cases and their tissue blocks were collected from medical records of the Department of Medicine and Oncology, Semmelweis University, Hungary, and histopathological archives located at the Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Hungary, respectively. In this study, we included the following clinicopathological data as variables: age, sex, ATA (2015) risk

score, TNM stage, and cancer stage based on the 8th edition of the American Joint Committee on Cancer (AJCC). The collected tissue samples were FFPE blocks from which hematoxylin–eosin-stained probe sections were performed to confirm tumor presence and histological type and to determine the percentage of tumor volume. Only those PTC samples which had a sufficient proportion of tumor burden at the site of interest within the Formalin-Fixed Paraffin-Embedded (FFPE) block relative to the overall tissue volume were involved in further processing. This assessment involved conducting a trial section on the FFPE block, which was then stained with hematoxylin and eosin and examined under a microscope by a pathologist to exclude samples containing less than 80% relative tumor volume. Also, the collection of the non-tumor control samples was optimized by targeting tissue areas as far from the PTC localizations as technically feasible, usually the contralateral lobe of the thyroid. (51)

Further preparation of the carefully selected tumor tissue blocks was carried out using macrodissection producing 4 pieces of 10 μ m thick sections per sample, which were subjected to molecular diagnostics. An area containing tumor as well as normal tissue was dissected from the same sample, thus resulting in 129×2 specimens. (51)

miRNAs were isolated from 258 tissue samples (related to the cancerous and normal samples of 129 PTC patients). Ten tissue sample pairs were excluded due to inapplicable isolate specimens; the remaining ones were then forwarded to sequencing. Via sequencing, expression patterns were examined in the context of 2656 different types of miRNAs in total. During the bioinformatic assessment following sequencing, one more sample pair was also excluded due to insufficient sequencing yield. After all necessary exclusions, tumor and control sample pairs related to a total of 118 PTC patients were included in the evaluation. (51)

II.2 Molecular processing (miRNA isolation, quality control (QC), miRNA quantification, and sequencing)

Zymo Quick RNA FFPE kit (Zymo Research, Irvine, CA, USA) was employed for the isolation of miRNAs from the prepared FFPE tissue sections. The process started with the removal of paraffin using proprietary deparaffinization solution, which was followed by rehydration of the tissue. The tissue was then subjected to proteinase K digestion at 55 °C for 2 h and subsequently at 65 °C for an additional 15 min to ensure thorough lysis. After digestion, the samples were treated with RNA lysis buffer, which facilitated the

selective binding of miRNAs to the kit's Zymo-Spin IICR column. The column was then washed multiple times to remove contaminants. To ensure the elimination of genomic DNA, the samples were treated with DNase. Finally, the miRNAs were eluted in 50 μ L of elution buffer. During molecular analysis, we performed RNA quality assessment. Initially, we determined RNA concentrations using the Qubit™ HS RNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on a Qubit™ 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). When necessary, the concentration of miRNAs was measured again using the Qubit™ microRNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) also on a Qubit™ 3.0 fluorometer. (51)

Then, we prepared miRNA libraries in the following multi-step process using NEXTFLEX® Small RNA-Seq Kit v4 (PerkinElmer Inc. Waltham, MA, USA). We started with an input of 50 ng of RNA, followed by the ligation of the NEXTFLEX® 3' adenylated adapters. After that, we removed the excess 3' adapters, then we ligated the NEXTFLEX® 5' adapters. This was succeeded by the reverse transcription, first-strand synthesis; post-synthesis, we conducted bead cleanup, then polymerase chain reaction (PCR) amplification was performed using barcoded primers (19 cycles). Lastly, we finished miRNA library preparation with size selection and cleanup. (51)

The next step was the quality control of the miRNA libraries involving DNA concentration measurement using the Quant-iT™ 1X HS dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on either a FLUOstar Omega fluorometer (BMG Labtech, Ortenberg, Germany) or a Qubit™ 3.0 fluorometer, along with assessment of the fragment sizes using the LabChip® GX Touch™ nucleic acid analyzer (PerkinElmer Inc. Waltham, MA, USA) with an HT DNA X-Mark Chip (CLS144006) (PerkinElmer Inc. Waltham, MA, USA) with the HT DNA NGS 3K Reagent Kit (PerkinElmer Inc. Waltham, MA, USA). (51)

For pooling the libraries, we calculated the molar concentrations based on the overall concentrations and the fragment sizes. Then, equal molar quantities were pooled from the libraries. The concentration of this pool was measured using the Quant-iT™ 1X HS dsDNA Assay Kit again and diluted to the final concentration of 2 nM. (51)

Finally, the sequencing was carried out on a NextSeq 2000 system (Illumina Inc., San Diego, CA, USA) using a P3 (1 $\times$ 50 cycles) kit. This setup allowed us to generate 2 $\times$ 40 bp paired-end reads. miRNAs showing expression levels below the set threshold of the

applied NGS platform both in the PTC and control samples were considered as not-expressed miRNAs. During the process, we maintained the seeding concentration at 650 pM to ensure optimal sequencing depth and quality. (51)

II.3 Data analysis via bioinformatics and statistical evaluation

The quality check of the raw reads was performed via FastQC v0.11.7 and MultiQC. Forward and reverse reads were merged via PEAR v0.9.11 and then quality trimmed with Trim Galore v0.6.10. The quality threshold was set to 30 and only reads between 18 and 30 base pairs (bp) in length were used for further analysis based on the literature. One sample pair was removed from the analysis due to insufficient sequencing yield compared to the other samples. Based on recommendations, Bowtie1 v1.3.1 was used for the alignment of the reads to the miRBase v22.1 *H. sapiens* miRNA (hsa-miRNA) database with the following parameters: -n 0 -l 8 --best --strata -m 1 -no-unal. Read counts were calculated for each miRNA using SAMtools v1.14. (51)

The expression levels of miRNAs can span several orders of magnitude, making the direct comparison of raw data challenging and less informative. To address this, we presented our data using logarithmic values, specifically $\log_2$ fold change for expression levels and $-\log_{10}P$ for p-values. We applied a threshold for significant differential expression with a minimum $\log_2$ fold change of ± 1 and a p -value below 0,05 for determining the statistical significance. By using a logarithmic transformation, we were able to mitigate the impact of extreme values, create a symmetrical view of up- and downregulation (both of which can be relevant), and make our results more visible. Principal component analysis (PCA) is another statistical tool we applied with which we were able to reduce data dimensionality by identifying the most important patterns that describe the data variability. PCA also reorients the data into principal components, which are new, uncorrelated variables ordered by their importance. (51)

Statistical analysis of the read counts was performed in the R v4.2.1 programming environment. Differential miRNA expression was calculated with the DESeq2 package. miRNAs were evaluated to be significantly differentially expressed if the absolute value of the estimated $\log_2$ fold change was higher than or equal to 1 and the Benjamini–Hochberg-corrected p -value was less than or equal to 0,05. (51)

III. Surgical strategies and preoperative assessment in differentiated thyroid cancer

III.1 Patients and setting

We performed a retrospective patient database analysis. We included patients who were referred to our tertiary referral center with differentiated thyroid cancer between January 2014 and October 2018 for radioiodine treatment evaluation. Surgical indications and operation plans were established in other centers in some cases. Although surgical indication could either be clinically benign or malignant thyroid disease, our patients were selected retrospectively based on histopathology. Inclusion criteria were: final histology of DTC (any subtype of papillary, follicular or Hürthle-cell carcinoma) and total/near total removal of the thyroid gland (either in one or more steps). Patients whose thyroid gland had not been removed (near) totally were excluded from the evaluation. Cancer diagnosis for every case was based on histological data from surgical specimens, using the WHO Classification 3rd and 4th edition. Staging was based on the TNM system 7th edition. For preoperative staging, clinical investigation was used (ultrasound in all cases [most of them with FNAB], and CT, MR, PET-CT in some cases). Mostly a selective lymph node dissection was performed, but in some cases, documentation was poor, and malignancy was not suspected preoperatively – resulting no lymph node dissection. No other inclusion or exclusion criteria were set. (87)

The analyzed patient data included age, past medical and familial history (neck and thoracic irradiation, thyroid cancer in relatives), preoperative tumor characteristics, type of surgery, surgical complications and postoperative histological findings. Preoperative tumor characteristics were: size on imaging, multifocality and/or bilateral tumor presence, lymph node (LN) and/or distant metastases, extrathyroidal extension, aggressive histologic variant with FNAB. Postoperative histological findings included: cancer type and subtype, pathological staging, angioinvasion, tumor capsule invasion, extrathyroidal extension, signs of incomplete tumor resection. (87)

A./ We retrospectively evaluated the different outcomes that could have been achieved using surgical approaches based on the ATA (2015) and ETA guidelines and these were compared to our practice. Clinical data from all patients were re-evaluated using both of these guidelines and we established the type of surgery that would have been indicated based on the preoperative data. In cases where one or both guidelines advocated for

lobectomy, the need for hypothetical reoperation was evaluated based on postoperative data. (87)

B./ We compared our institute's practice to both the ATA and ETA guidelines by analyzing all cases where one step total/near total thyroidectomy was performed despite one or the other association's guideline recommending lobectomy based on the preoperative data.

We evaluated the need for completion thyroidectomy after a hypothetical lobectomy based on the available postoperative histological data.

C./ We assessed the accuracy of our preoperative evaluation in predicting the optimal surgical course when compared to postoperative histological data. Positive and negative predictive values (PPV and NPV, respectively), sensitivity and specificity were calculated. Cohen's kappa was calculated to evaluate concordance between preoperative ATA or ETA recommendations and the histopathology based correct course of action.

We examined the utility of preoperative ultrasound and clinical findings in predicting the optimal surgical strategy. These included tumor diameter, age, sex, and presence of Hashimoto thyroiditis. The relevance of tumor size in predicting individual postoperative high-risk features, such as bilateral tumor, angioinvasion, extrathyroid extension, incomplete surgical resection, aggressive histology and lymph node metastasis, was also evaluated.

D./ We compared the complication rate in different types of surgeries. (87)

III.2 Statistical methods

Data are given as mean $\pm$ SD for continuous and frequencies for discrete variables. Between-group differences with categorical predictors and outcomes (e.g. complication rates of different surgical strategies) were assessed using Chi-square and Fisher's exact test. In cases with one or more independent scale variable (e.g. predictive value of tumor diameter on surgical outcome), binary logistic regression was used to build the appropriate model. Unless otherwise noted *p*-values less than 0.05 are considered statistically significant. Analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp. Released 2015 Armonk, New York, USA.). (87)

III.3 Ethical approval

The study was approved by Semmelweis University Regional and Institutional Committee of Science and Research Ethics (Egészségügyi Tudományos Tanács, Tudományos és Kutatásetikai Bizottság (under case number IV/4097–2/2020/EKU)). Since this retrospective cohort study was performed on an anonymous database, the informed consent was waived. (87)

4. RESULTS

I. Investigation of the shared genetic background of breast and thyroid cancer

Table 2 displays patients' clinical characteristics. Breast cancer (BC) only patients had a slightly younger age at the time of breast cancer diagnosis compared to the synchronous/metachronous cancer group (47,7 vs 54,4, $p = 0,079$). All other clinical parameters evaluated were similar in the BC and BC-TC groups (as BC-TC group refers to the synchronous/metachronous breast- and thyroid cancer patients). It is worth noting that no difference was found in the frequency of the different BC treatment modalities (irradiation, hormone or chemotherapy). (20)

Table 2. Patient characteristics at BC diagnosis and at follow-up (20)

	BC only		BC and TC		p-value
	Mean / No of cases	SD / %	Mean / No of cases	SD / %	
No. of patients	15		19		
Age at follow-up	67,3	9,2	62,3	10,5	0,16
BC clinical characteristics					
Age at BC diagnosis	47,7	10,2	54,4	11,2	0,079
BC grade					0,363
1	3	37,5%	3	20%	1
2	1	12,5%	6	40%	0,104
3	4	50%	6	40%	1
BC T stage					0,393
1	4	36,4%	7	58,3%	0,715
2	6	54,5%	5	41,7%	0,475
4	1	9,1%	0	0%	0,441
Lymph node metastasis	5	33,3%	8	42,1%	0,728
Vascular invasion	2	22,2%	2	15,4%	1
HER2-positivity	6	40%	4	21%	0,276
ER-positivity	11	73%	10	52%	0,296
PR-positivity	8	53%	8	42%	0,730
Invasive ductal carcinoma	13	86,7%	16	84,2%	1
Distant metastasis	3	20%	2	10,5%	0,634
BC radiation therapy	14	93,2%	17	89,5%	1
BC chemotherapy	10	66,7%	9	60%	1
BC hormone therapy	11	73,3%	10	62,5%	0,704
BC targeted therapy	1	6,7%	2	12,5%	1
BC relapse	4	26,7%	3	15,8%	0,672
TC clinical characteristics					
Age at TC diagnosis	-	-	53,5	15	-
Papillary histology	-	-	18	94%	-
Follicular histology	-	-	1	5,3%	-
Lymph node metastasis	-	-	4	21%	-

The results of the genetic analyses for the BC-TC patients and the control group are presented in Tables 3 and 4 respectively. Details of the statistical analysis are reported in Table 5.

Table 3. List of any cancer (and breast cancer) related genes and unique variants identified by individual patients in the BC-TC group. The strength of known association of each variant with oncogenesis is marked as known (**), potential (*) or no (no marker). (20)

BC-TC patients (no. of SNPs)	Gene (<i>known breast cancer gene</i>)	SNP	Effect of mutation *- potential **- known association
T+1 (4)	POLE	c.1738C>A	p.His580Asn*
	BUB1	c.677C>T	p.Ala226Val*
	WRN	c.355+4G>C	intronic*
	ERCC2	c.545C>T	p.Ala182Val*
T+2 (4)	SMARCA4	c.918G>C	p.Gln306His*
	TSC1	c.3109_3110insGCA	p.Gly1037_Ser1038insSer*
	BIVM-ERCC5,ERCC5	c.1954C>G, c.592C>G	p.Pro652Ala, p.Pro198Ala*
	NBN	c.511A>G	p.Ile171Val*
T+3 (5)	ATR	c.2924T>C	p.Leu975Ser*
	KIT	c.2695A>G	p.Met899Val*
	BMPRI1A	c.563G>A	p.Arg188His*
	<i>PALB2</i>	c.522A>G	p.(=)*
	HOXB13	c.251G>A	p.Gly84Glu**
T+4 (3)	<i>BRCA1</i>	c.3925A>C	p.Asn1309His*
	CDKN2A	c.- 15_8delGGCGGCGG GGAGCAGCATGGAG CC	p.Glu2_Pro3del**
	CDK4	c.625C>T	p.Arg209Cys
T+5 (5)	SMAD4	c.845A>C	p.His282Pro*
	XPC	c.155C>T	p.Ser52Leu*
	VPS45	c.566A>G	p.Glu189Gly*
	FANCM	c.527C>T	p.Thr176Ile
	<i>ATM</i>	c.4388T>G	p.Phe1463Cys
T+6 (4)	ATR	c.4357A>G	p.Ile1453Val*
	<i>ATM</i>	c.8983C>A	p.Leu2995Ile*
	<i>BRCA2</i>	c.8755-1G>A	intronic**
	<i>BRCA1</i>	c.692C>T	p.Thr231Met*
T+7 (6)	MUTYH	c.1276C>T	p.Arg426Cys
	ATR	c.4912C>T	p.Gln1638Ter**
	ATR	c.1546A>G	p.Thr516Ala*
	NSD1	c.3805T>C	p.Ser1269Pro*
	<i>BRCA2</i>	c.6968A>G	p.His2323Arg*
	<i>CHEK2</i>	c.614C>T	p.Thr205Ile*

Table 3. (continued)

