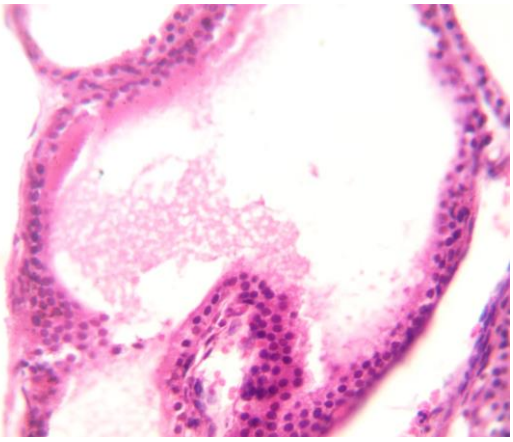


THYROGLOBULIN: WHEN IS IT A TUMOR MARKER?

ABSTRACT

Thyroglobulin is produced in the thyroid and is a driver of T3/T4 production. It has been used as a cancer marker in patients undergoing a thyroidectomy. However, with increasing lobectomies in more indolent thyroid cancers the use of TG may be problematic. We explore this issue as a possible parallel to the similar ones with PSA in prostate cancer.

Terrence McGarty

TGL 176, May 2020

Notice

This document represents the personal opinion of the author and is not meant to be in any way the offering of medical advice or otherwise. It represents solely an analysis by the author of certain data which is generally available. The author furthermore makes no representations that the data available in the referenced papers is free from error. The Author also does not represent in any manner or fashion that the documents and information contained herein can be used other than for expressing the opinions of the Author. Any use made and actions resulting directly or otherwise from any of the documents, information, analyses, or data or otherwise is the sole responsibility of the user and The Author expressly takes no liability for any direct or indirect losses, harm, damage or otherwise resulting from the use or reliance upon any of the Author's opinions as herein expressed. There is no representation by The Author, express or otherwise, that the materials contained herein are investment advice, business advice, legal advice, medical advice or in any way should be relied upon by anyone for any purpose. The Author does not provide any financial, investment, medical, legal or similar advice in this document or in its publications on any related Internet sites.

Furthermore, this document contains references to and quotes from papers and documents under the premise of Fair Use in order to present ideas and understandings in context. The Author has attempted to make any and all references to such material separate from those of the author per se and has referenced the source expressly in all cases. These documents are for the dissemination of ideas and have no commercial intent. The Author would appreciate any communications relating to these documents and these should be sent to:

tmcgarty@telmarc.com.

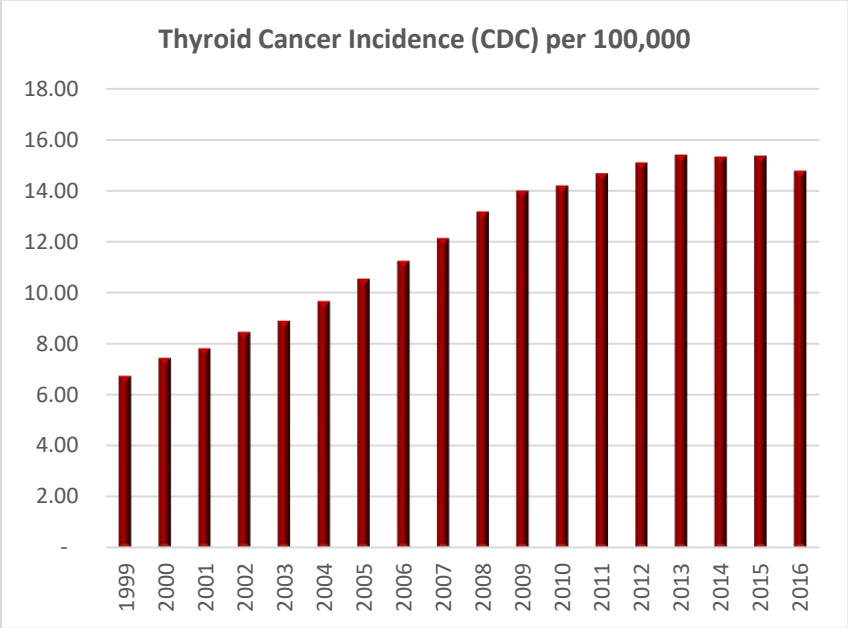
Contents

1 INTRODUCTION

Thyroid cancer has an increasing incidence yet it is often discovered in a more indolent state Thus, exhibiting very low morbidity and mortality[1]. Microcarcinomas are often found as a result of an incidental examination. Highly encapsulated microcarcinomas of the follicular variant of a papillary thyroid Carcinomas, FVPTC, are often quite indolent and are treated with lobectomies if the other lobe shows no sign of a malignancy. Follow up involves measurement of TSH, T4 and thyroglobulin (TG), as well as ultrasound scans of the remaining gland and lymph nodes.

There appears to be some controversy as to the TG levels post lobectomy. In the case of a thyroidectomy, TG levels are often indicative of a metastasis. However, with a lobectomy they may be at best suggestive if not confusing[2].

The current incidence is shown below:



1.1 THE ISSUE

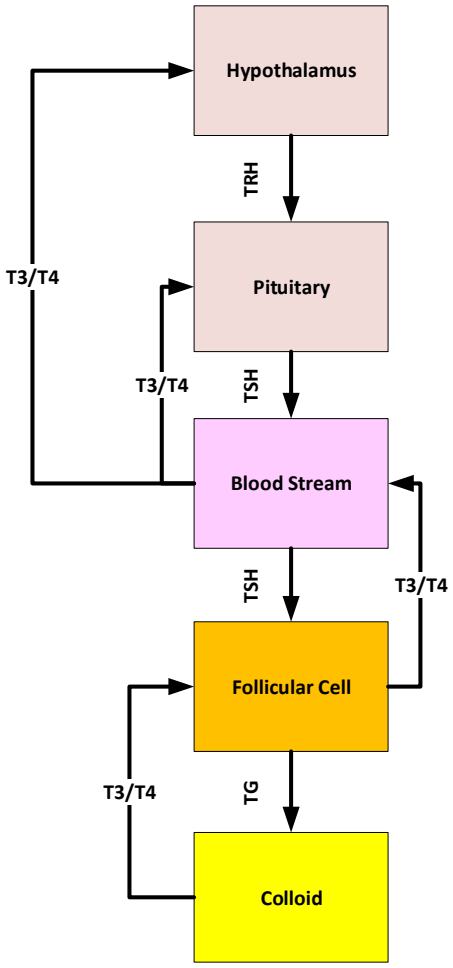
Let us consider the simple view below of the thyroid network. The hypothalamus releases TRH picked up by the pituitary which in turn releases TSH which works its way to the thyroid. There TSH stimulates TG production in a thyroid follicular cell, any thyroid follicular cell. TG then moves to the colloid in a thyroid gland where it is used to produce T3 and T4 which work their

[1] https://www.researchgate.net/publication/331935614_Thyroid_Cancer_Seek_and_Ye_Shall_Find

[2] https://www.researchgate.net/publication/335404502_Thyroid_Cancer_and_Genetic_Differentiation

way back through the follicular cell and into the blood stream and then back to the pituitary and hypothalamus as a simple feedback mechanism.

Namely, the more T3 and T4 the more suppressed TRH and TSH become. Lower TSH means lower TG and in turn lower T3 and T4. Stasis is achieved.



Now the question we are posing is; if stasis exists before a lobectomy, then after a