BC-TC patients (no. of SNPs)	Gene (<i>known breast cancer gene</i>)	SNP	Effect of mutation *- potential **- known association
T+8 (4)	ATR	c.3424A>G	p.Ser1142Gly*
	PDGFRA	c.842C>T	p.Thr281Met*
	TINF2	c.488C>G	p.Pro163Arg*
	FLCN	c.592G>A	p.Asp198Asn
T+9 (1)	TERT	c.1234C>T	p.His412Tyr
T+10 (6)	TSC2	c.3820T>C	p.Ser1274Pro*
	FANCI	c.2011A>G	p.Ile671Val
	FANCI	c.2604A>C	p.Glu868Asp
	MFS3	c.1033C>T	p.Arg345Cys*
	<u>BRCA2</u>	c.1483G>C	p.Ala495Pro*
	<u>BRCA2</u>	c.4409_4410delTA	p.Ile1470fs**
T+11 (2)	PTCH1	c.4324C>T	p.Arg1442Trp
	<u>CDH1</u>	c.32T>C	p.Leu11Pro*
T+12 (5)	PMS1	c.2783T>C	p.Leu928Pro*
	<u>ATM</u>	c.1273G>A	p.Ala425Thr*
	<u>ATM</u>	c.1300C>T	p.Pro434Ser*
	<u>BRCA2</u>	c.9038C>T	p.Thr3013Ile
	STK11	c.413A>G	p.Glu138Gly*
T+13 (2)	MUTYH	c.536A>G	p.Tyr179Cys**
	SLX4	c.2359G>A	p.Glu787Lys
T+14 (3)	FANCG	c.634G>A	p.Ala212Thr*
	<u>CHEK2</u>	c.1556G>T	p.Arg519Leu*
	KIF1B	c.2680G>A	p.Val894Met*
T+15 (2)	RECQL	c.1360C>T	p.Arg454Cys*
	<u>CHEK2</u>	c.444+1G>A	intronic**
T+16 (4)	TSC1	c.2418G>A	p.Met806Ile*
	POLD1	c.835_837delGAG	p.Glu279del*
	PDGFRA	c.1099G>A	p.Val367Met*
	DIS3L2	c.1447C>G	p.Arg483Gly*
T+17 (3)	RECQL4	c.3062G>A	p.Arg1021Gln
	SLX4	c.3890G>A	p.Gly1297Glu
	SLX4	c.179A>C	p.Gln60Pro
T+18 (4)	MSH6	c.3226C>T	p.Arg1076Cys**
	<u>ATM</u>	c.7290T>G	p.His2430Gln*
	MAX	c.25G>T	p.Val9Leu*
	<u>BRCA1</u>	c.181T>G	p.Cys61Gly**
T+19 (0)	-	-	

Table 4. List of any cancer (and breast cancer) related genes and unique variants identified by individual patients in the control group. The strength of known association of each variant with oncogenesis is marked as known (**), potential (*) or no (no marker). (20)

BC only patients (no. of SNPs)	Gene (<i>known breast cancer gene</i>)	SNP	Effect of mutation *- potential **- known association
T-1 (3)	<u>ATM</u>	c.8965C>G	p.Gln2989Glu*
	MUTYH	c.1435G>A	p.Glu479Lys*
	FANCB	c.2435A>G	p.Tyr812Cys*
T-2 (2)	MSR1	c.919G>T	p.Asp307Tyr*
	<u>ATM</u>	c.6067G>A	p.Gly2023Arg
T-3 (4)	RAD50	c.1741C>T	p.His581Tyr*
	MITF	c.1255G>T	p.Glu419Ter**
	WRN	c.95A>G	p.Lys32Arg
	APC	c.7490C>T	p.Ser2497Leu
T-4 (6)	ELANE	c.341T>C	p.Leu114Ser*
	RB1	c.10A>C	p.Lys4Gln*
	WRN	c.1149G>T	p.Leu383Phe
	ERCC4	c.1135C>T	p.Pro379Ser
	MSR1	c.667T>A	p.Ser223Thr*
	WRN	c.2983G>A	p.Ala995Thr
T-5 (1)	TSC1	c.1390G>A	p.Gly464Ser*
T-6 (1)	<u>CHEK2</u>	c.190G>A	p.Glu64Lys**
T-7 (3)	MSH2	c.2187G>A	p.Met729Ile*
	RAD50	c.734A>G	p.Glu245Gly*
	FANCA	c.2658G>C	p.Glu886Asp*
T-8 (2)	<u>CDH1</u>	c.344C>T	p.Thr115Met*
	MET	c.2962C>T	p.Arg988Cys
T-9 (6)	MDH2	c.415G>A	p.Val139Ile*
	FANCA	c.1874G>C	p.Cys625Ser
	ATR	c.7303A>G	p.Ile2435Val*
	TERT	c.2726T>C	p.Val909Ala*
	<u>PALB2</u>	c.2483G>A	p.Cys828Tyr*
	FANCB	c.454T>C	p.Phe152Leu*
T-10 (3)	RECQL4	c.616A>C	p.Lys206Gln*
	BLM	c.3536C>T	p.Thr1179Ile*
	FANCC	c.632C>G	p.Pro211Arg
T-11 (3)	MITF	c.1334C>A	p.Thr445Lys
	WRN	c.1211T>C	p.Ile404Thr
	SLX4	c.179A>C	p.Gln60Pro
T-12 (1)	FANCI	c.824T>C	p.Ile275Thr

Table 4. (continued).

BC only patients (no. of SNPs)	Gene (<i>known breast cancer gene</i>)	SNP	Effect of mutation *- potential **- known association
T-13 (3)	BUB1	c.307A>G	p.Ile103Val
	FANCA	c.1874G>C	p.Cys625Ser
	AXIN2	c.1994delG	p.Gly665fs*
T-14 (3)	RECQL4	c.941C>T	p.Pro314Leu
	SLX4	c.4963A>G	p.Arg1655Gly
	RAD51C	c.130T>C	p.Ser44Pro
T-15 (1)	PMS1	c.1609G>A	p.Glu537Lys*

Table 5. Comparison of the two patient groups by the number of genetic variants (20)

<i>Genes associated with BC</i>	BC only		BC and TC		p-value
	Mean	Std. error	Mean	Std. error	
No. of SNPs with potential/ known association with BC	0,27	0,12	0,84	0,22	0,029
No. of SNPs with potential/ known association with BC (weighted scores)	0,17	0,08	0,53	0,15	0,039
<i>112 cancer risk genes</i>	BC only		BC and TC		p-value
	Mean	Std. error	Mean	Std. error	
No. of SNPs with no known association with oncogenesis	1,20	0,30	0,74	0,20	0,206
No. of SNPs with a potential association with oncogenesis	1,40	0,39	2,32	0,37	0,096
No. of SNPs with a known association with oncogenesis	0,20	0,11	0,47	0,14	0,131
No. of SNPs with potential/ known association with oncogenesis	1,60	0,36	2,79	0,39	0,032
No. of SNPs with potential and known association with oncogenesis (weighted scores)	0,90	0,18	1,63	0,23	0,017

We found a significantly lower number of SNPs with potential or known association with breast cancer in the BC only group (mean difference -0.58 , BCa 95% CI $[-1.09, -0.06]$, $t(27) = -2.30$, $p = 0.029$, $g = 0.74$ for unweighted and mean difference -0.36 , BCa 95% CI $[-0.70, -0.02]$, $t(27,26) = -2.17$, $p = 0.039$, $g = 0.70$ for weighted scores). (20)

The individual number of SNPs with no known genetic association with cancer was nominally higher in the BC only group (mean difference 0,46, BCa 95% CI [- 0,27, 1,20], $t(25,56) = 1,30$, $p = 0,206$, $g = 0,39$), while the number of potentially associated SNP or those with a known association was nominally lower in the BC only group (mean difference - 0,92, BCa 95% CI [- 2,01, 0,17], $t(30,98) = - 1,72$, $p = 0,096$, $g = 0,59$ and mean difference - 0,27, BCa 95% CI [- 0,63, 0,09], $t(31,37) = - 1,55$, $p = 0,131$, $g = 0,51$, respectively). (20)

When we lumped together SNPs with potential or known association with carcinogenesis to increase statistical power, we found significantly higher values in the synchronous/metachronous cancer group compared to the BC only group using both unweighted (mean difference - 1,19, BCa 95% CI [- 2,27, - 0,11], $t(31,88) = - 2,24$, $p = 0,032$, $g = 0,76$) and weighted (mean difference - 0,73, BCa 95% CI [- 1,32, - 0,14], $t(31,80) = - 2,51$, $p = 0,017$, $g = 0,83$) scores. (20)

II. MicroRNA profiling in papillary thyroid cancer

II.1 Study population

From the initial pool of 129 PTC patients, 118 were eligible for inclusion, yielding 236 samples (118 cancerous, 118 non-cancerous tissues) for analysis of the expression pattern of 2656 types of miRNAs. The tumor samples included in our study contained the following histological subtypes of PTC: 96 conventional (81,35%), 16 follicular subtypes (13,56%), 4 oncocytic (3,39 %), 1 columnar cell (0,85%), and 1 Warthin-like (0,85%). (51)

II.2 Descriptive analysis

The descriptive analysis of miRNA expression profiles in PTC patients provided a detailed landscape of miRNA dysregulation of the disease. We listed miRNAs with significant expression dysregulation in PTC in Figure 4 based on our sequencing data. Subsequent analysis following isolation revealed significant differences in the expression of 30 individual miRNAs in total, 27 of them being over-represented (e.g., miR-551b-3p, miR-146b-3p, miR-9983-3p, miR-221-3p, miR-222-3p, and miR-375-3p) and 3 of them being under-represented (miR-206, miR-873-3p, and miR-204-3p) in PTC compared to the healthy parts of the thyroid. As mentioned above, we presented the results indicating not only statistical significance but individual fold changes as well. MiR-551b-3p, miR-

146b-3p, miR-146b-5p, miR-221-3p, miR-375-3p, miR-873-3p, and miR-204-3p are the most noteworthy. It is also worth mentioning that 582 of the studied miRNA types were not expressed in thyroid tissue at all. (51)

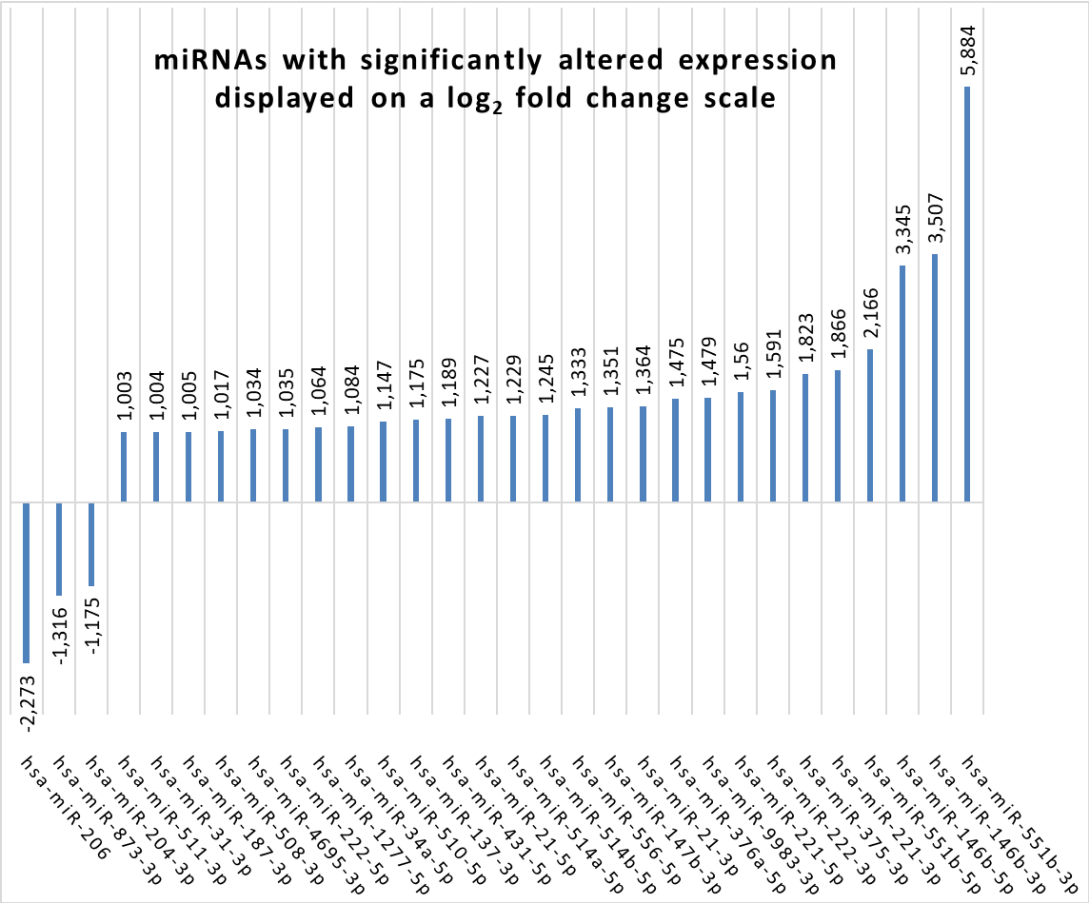


Figure 4. The magnitude and significance of the expression deviations between tumor and non-tumor tissue samples corresponding to the “top 30 miRNAs”. The log₂ fold change indicates the average expression level changes of the listed miRNAs. Statistical significance was assessed using false discovery rate corrected p-values, with significance set at p < 0,05. The bars of this chart present the log₂ fold change of the top 30 miRNAs (named on the horizontal axis), as depicted their significantly different expression profiles between cancer and control groups. Bars indicating upward from the zero line are showing overexpression of the particular miRNA in tumor tissue, while those indicating downward are underexpressed. (51)

We also conducted a miRNA expression comparison between the conventional and follicular subtypes within our PTC tumor sample cohort. We were unable to detect any

significant miRNA expression differences between these two subtypes in the context of the “top miRNAs”. We did not perform further analysis of other subtypes due to their low frequency in the study population. (51)

II.3 Association analysis between miRNA expressions and states of selected clinicopathological variables

In our extensive analysis of miRNA expression data, we identified a total of 352 significant ($p < 0,05$) miRNA expression differences between different states of the examined clinicopathological variables—such as age, sex, ATA risk score, as well as TNM and AJCC (eighth edition) stages—of the study cohort. We investigated the miRNA expression deviations and their connection to clinicopathological variables in the cases of both tumor and adjacent healthy control tissues. (51)

Notably, 36 of the miRNA expression deviations pertained to tumor samples, while the majority, 316, were related to control samples. Our analysis also revealed a quite balanced distribution in the context of the direction of miRNA expressions, with 165 links being caused by miRNA overexpression and 187 by underexpression. Furthermore, 31 of these “miRNA expression–state of variable” associations were highly intense, exhibiting extreme log2 fold change values either above 10 or below -10 , shown in Table 6. Among these stronger associations, it is worth highlighting those miRNAs with the most prominent links to ATA risk: miR-6880-5p (direct, in tumor), miR-6753-5p (inverse, in tumor), miR-3648 (inverse, in control), and miR-6862-3p (inverse, in control); and those showing link to TNM score: miR-6753-5p (inverse, in tumor), miR-6805-5 (inverse, in control), miR-519c-3 (inverse, in control), and miR-6862-3p (inverse, in control). Note that downregulation of miR-6862-3p in healthy thyroid tissue adjacent to PTC is associated with greater ATA risk score, TNM score, and clinical stage as well. (51)

Table 6. The 31 most relevant associations between significant miRNA expression differences and the clinicopathological variables examined (such as age, sex, ATA risk, and stages). Each represent not only a significant change in miRNA expression ($p < 0,05$), but also a log2 fold ± 10 change relative to at least one clinicopathological variable. The (+) or (–) signs indicate miRNA overexpression or underexpression, respectively. (E.g. miR-6880-5p overexpression in tumor tissue was more associated with ATA risk 3, than with ATA risk 2.)

Clinicopathological state	miRNA expression difference in tumor tissue [overexpression (+) or underexpression (–)]	miRNA expression difference in control tissue [overexpression (+) or underexpression (–)]
ATA risk 3 vs. 2	6880-5p (+) 4690-3p (+) 2115-5p (+) 196a-1-3p (+)	3648 (–) 3675-5 (–)
ATA risk 3 vs. 1	–	6862-3p (–) 3648 (–)
ATA risk 2 vs. 1	6753-5p (–) 4690-3p (–) 2115-5p (–) 196a-1-3p (–)	3675-5 (+)
TNM T2 vs. T1b	6753-5p (–) 2115-5p (+)	6805-5 (–) 3675-5 (–) 6891-5 (–) 3648 (+)
TNM T2 vs. T1a	–	6805-5 (–)
TNM T1b vs. T1a	2115-5p (–) 6880-5p (+)	3648 (–) 6891-5 (+) 3675-5 (+)
TNM N1b vs. N1a	–	6862-3p (–)
TNM N1b vs N0	–	6862-3p (–) 519-c3 (–) 3675-5 (+)
Stage IVb vs. III	–	150-3p (+)
Stage IVb vs. I	–	4785 (+)

III. Surgical strategies and preoperative assessment in differentiated thyroid cancer

We identified 261 potential patients in our database that satisfied inclusion criteria. Two of them were excluded based on anaplastic or medullary histology. Out of the 259 patients, 76,4% were female and 23,6% were male. In 86,9% of cases papillary thyroid cancer, in 10,0% follicular and in 3,1% Hürtle cell histologic type was established. A further 11 patients were excluded from further analysis as they underwent lobectomy without completion. However, 248 met the inclusion criteria, where applicable, 259 well-differentiated thyroid cancer patients were included in the calculations, e.g. post-lobectomy complications. (87)

A./ From the final 248 patients, 152 (61,3%) and 23 (9,3%) cases would have been recommended for initial lobectomy according to ATA and ETA, respectively. Based on postoperative histological data, these hypothetical lobectomies would have been inadequate in 94 (61,8%) and 15 (65,2%) cases (ATA and ETA, respectively) leading to completion thyroidectomy. Following a hypothetical lobectomy, the most common indications for repeated surgery would have been: previously unknown lymph node metastases, incomplete surgical resection (R1 resection or positive surgical margin), extrathyroidal or vascular invasion and bilateral tumor. Bilateral tumor would have been discovered later, so a later reoperation would have had to be performed. (87)

Among our patients ATA would have recommended total thyroidectomy in 38,7% (96 patients) of the cases. The most frequent indication for primary total thyroidectomy according to ATA guidelines was tumor size above 4 cm. In contrast to this, 90,7% (225 patients) of our patients were candidates for total thyroidectomy using the ETA guideline. In cases where the guidelines advocated for initial (near) total thyroidectomy, this decision would have been justified in 81 of 96 (84,4%) (ATA) and 161 of 225 (71,6%) (ETA) of cases. In other words, we found one or more histological risk factors in 84,4 and 71,6% of the surgical specimens in these patients. (87)

There was a statistically significant difference between the ATA and ETA guidelines in the frequency of recommending initial lobectomy. The hypothetical need for subsequent reoperation, however, did not appear to be different – with the absolute number of reoperations being much lower (15 vs. 94 of 259 patients). In cases where the individual guidelines called for initial thyroidectomy, the ATA guideline had a higher positive

predictive value in identifying cases with high-risk histology. Data is shown in detail in Table 7. (87)

Table 7. Hypothetical indications and outcomes in patients whose thyroid gland were removed in one or two steps, according to the 2 main social guidelines, based on the pre- and postoperative findings. (87)

	ATA		ETA		p-value
	N	%	N	%	
Lobectomy	152	61,30%	23	9,30%	<0,001
Total thyroidectomy	96	38,70%	225	90,70%	
Lobectomy completion needed	94	61,80%	15	65,20%	0,756
Lobectomy only sufficient	58	38,20%	8	34,80%	
After total thyroidectomy:					
risk factor(s) positive	81	84,40%	161	71,60%	0,021
risk factor negative	15	15,60%	64	28,40%	

Compared to ATA recommendations, we performed primary total thyroidectomies more frequently (38,7% vs. 73,8%). However, ETA would have recommended even more one step total thyroidectomies (90,7% vs. 73,8%). Lobectomies were followed by a second surgery in most of our cases (91,2%). (87)

If ATA would have recommended total thyroidectomy and we followed this recommendation in 81,3% (n=78) of these cases, this was proved to be eligible in 84,4% of cases. If ATA would have recommended lobectomy and we have performed accordingly, at least one risk factor was shown in the histology result in 69,0% of cases. In cases where ETA would have recommended total thyroidectomy and in 77,8% of cases we have done so, resulting a 73,7% postoperative risk factor ratio – compared to the 71,6% if we had proceeded entirely according to ETA. Postoperative risk factor ratio is calculated by number of cases where at least 1 postoperative risk factor was present divided by number of all cases. Interestingly, if ETA indicates lobectomy, and we have performed the surgery accordingly (39,1%), the ratio of the postoperative risk factor was found to be 88,9%. (87)

Indicators of ATA have turned out to be superior to ours: positive predictive values were 84,4% vs. 71,7%; negative predictive values were 38,2% vs. 32,3%; false positive rates were 15,6% vs. 28,3%; false negative rates were 61,8% vs. 67,7%, respectively. (87)

B./ Primary total thyroidectomy was undertaken at our institute in 110 of the 152 cases where the ATA guideline would have recommended lobectomy. Postoperative histological results validated this approach in 60,0% of cases. The identified risk factors are shown in Table 8. The distribution of these risk factors among the 66 of our patients who underwent primary total thyroidectomy despite the ATA guideline recommending lobectomy was the following: 38 patients had 1 risk factor, 15, 10 and 3 patients presented with 2, 3 and 4 risk factors respectively. Undiscovered lymph node metastasis would have been the leading cause (47,0%) of consequent reoperation had these patients undergone primary lobectomy. Other factors were incomplete surgical resection (34,8%) and extrathyroidal extension (27,3%, mainly microscopic). Furthermore, in 12 of 110 cases (10,9%), a contralateral tumor would have remained undiscovered. Two, 3 and 4 concurrent risk factors were discovered in 22,7, 15,2 and 4,6% of these patients, respectively. (87)

Table 8. Frequency of postoperative risk factors in those, who reasonably underwent total thyroidectomy. Patients may have multiple postoperative risk factors. (87)

Postoperative risk factors in n=66 patients	Men (n=16)		Women (n=50)		Total (n=66)	
Lymph node metastasis	8	50,0%	23	46,0%	31	47,0%
Positive surgical margin	6	37,5%	17	34,0%	23	34,8%
Extrathyroidal extension	4	25,0%	14	28,0%	18	27,3%
Angioinvasion	4	25,0%	13	26,0%	17	25,8%
Bilateral tumor	5	31,3%	7	14,0%	12	18,2%
Aggressive variant	1	6,3%	8	16,0%	9	13,6%
Total	28	25,4%	82	74,6%	110	100,0%

Total thyroidectomy was performed at our institute in 14 of the 23 cases where the ETA guideline would have advocated for lobectomy. This decision was confirmed by postoperative risk factor assessment in half of the cases. Of these, incomplete surgical resection and previously undiscovered lymph node metastases were once again the most common findings (71,4 and 42,9% respectively). (87)

C./ We compared the accuracy of preoperative clinical work-up (US and FNA) in predicting postoperative histological findings. While specificity and PPV were mostly

acceptable (79,5–99,1% and 44,4–100,0% respectively), sensitivity was uniformly low in all areas including prediction of lymph node metastasis (48,9%), identifying extrathyroidal (5,9%) or bilateral tumor (32,0%) presence, or establishing the presence of aggressive tumor variants (28%). Cohen's kappa was 0,04 and 0,20 for ETA and ATA criteria respectively. These values reflect the low agreement between pre- and postoperative findings with the modest superiority for ATA criteria. (87)

We examined the predictive value of preoperative clinical parameters on the type of surgical intervention in the subgroup of cases where primary total thyroidectomy was performed at our institute despite the ATA guideline advocating for initial lobectomy ($n = 110$). Of the preoperative parameters examined, only tumor size had a significantly positive ($p = 0,001$) and patient age a nearly significantly negative ($p = 0,057$) effect on the presumed need for total thyroidectomy if the ATA guideline had been followed. Model fit was moderate ($R^2 = 0,20$ (Nagelkerke)). Results from the logistic regression are presented in Table 9. (87)

Table 9. Effect of preoperative clinical parameters on disease severity defined by the histologically verified need for total thyroidectomy. $R^2=0.20$ (Nagelkerke). See text for details. (87)

	OR	95% CI for Exp(B)	p
Nodule size (mm)	1,098	1,037 – 1,161	0,001
Patient age (years)	0,972	0,944 – 1,001	0,057
Gender	1,002	0,335 – 2,995	0,998
Hashimoto (Y/N)	0,574	0,225 – 1,466	0,246

We examined the value of tumor size in predicting individual postoperative risk factors using data from all our patients ($n = 259$). Results from the logistic regression are shown in Table 10. After adjustment for multiple testing, preoperative tumor size significantly correlated with the presence of vascular invasion. There was a near significant association with incomplete surgical resection. However, the size of the primary malignancy, was not a significant predictor for the presence of a contralateral tumor, aggressive histology, extrathyroidal extension or undiagnosed lymph node metastasis. (87)

Table 10. The effect of tumor size (mm) on the presence individual postoperative risk factors. After adjustment for multiple testing using the Bonferroni method, p values $< 0,008$ are considered statistically significant. (87)

Outcome	OR	95% CI for Exp(B)	p
Contralateral tumor	0,994	0,970 - 1,019	0,653
Aggressive histology	1,014	0,987 - 1,042	0,307
Extrathyroidal extension	1,019	0,996 - 1,042	0,115
Positive surgical margins	1,030	0,007 - 1,052	0,009
Lymph node metastasis	1,001	0,982 - 1,021	0,893
Vascular invasion	1,047	1,023 - 1,071	0,000087

D./ From the pool of all 259 patients with DTC, 38 (14,7%) patients suffered a total of 44 surgical complications. Amongst these, hypocalcaemia and temporary, unilateral recurrent laryngeal nerve palsy were the most common. Complication rate was higher with primary total thyroidectomy (15,2%) vs. lobectomy (4,4%) ($p = 0.02$). (87)

We also assessed complication rates separately in the subgroup of cases where total thyroidectomy had been performed and its need was retrospectively validated by postoperative histological findings ($n = 173$). Both the overall rate of complications and the frequency of specific complications were similar in patients who underwent total thyroidectomy in one step versus those who had lobectomy followed by completion thyroidectomy (18,8% vs. 19,5%, Exact p -value = 1,00). (87)

5. DISCUSSION

I. Increased oncogenic SNP burden in synchronous/metachronous thyroid- and breast cancer patients

In our study, investigating the common genetic factors associated with the co-occurrence of thyroid and breast cancer, we found a significantly higher burden of established and potential germline cancer risk variants among subjects with a history of metachronous thyroid and breast cancer compared to patients with a history of breast cancer only. The trend was similar, and effect sizes were still considerable when we assessed the number of SNPs with known and potential cancer risk separately. However, in these cases, probably due to lack of power, statistical significance was not reached. Our results are compatible with the hypothesis that the sporadic co-occurrence of thyroid and breast cancer is of multigenetic origin and probably related to the burden of the carcinogenic SNPs rather than an individual gene alteration. (20)

Comparison of clinical features of breast cancer, such as onset, stage, histology and treatment also yielded no significant differences (87). There was a non-significant tendency for later age of breast cancer onset in the study group which is contrary to previous findings (92).

The metachronous and synchronous occurrence of breast and thyroid cancer has been first described nearly four decades ago (10). The relationship between the two tumor types has since been found to be bidirectional. In recent meta-analyses, the odds ratio for thyroid cancer following breast cancer treatment was 1,55 while the odds ratio of developing BC after TC was reported between 1,18–1,32 (12, 13).

Studies exploring the cause behind this association emphasize either the role of common underlying hormonal, environmental and lifestyle factors, or the role of the first malignancy in the development of the second. The potential role of cancer treatment and the increased surveillance of cancer survivors fall in this latter category. (87)

With breast cancer being the most common type of malignancy in women (93) and with the increasing incidence of thyroid cancer worldwide (94, 95), the importance of this topic is clear.

I.1 Female sex hormones

One potential hypothesis explaining this bidirectional relationship implicates common hormonal factors and is rooted in the overwhelming predominance of both cancer types in reproductive-aged women (13). The potential importance of hormonal effects is supported by the interesting observation that the incidence of thyroid cancer is increased in other sex hormone-related diseases such as myoma and benign breast diseases.

Estrogen has been implicated in the pathogenesis of both breast and, to a lesser degree, thyroid cancer (15, 96-98). Estrogen, progesterone, androgen and HER-2 receptors are often present on the epithelium of thyroid cancer and breast cancer cells (99-102). HER2 positive status may also be associated with poorer prognosis, based on low evidence data (103). In vitro and animal studies also point towards the potential oncogenic effect of estrogens in thyroid cells (99, 104).

Also, in RET/PTC fusions or BRAF and RAS mutations, estrogen can stimulate MAPK and PI3K pathways (100). Expression of ER α is 8-fold higher in thyroid progenitor cells, what are the presumptive source cells of thyroid cancer (15, 105). In a recent study, PTC proliferation, migration and invasion was facilitated by estrogen, via the activation of ER α /KRT19 signaling axis. KRT19 (keratin 19), an intermediate filament protein, is highly expressed in various cancers, including breast and thyroid cancer.

It is also possible that the hormonal milieu of pregnancy (hCG, increased TRH and prolactin) would increase the thyroid and the estrogen-like effects of thyroid hormones, hence the risk of both cancer types (106).

Selective estrogen receptor modulators (SERMs) are widely used in breast cancer treatment. While there is some evidence that these drugs due to their partial estrogen effect increase TSH secretion and thyroid cell proliferation, their potential role in thyroid cancer development has not yet been studied (107).

The similar frequency of hormone therapy in the BC only, and the BC-TC groups in our study point toward a limited role of sex hormones in the development of co-occurring breast and thyroid cancer. (87)

I.2 Thyroid hormones

An increased TSH secretion in response to estrogens has also been suggested as a potential pathomechanism for thyroid cancer development (12), as serum TSH levels

have been shown to correlate with thyroid cancer risk (108). TSH and thyroid hormones also have been implicated in breast carcinogenesis by in vitro animal and observational studies. T3 seems to stimulate proliferation in breast cancer cells in vitro at least in part through interactions with the estrogen signaling system (109, 110). Tumor suppressor pathways downstream of the TR β nuclear thyroid receptor and oncogenic pathways downstream of the membrane receptor α V β 3 have been suggested to mediate the role of thyroid hormones in carcinogenesis (111-115), other studies have failed to replicate these findings.

Furthermore, observational studies in humans found a decreased breast cancer incidence in hypothyroid and an increased incidence in hyperthyroid patients (15, 114-119). Contradictory data suggest that hyperthyroidism and hypothyroidism may also increase the risk of breast cancer: thyroid hormones can affect the signaling pathways of estrogen, progesterone, and HER-2 receptors. Thyroid receptor (TR) is also present in BC cells (120).

The association of autoimmune thyroid diseases (Hashimoto thyroiditis and Graves' disease) and breast cancer have also been theorised (15, 121, 122). The role of autoimmunity may be suggested by the observation that breast cancer and thyroid cancer are more common in autoimmune thyroid disease. Based on this theory, the abnormal immune system causing the autoimmunity, and could not recognising the cancerogenic cells. Thyreoperoxidase can also be expressed in breast cancer cells or in the peritumoral tissues. Accordingly, serum anti-TPO or Anti-Thyreoglobulin positivity may be more frequent in breast cancer patients (121). Anti-TSH-receptor antibodies posed an increased risk of breast cancer in a recent meta-analysis (122).

Endocrine disrupting chemicals (EDC) have been linked to numerous types of hormone dependent malignancies including both thyroid and breast cancer (123-125).

I.3 Lifestyle factors: obesity, iodine, diabetes

The role of lifestyle factors can not be discounted (15, 126, 127). It is well known that obesity predisposes to at least 13 types of malignancy, including thyroid and breast cancer (128-131). Obesity is also associated with higher-stage disease in both thyroid and breast cancer (126, 132) and decreased treatment efficacy in breast cancer (133). In parallel, overweight and obesity were substantial independent risk factors for extrathyroidal extension, tumor size and multifocality in PTC among male patients (126).

Comparable data were also reported for type 2 diabetes (121). T2DM (Type 2 Diabetes Mellitus) posed a 1,38 relative risk (95% CI: 1,13-1,67) to thyroid cancer in women (134) and an 1,08-1,20 relative risk for breast cancer (135). T2DM was associated with higher stages (III/IV) and larger tumor size (>20 mm) and lymph node metastasis in breast cancer patients (136).

Japanese women with a richer iodine-containing diet have a reduced risk of breast cancer (15). This may have links to the fact that NIS (Sodium Iodide Symporter) is expressed in lactating breast and 80% of breast cancers (122, 137, 138). In parallel, iodine-rich foods may also be a protective factor against thyroid cancer (139), but large, prospective studies adjusted for other lifestyle factors, dietary patterns and geological factors are still lacking.

Vitamin D deficiency can also contribute to the higher risk of several malignancy, including thyroid cancer and breast cancer (140-143). Vitamin D receptor known to be expressed both in BC and PTC (144, 145).

I.4 Treatment of the first cancer

Other theories suggest the role of prior cancer treatment in the development of the second primary malignancy. Radio- and hormone therapy for breast cancer and I-131 treatment for thyroid tumors have both been implicated in carcinogenesis (15, 107, 146, 147).

Despite several improvements minimizing radiation scatter to surrounding tissues, there is a definitely increased risk for certain malignancies following external beam radiation for breast cancer (148). While some reports are conflicting (149), most available data do not substantiate such a connection between thyroid cancer and previous adjuvant breast irradiation (150-153). Our data with similar frequency of radiation therapy for breast cancer in the investigated groups argue against the role of external or internal radiation in the development of synchronous/metachronous breast and thyroid cancer (87).

However, there are no data available in the literature on the risk of a second primary thyroid cancer after chemotherapy for breast cancer (15).

The issue of high-dose I-131 radioiodine therapy (RAI) is particularly noteworthy. In Hou's publication (5), I-131 therapy was not found to increase the risk of a second primary tumor, but for example Kim 2023 (154) described a linear dose-dependent increase in risk of a second primary tumor after RAI at a cumulative dose of 3,7 GBq (HR= 1,08).

Notably, the risk of a second primary malignancy was significantly increased above a dose of 3,7 GBq, and a strong linear correlation was observed between cumulative RAI dose and the risk of SPM. In this study, the risk of myeloid leukemia, salivary gland, trachea, lung/bronchus, uterine and prostate cancers was significantly increased after RAI therapy. In contrast to the above, another publication (155) found no association between RAI treatment and the increased incidence of second primary malignancies. In a critical review by Relnecke and colleagues (156), the relative risk of RAI ranged from 1,14 to 1,84, although the evidence for this was considered low to very low. The authors concluded that, based on the low-quality evidence, the overall excess risk of RAI for second primary malignancies cannot be excluded, but the effect is not expected to be significant.

In 2022, Pasqual (14) published a major study investigating the effect of RAI on second primary malignancies in children and young adults with long follow-up. Their results showed an increased incidence of second primary malignancies in all organs except the bladder, liver and cervix. The relative overall risk for SPM was 1,47 for survivors beyond 20 years and 1,46 for breast cancer. In the case of solid organs, the risk increased particularly after 20 years, which was even more pronounced in the case of breast cancer. Based on their results, RAI was estimated to be responsible for 14% of hematological malignancies, 6% of solid malignancies and 5% of breast cancers among thyroid cancer survivors treated with radioiodine in children and young adults (up to 45 years of age). The study confirms that the risk of SPM associated with radioiodine treatment is more pronounced in younger age groups. This finding is supported by the results of a large retrospective study (157), where the risk of second primary breast cancer was increased by radioiodine treatment in adolescents and young adults to a greater extent than in middle-aged patients (HR= 1,65 vs. 1,18).

Due to the short follow-up period, it was not possible to investigate the risk of RAI on second primary malignancies.

1.5 Surveillance bias

It is also possible that the increased surveillance of cancer survivors could lead to earlier recognition or even overdiagnosis of a second primary malignancy (87). This seems to be especially true for differentiated thyroid cancer (158). In a meta-analysis of 6286 autopsies, after examination of the whole thyroid gland there was a pooled subclinical

PTC incidence of 12,9% (95% CI: 7,8-16,8) (159). This study shows no difference in subclinical PTC prevalence between men and women regarding the age-specific prevalence neither difference between prevalence in age groups. Furthermore, another meta-analysis found high rate of adverse histological features in subclinical DTCs (160). The pooled prevalence of incidentally discovered DTC was 8,5%, from which the frequency of multifocal, bilateral, minimal extrathyroidal extensive, LN metastatic, vascular invasive were 28,2%, 18,0%, 24,5%, 11,0%, 16,0% respectively. Notably, from the 722 subjects, 1 had occult distant metastasis, and 2 had undiagnosed fatal metastatic disease.

However, as there are no screening programs for breast cancer among thyroid cancer survivors or vice versa, surveillance bias is unlikely to account for the preferential association of these tumor types. (87)

1.6 Genetics

Cowden syndrome (CS) and Cowden-like syndromes (CLS) are characterised by hamartomas and an extremely increased risk for several types of malignancies including breast cancer, thyroid cancer, endometrial cancer, colorectal cancer, and melanoma with standardized incidence ratios for breast and thyroid cancer in the range of 6 to 9 (17). CS and most forms of CLS have an autosomal dominant inheritance. However, these conditions, referred to as PTEN hamartoma tumor syndromes, make up only a small fraction of synchronous/metachronous thyroid and breast cancer cases (87).

This is consistent with our finding that no patient in our samples had a mutation in the PTEN gene. Thus, the existence of other shared genetic risk factors is highly probable. To the best of our knowledge, genetic analyses exploring this notion have not been conducted and genetic factors underlying sporadic metachronous cancer cases are yet to be elucidated.

Epigenetic changes in lncRNAs (long non-coding RNA), in particular MANCR and overexpression of VEGF or hypermethylation of KLLN gene may also play a role in the association of thyroid and breast cancer (15, 161, 162).

The main strengths of this study were the state-of-the-art genetic analysis using NGS technology, and the fact that both cases and controls came from the same source population. The main limitation of our research was the relatively low number of patients

limiting statistical power even in analyses of genetic scores. Furthermore, our study was unable to identify or investigate any single genetic risk locus behind synchronous/metachronous cancer development. Two types of bias could also have affected our findings. First, participants were recruited years after diagnosis potentially leading to survivor bias. Second, the exclusion of controls with less than 12 years of follow-up lead to an age difference between our study groups that is contrary to previous findings (92) and probably reflects selection bias. However, both of these selections were deemed to be necessary to minimize contamination of the control group with thyroid cancer. (87)

II. Altered miRNA expression in papillary thyroid cancer

In the study investigating miRNA profile of papillary thyroid cancer, we identified 30 miRNAs that showed significant up- or downregulation compared to healthy thyroid tissue. As far as we know, this is the first study describing the association of PTC with the overexpression of miR-9983-3p, miR-4695-3p, miR-1277-5p, miR147b-3p, miR-511-3p, and miR-137-3p. MiR-551b-3p was overexpressed in our cancer samples, suggesting its oncogenic role in PTC, unlike in other cancer types discussed in previous research. In consensus with the literature, we also confirmed the overexpression of miR-221, miR-222, and miR-146b, among others. (51)

Our findings may significantly contribute to improving diagnostic accuracy in thyroid nodules with uncertain cytological readings by supporting the future integration of miRNA-based testing alongside DNA-based molecular testing of cytological samples. In addition, not yet published associations between the miRNA pattern of PTC and clinicopathological variables were revealed as well. (51)

Despite being a well-researched topic, the exact pathogenesis of PTC remains unknown, with most of the current, mutation-based models providing only a partial understanding of the disease (163). Therefore, deeper insights into non-conventional tumor formation caused by miRNA regulatory mechanisms on gene expression are required for the improvement of cancer diagnostics and therapeutics (25, 164). The role of miRNAs in the development of PTC is rather complex, but association with many signaling molecules such as tumor protein p53 (TP53) (48), cyclin-dependent kinase inhibitor 1B (CDKN1B) (48), insulin-like growth factor binding protein 5 (IGFBP5) (66),

transforming growth factor beta (TGF- β) (165), zinc and ring finger 3 (ZNRF3) (63), the RB1 gene (44), as well as serum thyroglobulin (TG) (30) levels is assumed.

Although the initial discovery of the disease as well as its treatment options are already quite effective with low rates of recurrence and complications, further improvement in the follow-up of the patients could still be achieved (166). In clinical practice, disease management is heavily dependent on risk assessments such as the 2015 ATA risk stratification system takes the *BRAFV600E* mutation into account to estimate the chance of disease recurrence (68). However, it lacks the inclusion of other genetic alterations and miRNA expression deviations, both of which could largely alter the long-term outcome of each clinical setting. For example, oncogenic cellular pathways like those related to *RET* or *NTRK* gene mutations can already be targeted by selective tyrosine kinase inhibitors (TKIs); some miRNAs such as miR-146b, miR-203a, miR-204, miR-221, or miR-222 are also suggested to be potential prognostic indicators (30, 62, 167, 168), although available data in this regard are still controversial (169). The latest studies on miRNAs suggest that an underlying correlation with *BRAF* mutations is possible as well (59, 63). It is also important to be aware that the currently used circulating biomarkers in DTC diagnostics and surveillance like TG levels have limitations, especially in the presence of TG antibodies (TGAb), which can heavily compromise the accuracy of TG measurements (170). This is why novel circulating biomarkers for diagnostics and surveillance are keenly researched (30, 31), with thyroid stimulating hormone receptor (TSHR) mRNA, TG mRNA, and certain miRNAs as candidates (170). Messenger RNAs are inherently unstable molecules; in addition, the sensitivity of circulating mRNAs is dependent on the timing of blood sampling, and the specificity of TG mRNA can easily be influenced by non-thyroid origins of the molecule and/or technical difficulties of the measurement (170). In this sense, the inclusion of specific miRNA expressions in risk assessment might be worth considering. (51)

Given their potential, our research aimed to detect and analyze the expression levels of a wide range of miRNA types in PTC, both in tumor tissues as well as in their adjacent healthy-tissue counterparts from the same patient's thyroid. Firstly, we established two cohorts of identical size (tumor and control) and performed the same molecular analysis on each of them. With this approach, we were able to identify PTC-specific miRNAs expressed in significantly higher or lower amounts than in the control samples. This is consistent with previous findings in which miRNAs acted as promising diagnostic

biomarkers distinguishing thyroid cancer from benign thyroid disease or healthy controls (30, 31, 62, 64, 171). By comparing the molecular dynamics within the same thyroid source tissue, we also had the opportunity to eliminate most of the biases related to patient selection and sample processing. (51)

Interestingly, we found that 30 individual miRNAs showed significant deviations from values in the control healthy tissue. Among these 30 miRNAs, we are the first to describe miR-9983-3p, miR-4695-3p, miR-1277-5p, miR147b-3p, miR-511-3p, and miR-137-3p in relation to PTC, although most of them have been previously mentioned in the context of other malignant diseases (172, 173). In the case of miR-9983-3p and miR-147b-3p, however, there is very little historical evidence regarding their roles in any cancer. Further studies on these novel PTC-associated miRNAs may help expand our knowledge of PTC. (51)

In addition, our results demonstrated PTC-related up- and downregulation of specific miRNAs, some of which have already been mentioned in previous studies, but some have attracted less attention so far. The most relevant one of such miRNAs is miR-551b-3p, which exhibited the most substantial fold change in PTC when compared to adjacent healthy thyroid tissue. Notably, an almost 60-fold overexpression of miR-551b-3p was seen within PTC tissue (Figure 4). Indeed, previous papers have reported on the irregular expression of miR-551b-3p in PTC (174, 175). It is noteworthy, however, that miR-551b-3p has been formerly recognized rather as a tumor suppressor in malignancies, such as gallbladder or gastric cancers (176, 177). It is also worth mentioning that in these studies (174-177), the number of patients involved was much lower ($n = 42-60$) than in our investigation. Consistent with its tumor-suppressive effects, miR-551b-3p was found to be underexpressed in these malignancies. However, our finding, indicating rather a significant overexpression in malignant thyroid tissue, suggests a potential oncogenic role for miR-551b-3p in relation with PTC. This observation underscores the dynamic nature of miRNA function; wherein certain miRNAs may manifest either oncogenic or tumor-suppressive properties depending on the specific malignancy under consideration. (51)

In agreement with previous data (59, 178) our results also demonstrate the significant over-representation related to miR-21, miR-146b, miR-221, and miR-222 within PTC. Therefore, the potential oncogenic role of these miRNAs can be underscored. Furthermore, miR-146b has been previously associated with epithelial–mesenchymal

transformation and the rather invasive features of PTC (63). Our data confirm miR-146b as a highly promising and novel diagnostic factor in the context of PTC. Additionally, our investigation provides further validation of the conspicuous overexpression of miR-221 and miR-222 as well. Dysregulation of these miRNAs, recognized as among the most established ones in thyroid cancer, continues to be implicated in the molecular landscape of PTC (60). This supports their significance as noteworthy factors in the pathogenesis of PTC, contributing to a more comprehensive understanding of the molecular mechanisms underlying thyroid cancer. (51)

As part of the study, we analyzed miRNA expression data in the context of different states of some clinicopathological variables related to the patients. We identified 352 significant “miRNA expression–state of variable” links, of which 31 were highly suggestive of being caused by underlying correlations between certain miRNA expression patterns and the presence of different clinicopathological states such as those related to ATA risk (e.g. miR-6880-5p, miR-6753-5p, miR-3648, and miR-6862-3p), TNM score (e.g. miR-6753-5p, miR-6805-5, miR-519c-3, and miR-6862-3p), and clinical stage (e.g. miR-6862-3p). MiR-6862-3p underexpression in the healthy adjacent thyroid tissue showed a higher state of all these three variables. This underscores the notion that some miRNAs might have a role in the development of PTC, its clinical behavior, and the prognosis of the disease through direct or indirect effects (e.g., expression dysregulation facilitated by age or sex). (51)

Our results not only enhance our understanding of PTC’s molecular foundations but also illuminate the possibilities of miRNAs as novel therapeutic targets and biomarkers (51).

However, our study has some limitations. First and foremost, though, our study was designed as original molecular research; although, we utilized tissue samples retrospectively from existing histological archives. Individual miRNA functions and their exact roles in molecular pathways were not investigated in this research; we only compared expression deviations of each miRNA between cancerous and healthy thyroid tissues. Also, we did not consider miRNA relations to mutational data such as *BRAFV600E*. This restriction was mainly due to the limited amount of tissue samples suitable for molecular processing available in our archives. Apart from age, sex, ATA risk score, and stage, we did not correct our results for other secondary variables known to be associated with the development or behavior of PTC such as clinical data (e.g.,

comorbidities, previous ionizing radiation exposure, and medications) or histopathological data (e.g., tumor size, PTC subtypes, histological aggressiveness, focality, and invasiveness). In addition, the statistical power to detect miRNA expression differences across less common PTC subtypes—such as oncocytic, columnar cell variant, etc.—was limited and could have been improved with a larger cohort. However, this was mainly due to the relatively high prevalence of the conventional subtype and a relatively low occurrence of other histological variants, which is consistent with other population-level observations (179, 180). It is also worth mentioning that our sample size is still larger than that used in many similar studies in this regard. Plus, this study focuses on individual miRNA expression variations and does not investigate inter-miRNA interactions or the combined effects of the miRNAs on PTC development. In addition, our data would have been even more reliable if we had been able to carry out repeated measurements of those miRNAs found to be significant in this study. However, this was not feasible due to the sample size limitations mentioned above. Also, we used FFPE tissue samples instead of fresh tissues or FNAB samples, which may impact the real clinical value of our results. Furthermore, we recognize that our study lacks functional validation experiments which would be crucial for gaining a deeper understanding of the relation between PTC pathogenesis and the identified differentially expressed miRNAs. Therefore, conducting functional validation tests (in vivo and/or in vitro) of the relevant miRNAs, such as knockdown animal experiments, would be highly beneficial. Lastly, an extension of our investigation in the future to multiple centers would definitely help verify our conclusions by avoiding potential patient selection biases from the same geographical region. (51)

III. Outcomes of surgical approaches in low-risk differentiated thyroid cancer

Despite the increasing incidence of thyroid cancer and the numerous possible etiologies of co-occurrence with breast cancer, thyroid cancer remains mostly indolent in behavior. The golden mean between the harms of overtreatment and the risks of undertreatment has not been established, as evidenced by differences in the first surgical approach to malignant thyroid nodules, especially in the case of low-risk nodules between 1 and 4 cm in size.

In our study, we compared the outcomes of different surgical strategies for preoperatively diagnosed differentiated thyroid cancer, using our institute's protocol and the potential

outcomes that could have been achieved with strict adherence to the ATA or ETA guidelines. We also evaluated the predictive power of preoperative ultrasound, cytological, and other clinical features in this context. Following these recommendations would have led to the same relative reoperation rate (absolute number of reoperations would have been much higher according to ATA guidelines and initial lobectomy would have been more frequent), and unrecognized lymph node metastases would have been the most common reason for completing thyroidectomies. Tumor size was not associated with the incidence of postoperative risk factors, excluding angioinvasion. Neither the tumor size, nor any other clinical data could predict adequately the optimal surgical approach. Our postoperative risk factor positivity ratio was very similar to that of ETA – this was found to be more eligible in lobectomies, but less in total thyroidectomies. However, predictive values of ATA were superior to our practice. (51)

This uncertainty of preoperative studies argues for a more radical surgical approach. A high number of primary total thyroidectomies were performed at our institution despite more lenient ATA recommendations. We advocate for near total thyroidectomy in more cases of thyroid cancer since the false negative ratio above 60% is considered to be unacceptable with particular regard to bilateral tumors.

Our postoperative risk factor positivity ratio was very similar to that of ETA – this was found to be more eligible in lobectomies, but less in total thyroidectomies. In a similar paper, published by DiMarco et al (77), from the pool of 275 FNAB-confirmed Bethesda V-VI cytology papillary thyroid cancers between 1 and 4 cm in size that were candidates for lobectomy according to ATA, 92,0% underwent complete thyroidectomy and the reoperation rate would have been 42,5%. In contrast to our study, angioinvasion (30,8%) and tumor invasion (23,9%) were the most frequent reason for the hypothetical completion thyroidectomy. Among our patients according to both ATA or ETA, the reoperation rate would have been nearly 50%. Neither bivariable analysis, nor multivariable logistic regression model found a significant predictive value for completion thyroidectomy among age, sex, tumor size, family history and FNAB result. Also in Kluijfhout's work (79), of the 149 low-risk differentiated thyroid cancer between 1 and 4 cm eligible for lobectomy according to ATA, 19,5% would have had to be reoperated to undergo adjuvant radioiodine treatment regarding the criteria set by ATA. If the relative indications (that are commonly used indications for radioiodine treatment

(RAI) although not included in ATA guideline) are considered, 49.0% would have had to be reoperated. (51)

ATA guidelines were based on findings that the tumor size of 1–4 cm in diameter alone should not influence the extent of surgery as it is not associated with survival (181, 182). However, confounding factors, such as aggressive variants, lymph node metastasis, positive surgical margin, male gender, lack of RAI do affect survival, and thus, those might have an impact on the decision making regarding the extent of surgery. Unfortunately, the preoperative assessment of these factors is markedly unreliable.

Our data suggest that growing tumor size is associated with an increasing risk for angioinvasion and positive surgical margin, however, the risk for bilateral tumor, lymph node metastasis, aggressive variants, and extrathyroidal extension do not decrease with smaller tumor diameter, thus, suggesting the need for total thyroidectomy. (51)

As the most permissive strategy, in an observational trial for following papillary thyroid microcarcinomas (183) it was found that follow-up alone could be a reasonable approach in patients without any unfavorable clinical or histological features. Thirty-two percent of the patients in the observational group had undergone surgery, and the rate of newly identified lymph node metastasis was increasing during the observational period. Other studies have shown that the recurrence rate is lower when total thyroidectomy is performed (184). Based on the aforementioned data, the more frequent consideration of primary total thyroidectomy over lobectomy might be advised. Psychological factors affecting patient preference should also be taken into consideration. In our practice patients usually prefer primary complete thyroidectomy and the higher chance of becoming completely tumor-free that is associated with this approach.

Lobectomy undoubtedly has a lower complication rate than total thyroidectomy. In our database, there were no difference between the adverse event rates in one- or two-step thyroidectomy groups. Nevertheless, the double anesthesia and hospital admission certainly carry additional risks and costs. Thus, if there is no difference in terms of complications as it is demonstrated by our data, primary total strumectomy could be more beneficial for the patients.

Although the same oncological outcome could be achieved by completion thyroidectomy, leaving undetected contralateral cancer foci, a second hospital admission, general anesthesia, more absence from work and more inconvenience could be prevented. RAI

treatment is aimed to decrease the recurrence rate in cases where unfavorable clinicopathological prognostic factors (high-risk for recurrence) are detected, and also to cure recurrence or metastasis (185, 186). Our findings indicate that at present it is not possible to determine these factors accurately without the complete removal of the thyroid gland. Furthermore, in cases of postoperative upstaging after lobectomy, a second completion strumectomy will be necessary, in order to perform a radioiodine therapy. The benefits of serum thyroglobulin (TG) and anti-thyroglobulin antibody (aTG) monitoring could only be utilized following total thyroidectomy and RAI treatment.

To determine the optimal surgical approach, and to decrease the need for unnecessary thyroidectomies, a new set of criteria should be established – but until then, we suggest the more frequent application of primary total thyroidectomy. Appending previously established predictive models with new features such as genetic and epigenetic data from FNA samples is one of the newly emerging promising methods (187-191).

This study has several advantages which include: a single institution of patient care with uniform diagnostic and therapeutic procedures, high number of cases per year, well documented pre- and post-op factors which all lead to well reconstructable cases. Among the limitations of our study, we must mention its retrospective setting. Due to the short follow-up time, we cannot present survival data which is a cornerstone of cancer studies. The high ratio of patients who underwent primary total thyroidectomy can decrease the statistical power when comparing outcomes of one- vs. two-step total thyroidectomies. Exclusion of patients submitted to lobectomy alone may be a source of selection bias. Sonographic assessment was provided by multiple observers at different hospitals on different ultrasound machines. No data on interobserver variability could be collected and no clear definition of sonographic extrathyroidal extension could be adopted. The judgement on extrathyroidal extension was solely on the single ultrasound observer. In our database lymph node metastases could include micrometastasis also (as some pathologic reports did not describe the size of the metastasis), and no data on the actual number of clinically relevant metastases was available. Cost-effectiveness could not be assessed, and the severity and the duration of complications are not well documented. The reconstruction may not be impeccable: the hypothetical adherence to different guidelines which we tried to model does not take into account all the detailed information that is evaluated in real life clinical scenarios. (51)

6. CONCLUSION

After differentiated thyroid cancer, the incidence of several malignant tumors becomes more common. This relationship is also true in reverse: after several malignant diseases, a second primary thyroid cancer is more frequent than expected (3). The possible casual relationships between these tumors are the subject of intensive research and are likely to be due to the interaction of numerous factors. These include genetic predisposition, lifestyle and environmental factors, treatment of the first tumor, and detection bias due to increased diagnostics. Regardless of which factor plays a greater role in each individual, long-term clinical follow-up by the practicing physician is necessary after thyroid cancer in order to detect any second primary malignancy at an early stage. Patients are advised to adhere strictly to the generally recommended cancer screening programs (especially mammography for women and colorectal cancer screening for both sexes) and scheduled medical appointments.

In our study investigating the shared genetic background of thyroid and breast cancer, we reported an increased burden of carcinogenic SNPs in people with both thyroid and breast cancer compared to individuals with breast cancer only, based on whole exome sequencing of 112 known hereditary cancer risk genes. We found no differences in clinicopathological parameters between the groups suggesting that both groups have similar presentation at the time of diagnosis of breast cancer. While our study was not powered to identify specific risk loci for metachronous cancer development, our findings further support the multigenetic etiology of co-occurring breast and thyroid cancer. Our findings do not directly contradict any of the other, previously detailed theories explaining the association between these tumor types. Nevertheless, our results do underline the need for further genetic research in this field, which are lacking at the moment. (20)

Our investigation on PTC–miRNA interactions involving the largest number of original molecular data resulting from PTC samples with matched controls ($n = 118$) and the most comprehensive set of analyzed miRNAs ($n = 2656$) provides strong evidence that the miRNA expression profiles manifest differently in tumorous and non-tumorous areas of the thyroid gland in the case of PTC by a significant margin. This could help improve the accuracy of DNA-based molecular testing for thyroid nodules with uncertain cytology and provide support for clinical decision-making. The data also suggest the essential role

of the identified key miRNAs in PTC pathogenesis, their contribution to different clinicopathological states. It is noteworthy that links between the expression levels of some miRNAs and values related to disease advancement (ATA risk, TNM, and clinical stage) can be found not only when analyzing PTC tissue itself but for the histopathologically healthy adjacent tissue as well. (51)

Most of the time, miRNAs labeled as significant were rather overexpressed in PTC cancer tissue; however, some of them showed a significantly reduced expression in PTC. Moreover, 582 miRNAs showed no expression in either tumor or control samples; however, these miRNAs could still turn out to be clinically significant in future studies of other types of thyroid cancer such as follicular, medullary, anaplastic, or even non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Our findings hold promise for the development of novel diagnostic and therapeutic strategies for PTC patients targeting molecular pathways related to specific miRNAs. Our results not only emphasize the importance of miRNA expression patterns in thyroid cancer research but also highlight that these small non-coding nucleic acids may contribute to personalized medicine in the future. Furthermore, it would also be worth investigating the potential of all the PTC-related miRNAs of this study as possible liquid biopsy biomarkers resulting from the serum as it is already successfully presented with a few miRNAs in PTC as well as with other markers in other malignancies (32, 192, 193). This could help diagnostics in the form of a routine laboratory test and could also provide a joint molecular diagnostic methodology for parallel research on different cancer types. (51)

Researching the outcome of different surgical approaches to previously diagnosed low-risk DTC, we may confirm that the decision to perform total thyroidectomy instead of lobectomy, despite ATA or ETA recommendations for the latter, was a posteriori warranted in the majority of our patients. It is also evident that current pre-operative criteria are inadequate to accurately determine the extent of initial surgery since our postoperative findings suggest frequent need for completion thyroidectomy. ETA has stricter criteria for lobectomies, so that the need for a second surgery is probably less frequent, however, the number of unnecessary total thyroidectomies are proportionately higher with this approach. Predicting the need for a total thyroidectomy is only one aspect of the recommendations, and we chose this variable for its ease of evaluation. We do not intend to state whether one guideline is superior to the other, but only to highlight the

unreliability of preoperative measurements in general. Since the rate of reoperation would have been similar following both the ATA and the ETA guidelines and there is no reliable predictive parameter available at present to determine the proper type of surgery, more frequent primary total thyroidectomy might be suggested until effective pre-operative set of criteria will be established. (87)

7. SUMMARY

1. In summary, we concluded, that thyroid cancer can be seen more frequently after numerous primary malignancies, and the risk of second primary malignancies after thyroid cancer is increased, than would be expected in the general population. We established, that the sporadic co-occurrence of thyroid and breast cancer is at least partly caused by an increased germline oncogene SNP burden. To our best knowledge, this association as a genetic predisposition has not been described previously in the literature.
2. Aiming to improve the diagnostic accuracy in distinguishing benign from malignant thyroid nodules with indeterminate cytology, we found a 30 kind of miRNA expressed differently in papillary thyroid cancer compared to intact thyroid tissue. This was the most comprehensive miRNA study in relation to PTC so far. Some of the identified associations have not been described before in the literature. Thus, this study provided the largest number of original molecular data derived from papillary thyroid cancer samples with matched controls and the most comprehensive set of analyzed miRNAs. We also demonstrated different miRNA profiles associated with different clinicopathological variables, providing a better insight of papillary thyroid cancer biologic behavior. These findings point toward novel diagnostic and moreover, therapeutic strategies for PTC patients.
3. We also established that lobectomy in preoperatively diagnosed low-risk thyroid cancer, according to either ATA or ETA guidelines, results in frequent completion thyroidectomy. This could be partly be explained by our finding that the preoperative assessment of such patients is far from reliable. The size of the malignant thyroid nodule associated weakly with the postoperative risk factors, only the presence of angioinvasion could be predicted based on the tumor size. As a consequence, a better, possibly genetic or epigenetic based preoperative set of criteria would be necessary, to decide more appropriately the initial surgical approach. Until that, we advocate for more one-step total thyroidectomy, avoiding the possible need for a second surgery.

8. REFERENCES

- (1) Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. *J Cancer Epidemiol*. 2013;2013:965212.
- (2) Furuya-Kanamori L, Bell KJL, Clark J, Glasziou P, Doi SAR. Prevalence of Differentiated Thyroid Cancer in Autopsy Studies Over Six Decades: A Meta-Analysis. *J Clin Oncol*. 2016 Oct 20;34(30):3672-3679.
- (3) Kiss A, Szili B, Bakos B, Tóbiás B, Balla B, Takács I, Lakatos P, Kósa J. A differenciált pajzsmirigyrák rizikója mint első és második primer tumor [Risk of differentiated thyroid cancer as the first and second primary tumor]. *Orv Hetil*. 2025 Feb 16;166(7):253-262. Hungarian.
- (4) Wang X, Zeng M, Ju X, Lin A, Zhou C, Shen J, Liu Z, Tang B, Cheng Q, Wang Y, Zhang J, Luo P. Correlation between second and first primary cancer: systematic review and meta-analysis of 9 million cancer patients. *Br J Surg*. 2024 Jan 3;111(1):znad377. Erratum in: *Br J Surg*. 2024 Jan 31;111(2):znae044.
- (5) Hou F, Cheng T, Yang CL, Sun XD, Yang ZX, Lv J, Liu C, Deng ZY. Risk prediction of second primary malignant tumor in primary differentiated thyroid cancer patients: a population-based study. *J Cancer Res Clin Oncol*. 2023 Oct;149(13):12379-12391.
- (6) Subramanian S, Goldstein DP, Parlea L, Thabane L, Ezzat S, Ibrahim-Zada I, Straus S, Brierley JD, Tsang RW, Gafni A, Rotstein L, Sawka AM. Second primary malignancy risk in thyroid cancer survivors: a systematic review and meta-analysis. *Thyroid*. 2007 Dec;17(12):1277-88.
- (7) Ruan X, Huang D, Zhan Y, Huang J, Huang J, Ng AT, Tsu JH, Na R. Risk of second primary cancers after a diagnosis of first primary cancer: A pan-cancer analysis and Mendelian randomization study. *Elife*. 2023 Nov 2;12:e86379.
- (8) Allen I, Hassan H, Sofianopoulou E, Eccles D, Turnbull C, Tischkowitz M, Pharoah P, Antoniou AC. Risks of second non-breast primaries following breast cancer in women: a systematic review and meta-analysis. *Breast Cancer Res*. 2023 Feb 10;25(1):18.
- (9) Allen I, Hassan H, Sofianopoulou E, Eccles D, Turnbull C, Tischkowitz M, Pharoah P, Antoniou AC. Risk of developing a second primary cancer in male breast cancer

survivors: a systematic review and meta-analysis. *Br J Cancer*. 2022 Nov;127(9):1660-1669.

(10) McTiernan A, Weiss NS, Daling JR. Incidence of thyroid cancer in women in relation to known or suspected risk factors for breast cancer. *Cancer Res*. 1987 Jan 1;47(1):292-5.

(11) An JH, Hwangbo Y, Ahn HY, Keam B, Lee KE, Han W, Park DJ, Park IA, Noh DY, Youn YK, Cho BY, Im SA, Park YJ. A Possible Association Between Thyroid Cancer and Breast Cancer. *Thyroid*. 2015 Dec;25(12):1330-8.

(12) Nielsen SM, White MG, Hong S, Aschebrook-Kilfoy B, Kaplan EL, Angelos P, Kulkarni SA, Olopade OI, Grogan RH. The Breast-Thyroid Cancer Link: A Systematic Review and Meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2016 Feb;25(2):231-8.

(13) Joseph KR, Edirimanne S, Eslick GD. The association between breast cancer and thyroid cancer: a meta-analysis. *Breast Cancer Res Treat*. 2015 Jul;152(1):173-181.

(14) Pasqual E, Schonfeld S, Morton LM, Villoing D, Lee C, Berrington de Gonzalez A, Kitahara CM. Association Between Radioactive Iodine Treatment for Pediatric and Young Adulthood Differentiated Thyroid Cancer and Risk of Second Primary Malignancies. *J Clin Oncol*. 2022 May 1;40(13):1439-1449. Erratum in: *J Clin Oncol*. 2022 Jul 10;40(20):2283.

(15) Lu M, Liu H, Zheng B, Sun S, Chen C. Links between Breast and Thyroid Cancer: Hormones, Genetic Susceptibility and Medical Interventions. *Cancers (Basel)*. 2022 Oct 19;14(20):5117.

(16) Tan H, Wang S, Huang F, Tong Z. Association between breast cancer and thyroid cancer risk: a two-sample Mendelian randomization study. *Front Endocrinol (Lausanne)*. 2023 May 23;14:1138149.

(17) Ngeow J, Stanuch K, Mester JL, Barnholtz-Sloan JS, Eng C. Second malignant neoplasms in patients with Cowden syndrome with underlying germline PTEN mutations. *J Clin Oncol*. 2014 Jun 10;32(17):1818-24.

(18) Ni Y, He X, Chen J, Moline J, Mester J, Orloff MS, Ringel MD, Eng C. Germline SDHx variants modify breast and thyroid cancer risks in Cowden and Cowden-like

syndrome via FAD/NAD-dependant destabilization of p53. *Hum Mol Genet.* 2012 Jan 15;21(2):300-10.

(19) Bolf EL, Sprague BL, Carr FE. A Linkage Between Thyroid and Breast Cancer: A Common Etiology? *Cancer Epidemiol Biomarkers Prev.* 2019 Apr;28(4):643-649.

(20) Bakos B, Kiss A, Árvai K, Szili B, Deák-Kocsis B, Tobiás B, Putz Z, Ármós R, Balla B, Kósa J, Dank M, Valkusz Z, Takács I, Tabák Á, Lakatos P. Co-occurrence of thyroid and breast cancer is associated with an increased oncogenic SNP burden. *BMC Cancer.* 2021 Jun 15;21(1):706.

(21) Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. *J Natl Cancer Inst.* 1994 Nov 2;86(21):1600-8.

(22) Durante C, Grani G, Lamartina L, Filetti S, Mandel SJ, Cooper DS. The Diagnosis and Management of Thyroid Nodules: A Review. *JAMA.* 2018 Mar 6;319(9):914.

(23) Alzahrani AS. Clinical use of Molecular Data in Thyroid Nodules and Cancer. *J Clin Endocrinol Metab.* 2023 Oct 18;108(11):2759–71.

(24) Labourier E, Shifrin A, Busseniers AE, Lupo MA, Manganelli ML, Andruss B, Wylie D, Beaudenon-Huibregtse S. Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology. *J Clin Endocrinol Metab.* 2015 Jul;100(7):2743-50.

(25) Silaghi CA, Lozovanu V, Silaghi H, Georgescu RD, Pop C, Dobrean A, Georgescu CE. The Prognostic Value of MicroRNAs in Thyroid Cancers-A Systematic Review and Meta-Analysis. *Cancers (Basel).* 2020 Sep 12;12(9):2608.

(26) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004 Jan 23;116(2):281-97.

(27) Ambros V. miRNAs Found by Genomics and Reverse Genetics [Internet] 2004. [(accessed on 1 January 2024)]. Available online: www.nature.com/nature.

(28) Mazeh H, Levy Y, Mizrahi I, Appelbaum L, Ilyayev N, Halle D, Freund HR, Nissan A. Differentiating benign from malignant thyroid nodules using micro ribonucleic acid amplification in residual cells obtained by fine needle aspiration biopsy. *J Surg Res.* 2013 Apr;180(2):216-21.

- (29) Rossing M, Kaczkowski B, Futoma-Kazmierczak E, Glud M, Klausen M, Faber J, Nygaard B, Kiss K, Sørensen CH, Nielsen FC, Bennedbæk FN, Friis-Hansen L. A simple procedure for routine RNA extraction and miRNA array analyses from a single thyroid in vivo fine needle aspirate. *Scand J Clin Lab Invest*. 2010 Dec;70(8):529-34.
- (30) Geropoulos G, Psarras K, Papaioannou M, Giannis D, Meitanidou M, Kapriniotis K, Symeonidis N, Pavlidis ET, Pavlidis TE, Sapalidis K, Ahmed NM, Abdel-Aziz TE, Eddama MMR. Circulating microRNAs and Clinicopathological Findings of Papillary Thyroid Cancer: A Systematic Review. *In Vivo*. 2022 Jul-Aug;36(4):1551-1569.
- (31) Ghafouri-Fard S, Shirvani-Farsani Z, Taheri M. The role of microRNAs in the pathogenesis of thyroid cancer. *Noncoding RNA Res*. 2020 Jun 20;5(3):88-98.
- (32) Ruiz-Pozo VA, Cadena-Ullauri S, Guevara-Ramírez P, Paz-Cruz E, Tamayo-Trujillo R, Zambrano AK. Differential microRNA expression for diagnosis and prognosis of papillary thyroid cancer. *Front Med (Lausanne)*. 2023 Apr 5;10:1139362.
- (33) Sassi Y, Avramopoulos P, Ramanujam D, Grüter L, Werfel S, Giosele S, Brunner AD, Esfandyari D, Papadopoulou AS, De Strooper B, Hübner N, Kumarswamy R, Thum T, Yin X, Mayr M, Laggerbauer B, Engelhardt S. Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. *Nat Commun*. 2017 Nov 20;8(1):1614.
- (34) Reddy S, Hu DQ, Zhao M, Blay E Jr, Sandeep N, Ong SG, Jung G, Kooiker KB, Coronado M, Fajardo G, Bernstein D. miR-21 is associated with fibrosis and right ventricular failure. *JCI Insight*. 2017 May 4;2(9):e91625.
- (35) De Martinis M, Ginaldi L, Allegra A, Sirufo MM, Pioggia G, Tonacci A, Gangemi S. The Osteoporosis/Microbiota Linkage: The Role of miRNA. *Int J Mol Sci*. 2020 Nov 24;21(23):8887.
- (36) Ghaffari M, Razi S, Zalpoor H, Nabi-Afjadi M, Mohebichamkhorami F, Zali H. Association of MicroRNA-146a with Type 1 and 2 Diabetes and their Related Complications. *J Diabetes Res*. 2023 Mar 3;2023:2587104.
- (37) Nielsen LB, Wang C, Sørensen K, Bang-Berthelsen CH, Hansen L, Andersen ML, Hougaard P, Juul A, Zhang CY, Pociot F, Mortensen HB. Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that

miR-25 associates to residual beta-cell function and glycaemic control during disease progression. *Exp Diabetes Res*. 2012;2012:896362.

(38) Xie Y, Zhang L, Gao Y, Ge W, Tang P. The Multiple Roles of Microrna-223 in Regulating Bone Metabolism. *Molecules*. 2015 Oct 23;20(10):19433-48.

(39) Plotkin LI, Wallace JM. MicroRNAs and osteocytes. *Bone*. 2021 Sep;150:115994.

(40) Li MP, Hu YD, Hu XL, Zhang YJ, Yang YL, Jiang C, Tang J, Chen XP. MiRNAs and miRNA Polymorphisms Modify Drug Response. *Int J Environ Res Public Health*. 2016 Nov 8;13(11):1096.

(41) Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer*. 2006 Apr;6(4):259-69.

(42) Ab Mutalib NS, Othman SN, Mohamad Yusof A, Abdullah Suhaimi SN, Muhammad R, Jamal R. Integrated microRNA, gene expression and transcription factors signature in papillary thyroid cancer with lymph node metastasis. *PeerJ*. 2016 Jun 15;4:e2119.

(43) Li X, Abdel-Mageed AB, Mondal D, Kandil E. MicroRNA expression profiles in differentiated thyroid cancer, a review. *Int J Clin Exp Med*. 2013;6(1):74-80.

(44) Le F, Luo P, Yang QO, Zhong XM. MiR-181a promotes growth of thyroid cancer cells by targeting tumor suppressor RB1. *Eur Rev Med Pharmacol Sci*. 2017 Dec;21(24):5638-5647.

(45) O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol (Lausanne)*. 2018 Aug 3;9:402.

(46) Shang R, Lee S, Senavirathne G, Lai EC. microRNAs in action: biogenesis, function and regulation. *Nat Rev Genet*. 2023 Dec;24(12):816-833.

(47) Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006 Nov;6(11):857-66.

(48) Toraih EA, Fawzy MS, Hussein MH, El-Labban MM, Ruiz EML, Attia AA, Halat S, Moroz K, Errami Y, Zerfaoui M, Kandil E. MicroRNA-Based Risk Score for Predicting Tumor Progression Following Radioactive Iodine Ablation in Well-

Differentiated Thyroid Cancer Patients: A Propensity-Score Matched Analysis. *Cancers (Basel)*. 2021 Sep 16;13(18):4649.

(49) Chen YT, Kitabayashi N, Zhou XK, Fahey TJ 3rd, Scognamiglio T. MicroRNA analysis as a potential diagnostic tool for papillary thyroid carcinoma. *Mod Pathol*. 2008 Sep;21(9):1139-46.

(50) Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *J Clin Endocrinol Metab*. 2008 May;93(5):1600-8.

(51) Armos R, Bojtor B, Papp M, Illyes I, Lengyel B, Kiss A, Szili B, Tobias B, Balla B, Piko H, Illes A, Putz Z, Kiss A, Toth E, Takacs I, Kosa JP, Lakatos P. MicroRNA Profiling in Papillary Thyroid Cancer. *Int J Mol Sci*. 2024 Aug 29;25(17):9362.

(52) Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell*. 2014 Oct 23;159(3):676-90.

(53) Bertol BC, Massaro JD, Debortoli G, Santos ALP, de Araújo JNG, Giorgenon TMV, Costa E Silva M, de Figueiredo-Feitosa NL, Collares CVA, de Freitas LCC, Soares EG, Neder L, Silbiger VN, Calado RT, Maciel LMZ, Donadi EA. BRAF, TERT and HLA-G Status in the Papillary Thyroid Carcinoma: A Clinicopathological Association Study. *Int J Mol Sci*. 2023 Aug 5;24(15):12459.

(54) Mohamad Yusof A, Jamal R, Muhammad R, Abdullah Suhaimi SN, Mohamed Rose I, Saidin S, Ab Mutalib NS. Integrated Characterization of MicroRNA and mRNA Transcriptome in Papillary Thyroid Carcinoma. *Front Endocrinol (Lausanne)*. 2018 Apr 16;9:158.

(55) Santiago K, Chen Wongworawat Y, Khan S. Differential MicroRNA-Signatures in Thyroid Cancer Subtypes. *J Oncol*. 2020 Jun 3;2020:2052396.

(56) Ricarte-Filho JC, Casado-Medrano V, Reichenberger E, Spangler Z, Scheerer M, Isaza A, Baran J, Patel T, MacFarland SP, Brodeur GM, Stewart DR, Baloch Z, Bauer AJ, Wasserman JD, Franco AT. DICER1 RNase IIIb domain mutations trigger widespread miRNA dysregulation and MAPK activation in pediatric thyroid cancer. *Front Endocrinol (Lausanne)*. 2023 Feb 21;14:1083382.

- (57) Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM, Fusco A. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer*. 2006 Jun;13(2):497-508.
- (58) He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A. The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci U S A*. 2005 Dec 27;102(52):19075-80.
- (59) Chou CK, Chen RF, Chou FF, Chang HW, Chen YJ, Lee YF, Yang KD, Cheng JT, Huang CC, Liu RT. miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extrathyroidal invasion and the BRAF(V600E) mutation. *Thyroid*. 2010 May;20(5):489-94.
- (60) Liang L, Zheng X, Hu M, Cui Y, Zhong Q, Wang S, Huang F. MiRNA-221/222 in thyroid cancer: A meta-analysis. *Clin Chim Acta*. 2018 Sep;484:284-292.
- (61) Gómez-Pérez AM, Cornejo Pareja IM, García Alemán J, Coín Aragüez L, Sebastián Ochoa A, Alcaide Torres J, Molina Vega M, Clu Fernández C, Mancha Doblas I, Tinahones FJ. New molecular biomarkers in differentiated thyroid carcinoma: Impact of miR-146, miR-221 and miR-222 levels in the evolution of the disease. *Clin Endocrinol (Oxf)*. 2019 Jul;91(1):187-194.
- (62) Chou CK, Yang KD, Chou FF, Huang CC, Lan YW, Lee YF, Kang HY, Liu RT. Prognostic implications of miR-146b expression and its functional role in papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2013 Feb;98(2):E196-205.
- (63) Deng X, Wu B, Xiao K, Kang J, Xie J, Zhang X, Fan Y. MiR-146b-5p promotes metastasis and induces epithelial-mesenchymal transition in thyroid cancer by targeting ZNRF3. *Cell Physiol Biochem*. 2015;35(1):71-82.
- (64) Khan R, Riaz A, Abbasi SA, Sadaf T, Baig RM, Mansoor Q. Identification of transcriptional level variations in microRNA-221 and microRNA-222 as alternate players in the thyroid cancer tumor microenvironment. *Sci Rep*. 2023 Sep 22;13(1):15800.
- (65) Pishkari S, Paryan M, Hashemi M, Baldini E, Mohammadi-Yeganeh S. The role of microRNAs in different types of thyroid carcinoma: a comprehensive analysis to find new miRNA supplementary therapies. *J Endocrinol Invest*. 2018 Mar;41(3):269-283.

- (66) Liu L, Wang J, Li X, Ma J, Shi C, Zhu H, Xi Q, Zhang J, Zhao X, Gu M. MiR-204-5p suppresses cell proliferation by inhibiting IGFBP5 in papillary thyroid carcinoma. *Biochem Biophys Res Commun*. 2015 Feb 20;457(4):621-6.
- (67) Schwertheim S, Sheu SY, Worm K, Grabellus F, Schmid KW. Analysis of deregulated miRNAs is helpful to distinguish poorly differentiated thyroid carcinoma from papillary thyroid carcinoma. *Horm Metab Res*. 2009 Jun;41(6):475-81.
- (68) Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016 Jan;26(1):1-133.
- (69) Park JL, Kim SK, Jeon S, Jung CK, Kim YS. MicroRNA Profile for Diagnostic and Prognostic Biomarkers in Thyroid Cancer. *Cancers (Basel)*. 2021 Feb 5;13(4):632.
- (70) Kent WD, Hall SF, Isotalo PA, Houlden RL, George RL, Groome PA. Increased incidence of differentiated thyroid carcinoma and detection of subclinical disease. *CMAJ*. 2007 Nov 20;177(11):1357-61.
- (71) Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. *N Engl J Med*. 2016 Aug 18;375(7):614-7.
- (72) Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013. *JAMA*. 2017 Apr 4;317(13):1338-1348.
- (73) Jukić T, Blažeković I, Franceschi M, Ovčariček PP, Butković MB, Dabelić N, Granić R, Punda M, Sonicki Z, Vagić D, Fröbe A, Kusić Z. Long-Term Outcome of Differentiated Thyroid Cancer Patients-Fifty Years of Croatian Thyroid Disease Referral Centre Experience. *Diagnostics (Basel)*. 2022 Mar 30;12(4):866.
- (74) Luster M, Aktolun C, Amendoeira I, Barczyński M, Bible KC, Duntas LH, Elisei R, Handkiewicz-Junak D, Hoffmann M, Jarząb B, Leenhardt L, Musholt TJ, Newbold K, Nixon IJ, Smit J, Sobrinho-Simões M, Sosa JA, Tuttle RM, Verburg FA, Wartofsky L, Führer D. European Perspective on 2015 American Thyroid Association Management

Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: Proceedings of an Interactive International Symposium. *Thyroid*. 2019 Jan;29(1):7-26.

(75) Ringel MD, Sosa JA, Baloch Z, Bischoff L, Bloom G, Brent GA, Brock PL, Chou R, Flavell RR, Goldner W, Grubbs EG, Haymart M, Larson SM, Leung AM, Osborne J, Ridge JA, Robinson B, Steward DL, Tufano RP, Wirth LJ. 2025 American Thyroid Association Management Guidelines for Adult Patients with Differentiated Thyroid Cancer. *Thyroid*. 2025 Aug;35(8):841-985.

(76) Bozec A, Dassonville O, Chamorey E, Poissonnet G, Sudaka A, Peyrottes I, Ettore F, Haudebourg J, Bussière F, Benisvy D, Marcy PY, Sadoul JL, Hofman P, Lassale S, Vallicioni J, Demard F, Santini J. Clinical impact of cervical lymph node involvement and central neck dissection in patients with papillary thyroid carcinoma: a retrospective analysis of 368 cases. *Eur Arch Otorhinolaryngol*. 2011 Aug;268(8):1205-1212.

(77) DiMarco AN, Wong MS, Jayasekara J, Cole-Clark D, Aniss A, Glover AR, Delbridge LW, Sywak MS, Sidhu SB. Risk of needing completion thyroidectomy for low-risk papillary thyroid cancers treated by lobectomy. *BJS Open*. 2019 Feb 6;3(3):299-304.

(78) Duh QY, Shen WT. CLINICAL IMPLICATIONS OF POSTOPERATIVE UPSTAGING OF DIFFERENTIATED THYROID CANCER BASED UPON PATHOLOGIC EVALUATION. *Endocr Pract*. 2018 Jan;24(1):124-125.

(79) Kluijfhout WP, Pasternak JD, Drake FT, Beninato T, Shen WT, Gosnell JE, Suh I, C L, Duh QY. Application of the new American Thyroid Association guidelines leads to a substantial rate of completion total thyroidectomy to enable adjuvant radioactive iodine. *Surgery*. 2017 Jan;161(1):127-133.

(80) Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W; European Thyroid Cancer Taskforce. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. *Eur J Endocrinol*. 2006 Jun;154(6):787-803. Erratum in: *Eur J Endocrinol*. 2006 Aug;155(2):385.

(81) Reed R, Strumpf A, Martz TG, Kavanagh KJ, Fedder KL, Jameson MJ, Shonka DC Jr. 2015 American Thyroid Association guidelines and thyroid-stimulating hormone suppression after thyroid lobectomy. *Head Neck*. 2021 Feb;43(2):639-644.

- (82) Schumm MA, Lechner MG, Shu ML, Ochoa JE, Kim J, Tseng CH, Leung AM, Yeh MW. Frequency of Thyroid Hormone Replacement After Lobectomy for Differentiated Thyroid Cancer. *Endocr Pract.* 2021 Jul;27(7):691-697.
- (83) Cooper D, Kaur R, Ayeni FE, Eslick GD, Edirimanne S. Hypothyroidism after hemithyroidectomy: a systematic review and meta-analysis. *Thyroid Res.* 2024 Jul 8;17(1):18.
- (84) Stempler M, Bakos B, Solymosi T, Kiss A, Ármós RL, Szili B, Mészáros S, Tőke J, Szűcs N, Reismann P, Péter P, Lakatos PA, Takács I. Analysis of factors influencing the dose of levothyroxine treatment in adequately controlled hypothyroid patients of different etiologies. *Heliyon.* 2024 Oct 19;10(20):e39639.
- (85) Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM; American Thyroid Association Task Force on Thyroid Hormone Replacement. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid.* 2014 Dec;24(12):1670-751.
- (86) Bakos B, Solymosi T, Szili B, Vincze Á, Mészáros S, Stempler M, Ármós R, Kiss A, Bakos A, Szűcs N, Reismann P, Tőke J, Pusztai P, Lakatos P, Takács I. Psychological factors and obesity, not thyroid biomarkers, predict thyroid-dependent quality of life in treated hypothyroidism: a cross-sectional study. *BMC Endocr Disord.* 2025 May 26;25(1):136.
- (87) Kiss A, Szili B, Bakos B, Ármós R, Putz Z, Árvai K, Kocsis-Deák B, Tobiás B, Balla B, Pikó H, Dank M, Kósa JP, Takács I, Lakatos P. Comparison of surgical strategies in the treatment of low-risk differentiated thyroid cancer. *BMC Endocr Disord.* 2023 Jan 26;23(1):23.
- (88) Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, Wendl MC, Kim J, Reardon B, Kwok-Shing Ng P, Jeong KJ, Cao S, Wang Z, Gao J, Gao Q, Wang F, Liu EM, Mularoni L, Rubio-Perez C, Nagarajan N, Cortés-Ciriano I, Zhou DC, Liang WW, Hess JM, Yellapantula VD, Tamborero D, Gonzalez-Perez A, Suphavitai C, Ko JY, Khurana E, Park PJ, Van Allen EM, Liang H; MC3 Working Group; Cancer Genome Atlas Research Network; Lawrence MS, Godzik A, Lopez-Bigas N, Stuart J, Wheeler D, Getz G, Chen K, Lazar AJ, Mills GB, Karchin R,

- Ding L. Comprehensive Characterization of Cancer Driver Genes and Mutations. *Cell*. 2018 Aug 9;174(4):1034-1035. Erratum for: *Cell*. 2018 Apr 5;173(2):371-385.e18.
- (89) Foulkes WD. Inherited Susceptibility to Common Cancers. *N Engl J Med*. 2008 Nov 13;359(20):2143–53.
- (90) Shah PD, Nathanson KL. Application of Panel-Based Tests for Inherited Risk of Cancer. *Annu Rev Genomics Hum Genet*. 2017 Aug 31;18(1):201–27.
- (91) Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, Jang W, Katz K, Ovetsky M, Riley G, Sethi A, Tully R, Villamarin-Salomon R, Rubinstein W, Maglott DR. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016 Jan 4;44(D1):D862-8.
- (92) Kuo JH, Chabot JA, Lee JA. Breast cancer in thyroid cancer survivors: An analysis of the Surveillance, Epidemiology, and End Results-9 database. *Surgery*. 2016 Jan;159(1):23-9.
- (93) Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017 Jan;67(1):7-30.
- (94) Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, Bai Y, Zhu C, Guo GL, Rothman N, Zhang Y. International patterns and trends in thyroid cancer incidence, 1973-2002. *Cancer Causes Control*. 2009 Jul;20(5):525-31.
- (95) Davies L, Welch HG. Current thyroid cancer trends in the United States. *JAMA Otolaryngol Head Neck Surg*. 2014 Apr;140(4):317-22.
- (96) Moleti M, Sturniolo G, Di Mauro M, Russo M, Vermiglio F. Female Reproductive Factors and Differentiated Thyroid Cancer. *Front Endocrinol (Lausanne)*. 2017 May 23;8:111.
- (97) Song ZM, Wang YD, Chai F, Zhang J, Lv S, Wang JX, Xi Y. Estrogen enhances the proliferation, migration, and invasion of papillary thyroid carcinoma via the ER α /KRT19 signaling axis. *J Endocrinol Invest*. 2025 Mar;48(3):653-670.
- (98) Joshi D, Patel J, Munshi M, Mistry Z, Prajapati A, Mukherjee A, Ramachandran AV, Parashar NC, Parashar G, Haque S, Tuli HS. Hormones as a double-edged sword:

the role of hormones in cancer progression and the potential of targeted hormone therapies. *Med Oncol.* 2024 Oct 14;41(11):283.

(99) Yane K, Kitahori Y, Konishi N, Okaichi K, Ohnishi T, Miyahara H, Matsunaga T, Lin JC, Hiasa Y. Expression of the estrogen receptor in human thyroid neoplasms. *Cancer Lett.* 1994 Aug 29;84(1):59-66.

(100) Derwahl M, Nicula D. Estrogen and its role in thyroid cancer. *Endocr Relat Cancer.* 2014 Oct;21(5):T273-83.

(101) Halada S, Casado-Medrano V, Baran JA, Lee J, Chinmay P, Bauer AJ, Franco AT. Hormonal Crosstalk Between Thyroid and Breast Cancer. *Endocrinology.* 2022 Jul 1;163(7):bqac075.

(102) Ruggeri RM, Campennì A, Giuffrè G, Giovanella L, Siracusa M, Simone A, Branca G, Scarfi R, Trimarchi F, Ieni A, Tuccari G. HER2 Analysis in Sporadic Thyroid Cancer of Follicular Cell Origin. *Int J Mol Sci.* 2016 Dec 6;17(12):2040.

(103) Qua Quarini E, Grillo F, Gervaso L, Arpa G, Fazio N, Vanoli A, Parente P. Prognostic and Predictive Roles of HER2 Status in Non-Breast and Non-Gastroesophageal Carcinomas. *Cancers (Basel).* 2024 Sep 13;16(18):3145.

(104) Rajoria S, Suriano R, Shanmugam A, Wilson YL, Schantz SP, Geliebter J, Tiwari RK. Metastatic phenotype is regulated by estrogen in thyroid cells. *Thyroid.* 2010 Jan;20(1):33-41.

(105) Xu S, Chen G, Peng W, Renko K, Derwahl M. Oestrogen action on thyroid progenitor cells: relevant for the pathogenesis of thyroid nodules? *J Endocrinol.* 2013 Jun 1;218(1):125-33.

(106) Kurata A. Differentiated thyroid cancer: Why does it affect predominantly women during the reproductive period and have higher incidence of mutual association with breast cancer? *Med Hypotheses.* 2019 Jan;122:5–7.

(107) Zidan J, Rubenstein W. Effect of Adjuvant Tamoxifen Therapy on Thyroid Function in Postmenopausal Women with Breast Cancer. *Oncology.* 1999;56(1):43–5.

(108) Haymart MR, Repplinger DJ, Levenson GE, Elson DF, Sippel RS, Jaume JC, Chen H. Higher serum thyroid stimulating hormone level in thyroid nodule patients is

associated with greater risks of differentiated thyroid cancer and advanced tumor stage. *J Clin Endocrinol Metab.* 2008 Mar;93(3):809-14.

(109) Hall LC, Salazar EP, Kane SR, Liu N. Effects of thyroid hormones on human breast cancer cell proliferation. *J Steroid Biochem Mol Biol.* 2008 Mar;109(1-2):57-66.

(110) Silva JM, Domínguez G, González-Sancho JM, García JM, Silva J, García-Andrade C, Navarro A, Muñoz A, Bonilla F. Expression of thyroid hormone receptor/erbA genes is altered in human breast cancer. *Oncogene.* 2002 Jun 20;21(27):4307-16.

(111) Kim WG, Cheng SY. Thyroid hormone receptors and cancer. *Biochim Biophys Acta.* 2013 Jul;1830(7):3928-36.

(112) Martínez-Iglesias O, Garcia-Silva S, Tenbaum SP, Regadera J, Larcher F, Paramio JM, Vennström B, Aranda A. Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis. *Cancer Res.* 2009 Jan 15;69(2):501-9.

(113) Carr FE, Tai PW, Barnum MS, Gillis NE, Evans KG, Taber TH, White JH, Tomczak JA, Jaworski DM, Zaidi SK, Lian JB, Stein JL, Stein GS. Thyroid Hormone Receptor- β (TR β) Mediates Runt-Related Transcription Factor 2 (Runx2) Expression in Thyroid Cancer Cells: A Novel Signaling Pathway in Thyroid Cancer. *Endocrinology.* 2016 Aug;157(8):3278-92.

(114) Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, Hsu L, Krishnamurthy S, Theriault RL, Hortobagyi GN. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer.* 2005 Mar 15;103(6):1122-8.

(115) Shi XZ, Jin X, Xu P, Shen HM. Relationship between breast cancer and levels of serum thyroid hormones and antibodies: a meta-analysis. *Asian Pac J Cancer Prev.* 2014;15(16):6643-7.

(116) Hardefeldt PJ, Eslick GD, Edirimanne S. Benign thyroid disease is associated with breast cancer: a meta-analysis. *Breast Cancer Res Treat.* 2012 Jun;133(3):1169-77.

(117) Jin J, Li J, Liu Y, Shi Q, Zhang B, Ji Y, Hu P. Thyroid Hormone Changes Correlate to Combined Breast Cancer with Primary Thyroid Cancer. *Breast Cancer (Dove Med Press).* 2024 Jan 9;16:15-22.

- (118) Muller I, Kilburn LS, Taylor PN, Barrett-Lee PJ, Bliss JM, Ellis P, Ludgate ME, Dayan CM. TPOAb and Thyroid Function Are Not Associated with Breast Cancer Outcome: Evidence from a Large-Scale Study Using Data from the Taxotere as Adjuvant Chemotherapy Trial (TACT, CRUK01/001). *Eur Thyroid J*. 2017 Jul;6(4):197-207.
- (119) Fang Y, Yao L, Sun J, Yang R, Chen Y, Tian J, Yang K, Tian L. Does thyroid dysfunction increase the risk of breast cancer? A systematic review and meta-analysis. *J Endocrinol Invest*. 2017 Oct;40(10):1035-1047.
- (120) Voutsadakis IA. The TSH/Thyroid Hormones Axis and Breast Cancer. *J Clin Med*. 2022 Jan 28;11(3):687.
- (121) Pan XF, Ma YJ, Tang Y, Yu MM, Wang H, Fan YR. Breast cancer populations may have an increased prevalence of thyroglobulin antibody and thyroid peroxidase antibody: a systematic review and meta-analysis. *Breast Cancer*. 2020 Sep;27(5):828-836.
- (122) Han M, Wang Y, Jin Y, Zhao X, Cui H, Wang G, Gang X. Benign thyroid disease and the risk of breast cancer: An updated systematic review and meta-analysis. *Front Endocrinol (Lausanne)*. 2022 Oct 12;13:984593.
- (123) Hoffman K, Lorenzo A, Butt CM, Hammel SC, Henderson BB, Roman SA, Scheri RP, Stapleton HM, Sosa JA. Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. *Environ Int*. 2017 Oct;107:235-242.
- (124) Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr Rev*. 2015 Dec;36(6):E1-E150.
- (125) Soto AM, Vandenberg LN, Maffini MV, Sonnenschein C. Does breast cancer start in the womb? *Basic Clin Pharmacol Toxicol*. 2008 Feb;102(2):125-33.
- (126) Huang X, Han Y, Yang J, Bu L, Cheng X, Zha X, Chen H, Huang Y, Qu S. Sex-dependent impact of obesity on aggressiveness of papillary thyroid cancer. *Hormones (Athens)*. 2024 Jun;23(2):217-225.
- (127) Isaksen IM, Dankel SN. Ultra-processed food consumption and cancer risk: A systematic review and meta-analysis. *Clin Nutr*. 2023 Jun;42(6):919-928.

- (128) Gunter MJ, Berrington de Gonzalez A. Is obesity a cause of all cancer types? *Lancet Reg Health Eur*. 2024 Oct 21;46:101110.
- (129) Sun M, da Silva M, Bjørge T, Fritz J, Mboya IB, Jerkeman M, Stattin P, Wahlström J, Michaëlsson K, van Guelpen B, Magnusson PKE, Sandin S, Yin W, Lagerros YT, Ye W, Nwaru B, Kankaanranta H, Lönnberg L, Chabok A, Isaksson K, Pedersen NL, Elmståhl S, Lind L, Hedman L, Häggström C, Stocks T. Body mass index and risk of over 100 cancer forms and subtypes in 4.1 million individuals in Sweden: the Obesity and Disease Development Sweden (ODDS) pooled cohort study. *Lancet Reg Health Eur*. 2024 Aug 20;45:101034.
- (130) Harvie M, Howell A, Evans DG. Can diet and lifestyle prevent breast cancer: what is the evidence? *Am Soc Clin Oncol Educ Book*. 2015:e66-73.
- (131) Ma J, Huang M, Wang L, Ye W, Tong Y, Wang H. Obesity and Risk of Thyroid Cancer: Evidence from a Meta-Analysis of 21 Observational Studies. *Med Sci Monit Int Med J Exp Clin Res*. 2015 Jan 22;21:283–91.
- (132) Warakomski J, Romuk E, Jarząb B, Krajewska J, Siemińska L. Concentrations of Selected Adipokines, Interleukin-6, and Vitamin D in Patients with Papillary Thyroid Carcinoma in Respect to Thyroid Cancer Stages. *Int J Endocrinol*. 2018 Dec 3;2018:4921803.
- (133) Lee K, Kruper L, Dieli-Conwright CM, Mortimer JE. The Impact of Obesity on Breast Cancer Diagnosis and Treatment. *Curr Oncol Rep*. 2019 Mar 27;21(5):41.
- (134) Yeo Y, Ma SH, Hwang Y, Horn-Ross PL, Hsing A, Lee KE, Park YJ, Park DJ, Yoo KY, Park SK. Diabetes mellitus and risk of thyroid cancer: a meta-analysis. *PLoS One*. 2014 Jun 13;9(6):e98135.
- (135) Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. *Obes Rev*. 2007 Sep;8(5):395–408.
- (136) Zhang F, de Haan-Du J, Sidorenkov G, Landman GWD, Jalving M, Zhang Q, de Bock GH. Type 2 Diabetes Mellitus and Clinicopathological Tumor Characteristics in Women Diagnosed with Breast Cancer: A Systematic Review and Meta-Analysis. *Cancers (Basel)*. 2021 Oct 5;13(19):4992.

- (137) Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, Deng HF, Amenta PS, Fineberg S, Pestell RG, Carrasco N. The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nat Med*. 2000 Aug;6(8):871-8.
- (138) Dong L, Lu J, Zhao B, Wang W, Zhao Y. Review of the possible association between thyroid and breast carcinoma. *World J Surg Oncol*. 2018 Jul 5;16(1):130.
- (139) Choi WJ, Kim J. Dietary factors and the risk of thyroid cancer: a review. *Clin Nutr Res*. 2014 Jul;3(2):75-88.
- (140) Dutta D, Sharma M, Aggarwal S, Mohindra R, Bhattacharya S, Kalra S. Vitamin D, Thyroid Autoimmunity and Cancer: An Interplay of Different Factors. *Indian J Endocrinol Metab*. 2019 Sep-Oct;23(5):507-513.
- (141) Maciejewski A, Lacka K. Vitamin D-Related Genes and Thyroid Cancer-A Systematic Review. *Int J Mol Sci*. 2022 Nov 7;23(21):13661.
- (142) Hu Y, Xue C, Ren S, Dong L, Gao J, Li X. Association between vitamin D status and thyroid cancer: a meta-analysis. *Front Nutr*. 2024 Jun 18;11:1423305.
- (143) de La Puente-Yagüe M, Cuadrado-Cenzual MA, Ciudad-Cabañas MJ, Hernández-Cabria M, Collado-Yurrita L. Vitamin D: And its role in breast cancer. *Kaohsiung J Med Sci*. 2018 Aug;34(8):423-427.
- (144) Voutsadakis IA. Vitamin D receptor (VDR) and metabolizing enzymes CYP27B1 and CYP24A1 in breast cancer. *Mol Biol Rep*. 2020 Dec;47(12):9821-9830.
- (145) Kim MJ, Kim D, Koo JS, Lee JH, Nam KH. Vitamin D Receptor Expression and its Clinical Significance in Papillary Thyroid Cancer. *Technol Cancer Res Treat*. 2022 Jan-Dec;21:15330338221089933.
- (145) Tunio MA, Al Asiri M, Bayoumi Y, Stanciu LG, Al Johani N, Al Saeed EF. Is thyroid gland an organ at risk in breast cancer patients treated with locoregional radiotherapy? Results of a pilot study. *J Cancer Res Ther*. 2015 Oct-Dec;11(4):684-9.
- (147) Zhang Y, Liang J, Li H, Cong H, Lin Y. Risk of second primary breast cancer after radioactive iodine treatment in thyroid cancer: a systematic review and meta-analysis. *Nucl Med Commun*. 2016 Feb;37(2):110-5.

- (148) Matesich SM, Shapiro CL. Second cancers after breast cancer treatment. *Semin Oncol*. 2003 Dec;30(6):740-8.
- (149) Grantzau T, Overgaard J. Risk of second non-breast cancer among patients treated with and without postoperative radiotherapy for primary breast cancer: A systematic review and meta-analysis of population-based studies including 522,739 patients. *Radiother Oncol*. 2016 Dec;121(3):402-413.
- (150) Li CI, Rossing MA, Voigt LF, Daling JR. Multiple primary breast and thyroid cancers: role of age at diagnosis and cancer treatments (United States). *Cancer Causes Control*. 2000 Oct;11(9):805-11.
- (151) Huang J, Walker R, Groome PG, Shelley W, Mackillop WJ. Risk of thyroid carcinoma in a female population after radiotherapy for breast carcinoma. *Cancer*. 2001 Sep 15;92(6):1411-8.
- (152) Lal G, Groff M, Howe JR, Weigel RJ, Sugg SL, Lynch CF. Risk of subsequent primary thyroid cancer after another malignancy: latency trends in a population-based study. *Ann Surg Oncol*. 2012 Jun;19(6):1887-96.
- (153) Kim SS, Kim SJ, Bae YT, Lee JY, Kim BH, Kim YK, Kim IJ. Factors associated with the development of new onset diffuse thyroid F18-fluorodeoxyglucose uptake after treatment of breast cancer in patients without a history of thyroid disease or thyroid dysfunction. *Thyroid*. 2012 Jan;22(1):53-8.
- (154) Kim KJ, Kim KJ, Choi J, Kim NH, Kim SG. Linear association between radioactive iodine dose and second primary malignancy risk in thyroid cancer. *J Natl Cancer Inst*. 2023 Jun 8;115(6):695-702.
- (155) Kim S, Bang JI, Boo D, Kim B, Choi IY, Ko S, Yoo IR, Kim K, Kim J, Joo Y, Ryoo HG, Paeng JC, Park JM, Jang W, Kim B, Chung Y, Yang D, Yoo S, Lee HY. Second primary malignancy risk in thyroid cancer and matched patients with and without radioiodine therapy analysis from the observational health data sciences and informatics. *Eur J Nucl Med Mol Imaging*. 2022 Aug;49(10):3547-3556.
- (156) Reinecke MJ, Ahlers G, Burchert A, Eilsberger F, Flux GD, Marlowe RJ, Mueller HH, Reiners C, Rohde F, van Santen HM, Luster M. Second primary malignancies induced by radioactive iodine treatment of differentiated thyroid carcinoma - a critical

review and evaluation of the existing evidence. *Eur J Nucl Med Mol Imaging*. 2022 Jul;49(9):3247-3256.

(157) Zhao X, Chen M, Qi X, Zhu H, Yang G, Guo Y, Dong Q, Yang Q. Association of Radioiodine for Differentiated Thyroid Cancer and Second Breast Cancer in Female Adolescent and Young Adult. *Front Endocrinol (Lausanne)*. 2022 Jan 28;12:805194.

(158) Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer "epidemic"--screening and overdiagnosis. *N Engl J Med*. 2014 Nov 6;371(19):1765-7.

(159) Arroyo N, Bell KJL, Hsiao V, Fernandes-Taylor S, Alagoz O, Zhang Y, Davies L, Francis DO. Prevalence of Subclinical Papillary Thyroid Cancer by Age: Meta-analysis of Autopsy Studies. *J Clin Endocrinol Metab*. 2022 Sep 28;107(10):2945-2952.

(160) Robenshtok E, Neeman B, Reches L, Ritter A, Bachar G, Kaminer K, Shimon I, Mizrahi A. Adverse Histological Features of Differentiated Thyroid Cancer Are Commonly Found in Autopsy Studies: Implications for Treatment Guidelines. *Thyroid*. 2022 Jan;32(1):37-45.

(161) Lu W, Xu Y, Xu J, Wang Z, Ye G. Identification of differential expressed lncRNAs in human thyroid cancer by a genome-wide analyses. *Cancer Med*. 2018 Aug;7(8):3935-3944.

(162) Wei X, Li Y, Zhang S, Ming G. Evaluation of thyroid cancer in Chinese females with breast cancer by vascular endothelial growth factor (VEGF), microvessel density, and contrast-enhanced ultrasound (CEUS). *Tumour Biol*. 2014 Jul;35(7):6521-9.

(163) Abdullah MI, Junit SM, Ng KL, Jayapalan JJ, Karikalan B, Hashim OH. Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. *Int J Med Sci*. 2019 Feb 28;16(3):450-460.

(164) Hu Y, Wang H, Chen E, Xu Z, Chen B, Lu G. Candidate microRNAs as biomarkers of thyroid carcinoma: a systematic review, meta-analysis, and experimental validation. *Cancer Med*. 2016 Sep;5(9):2602-14.

(165) Geraldo MV, Yamashita AS, Kimura ET. MicroRNA miR-146b-5p regulates signal transduction of TGF- β by repressing SMAD4 in thyroid cancer. *Oncogene*. 2012 Apr 12;31(15):1910-22.

- (166) McLeod DSA, Zhang L, Durante C, Cooper DS. Contemporary Debates in Adult Papillary Thyroid Cancer Management. *Endocr Rev.* 2019 Dec 1;40(6):1481-1499.
- (167) Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE, Nikiforova MN. MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. *Ann Surg Oncol.* 2011 Jul;18(7):2035-41.
- (168) Stojanović S, Šelemetjev S, Đorić I, Janković Miljuš J, Tatić S, Živaljević V, Išić Denčić T. BRAFV600E, BANCER, miR-203a-3p and miR-204-3p in Risk Stratification of PTC Patients. *Biomedicines.* 2023 Dec 18;11(12):3338.
- (169) Sheu SY, Grabellus F, Schwertheim S, Handke S, Worm K, Schmid KW. Lack of correlation between BRAF V600E mutational status and the expression profile of a distinct set of miRNAs in papillary thyroid carcinoma. *Horm Metab Res.* 2009 Jun;41(6):482-7.
- (170) Nixon AM, Provatopoulou X, Kalogera E, Zografos GN, Gounaris A. Circulating thyroid cancer biomarkers: Current limitations and future prospects. *Clin Endocrinol (Oxf).* 2017 Aug;87(2):117-126.
- (171) Stokowy T, Wojtas B, Jarzab B, Krohn K, Fredman D, Dralle H, Musholt T, Hauptmann S, Lange D, Hegedüs L, Paschke R, Eszlinger M. Two-miRNA classifiers differentiate mutation-negative follicular thyroid carcinomas and follicular thyroid adenomas in fine needle aspirations with high specificity. *Endocrine.* 2016 Nov;54(2):440-447.
- (172) Zang Y, Zhu J, Li Q, Tu J, Li X, Hu R, Yang D. miR-137-3p Modulates the Progression of Prostate Cancer by Regulating the JNK3/EZH2 Axis. *Onco Targets Ther.* 2020 Aug 10;13:7921-7932.
- (173) Mazurek M, Mlak R, Homa-Mlak I, Powrózek T, Brzozowska A, Kwaśniewski W, Opielak G, Małecka-Massalska T. High miR-511-3p Expression as a Potential Predictor of a Poor Nutritional Status in Head and Neck Cancer Patients Subjected to Intensity-Modulated Radiation Therapy. *J Clin Med.* 2022 Feb 2;11(3):805.
- (174) Qiao DH, He XM, Deng X, Ji YC, Yang H, Cheng L, Zhou XY. Aberrant expression of five miRNAs in papillary thyroid carcinomas. *J Clin Lab Anal.* 2021 Sep;35(9):e23907.

- (175) Swierniak M, Wojcicka A, Czetwertynska M, Stachlewska E, Maciag M, Wiechno W, Gornicka B, Bogdanska M, Koperski L, de la Chapelle A, Jazdzewski K. In-depth characterization of the microRNA transcriptome in normal thyroid and papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2013 Aug;98(8):E1401-9.
- (176) Ji T, Gao L, Yu Z. Tumor-suppressive microRNA-551b-3p targets H6PD to inhibit gallbladder cancer progression. *Cancer Gene Ther*. 2021 Jun;28(6):693-705.
- (177) Bai SY, Ji R, Wei H, Guo QH, Yuan H, Chen ZF, Wang YP, Liu Z, Yang XY, Zhou YN. Serum miR-551b-3p is a potential diagnostic biomarker for gastric cancer. *Turk J Gastroenterol*. 2019 May;30(5):415-419.
- (178) Kondrotienė A, Daukša A, Pamedytė D, Kazokaitė M, Žvirblienė A, Daukšienė D, Simanavičienė V, Klimaitė R, Golubickaitė I, Stakaitis R, Šarauskas V, Verkauskienė R, Žilaitienė B. Papillary Thyroid Carcinoma Tissue miR-146b, -21, -221, -222, -181b Expression in Relation with Clinicopathological Features. *Diagnostics (Basel)*. 2021 Mar 2;11(3):418.
- (179) Lam AK, Lo CY, Lam KS. Papillary carcinoma of thyroid: A 30-yr clinicopathological review of the histological variants. *Endocr Pathol*. 2005 Winter;16(4):323-30.
- (180) Almukhtar ZK. Histopathological Types of Papillary Thyroid Carcinoma: Clinicopathologic Study. *Open Access Maced J Med Sci [Internet]*. 2022 Jan. 9 [cited 2025 Sep. 8];10(A):79-83. Available from: <https://oamjms.eu/index.php/mjms/article/view/8059>.
- (181) Adam MA, Pura J, Gu L, Dinan MA, Tyler DS, Reed SD, Scheri R, Roman SA, Sosa JA. Extent of surgery for papillary thyroid cancer is not associated with survival: an analysis of 61,775 patients. *Ann Surg*. 2014 Oct;260(4):601-5; discussion 605-7.
- (182) Barney BM, Hitchcock YJ, Sharma P, Shrieve DC, Tward JD. Overall and cause-specific survival for patients undergoing lobectomy, near-total, or total thyroidectomy for differentiated thyroid cancer. *Head Neck*. 2011 May;33(5):645-9.
- (183) Ito Y, Miyauchi A, Inoue H, Fukushima M, Kihara M, Higashiyama T, Tomoda C, Takamura Y, Kobayashi K, Miya A. An observational trial for papillary thyroid microcarcinoma in Japanese patients. *World J Surg*. 2010 Jan;34(1):28-35.

- (184) DeGroot LJ, Kaplan EL, McCormick M, Straus FH. Natural history, treatment, and course of papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 1990 Aug;71(2):414-24.
- (185) Koh JM, Kim ES, Ryu JS, Hong SJ, Kim WB, Shong YK. Effects of therapeutic doses of ¹³¹I in thyroid papillary carcinoma patients with elevated thyroglobulin level and negative ¹³¹I whole-body scan: comparative study. *Clin Endocrinol (Oxf)*. 2003 Apr;58(4):421-7.
- (186) Robbins RJ, Schlumberger MJ. The evolving role of (¹³¹)I for the treatment of differentiated thyroid carcinoma. *J Nucl Med*. 2005 Jan;46 Suppl 1:28S-37S.
- (187) Cha YJ, Koo JS. Next-generation sequencing in thyroid cancer. *J Transl Med*. 2016 Nov 21;14(1):322.
- (188) Dobrescu R, Badiu C. Actualities in genetics of differentiated thyroid cancer. *Acta Endocrinol (Buchar)*. 2020 Jan-Mar;16(1):118-120.
- (189) Trimboli P, Scappaticcio L, Treglia G, Guidobaldi L, Bongiovanni M, Giovanella L. Testing for BRAF (V600E) Mutation in Thyroid Nodules with Fine-Needle Aspiration (FNA) Read as Suspicious for Malignancy (Bethesda V, Thy4, TIR4): a Systematic Review and Meta-analysis. *Endocr Pathol*. 2020 Mar;31(1):57-66.
- (190) Xing M. Genetic-guided Risk Assessment and Management of Thyroid Cancer. *Endocrinol Metab Clin North Am*. 2019 Mar;48(1):109-124.
- (191) Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, Kim M, Tufaro A, Ladenson P, Zeiger M, Tufano R. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *J Clin Oncol*. 2009 Jun 20;27(18):2977-82.
- (192) Martini M, de Pascalis I, D'Alessandris QG, Fiorentino V, Pierconti F, Marei HE, Ricci-Vitiani L, Pallini R, Larocca LM. VEGF-121 plasma level as biomarker for response to anti-angiogenetic therapy in recurrent glioblastoma. *BMC Cancer*. 2018 May 10;18(1):553.
- (193) Papaioannou M, Chorti AG, Chatzikyriakidou A, Giannoulis K, Bakkar S, Papavramidis TS. MicroRNAs in Papillary Thyroid Cancer: What Is New in Diagnosis and Treatment. *Front Oncol*. 2022 Feb 3;11:755097.

9. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

Related to the dissertation:

- Bakos B, Kiss A, Árvai K, Szili B, Deák-Kocsis B, Tobiás B, Putz Z, Ármós R, Balla B, Kósa J, Dank M, Valkusz Z, Takács I, Tabák Á, Lakatos P. Co-occurrence of thyroid and breast cancer is associated with an increased oncogenic SNP burden. BMC Cancer. 2021 Jun 15;21(1):706.

IF: 4,638

- Kiss A, Szili B, Bakos B, Ármós R, Putz Z, Árvai K, Kocsis-Deák B, Tobiás B, Balla B, Pikó H, Dank M, Kósa JP, Takács I, Lakatos P. Comparison of surgical strategies in the treatment of low-risk differentiated thyroid cancer. BMC Endocr Disord. 2023 Jan 26;23(1):23.

IF: 2,8

- Armos R, Bojtor B, Papp M, Illyes I, Lengyel B, Kiss A, Szili B, Tobias B, Balla B, Piko H, Illes A, Putz Z, Kiss A, Toth E, Takacs I, Kosa JP, Lakatos P. MicroRNA Profiling in Papillary Thyroid Cancer. Int J Mol Sci. 2024 Aug 29;25(17):9362.

IF: 4,9

Others:

- Kiss A, Szili B, Bakos B, Tóbiás B, Balla B, Takács I, Lakatos P, Kósa J. A differenciált pajzsmirigyrák rizikója mint első és második primer tumor [Risk of differentiated thyroid cancer as the first and second primary tumor]. Orv Hetil. 2025 Feb 16;166(7):253-262. Hungarian.

IF: 0,9 (2024)

- Lengyel B, Armos R, Bojtor B, Kiss A, Tobias B, Piko H, Illes A, Horvath E, Putz Z, Takacs I, Kosa JP, Lakatos P. Vitamin D in Endocrine Disorders: A Broad Overview of Evidence in Musculoskeletal, Thyroid, Parathyroid, and Reproductive Disorders. Pharmaceuticals (Basel). 2025 Dec 26;19(1):54.

IF: 4,8 (2024)

- Bojtor B, Vaszilko M, Armos R, Tobias B, Podani J, Szentpeteri S, Balla B, Lengyel B, Piko H, Illes A, Kiss A, Putz Z, Takacs I, Kosa JP, Lakatos P. Analysis of SIRT1 Gene SNPs and Clinical Characteristics in Medication-Related Osteonecrosis of the Jaw. *Int J Mol Sci*. 2024 Mar 25;25(7):3646.

IF: 4,9

- Stempler M, Bakos B, Solymosi T, Kiss A, Ármós RL, Szili B, Mészáros S, Tőke J, Szücs N, Reismann P, Péter P, Lakatos PA, Takács I. Analysis of factors influencing the dose of levothyroxine treatment in adequately controlled hypothyroid patients of different etiologies. *Heliyon*. 2024 Oct 19;10(20):e39639.

IF: 3,6

- Bakos B, Solymosi T, Szili B, Vincze Á, Mészáros S, Stempler M, Ármós R, Kiss A, Bakos A, Szücs N, Reismann P, Tőke J, Pusztai P, Lakatos P, Takács I. Psychological factors and obesity, not thyroid biomarkers, predict thyroid-dependent quality of life in treated hypothyroidism: a cross-sectional study. *BMC Endocr Disord*. 2025 May 26;25(1):136.

IF: 3,3 (2024)

- Bojtor, Bence; Balla, Bernadett; Vaszilko, Mihály; Ármós, Richárd; Tóbiás, Bálint; Szentpéteri, Szófia; Lengyel, Balázs; Pikó, Henriett; Illés, Anett; Putz, Zsuzsanna; Kiss, András; Takács, István; Kósa, János; Lakatos, Péter. A gyógyszer indukálta állcsontnekrózis genetikai háttere. In: *ORVOSTOVÁBBKÉPZŐ SZEMLE* 2024. 31. évf. 4. sz., p. 72-78.

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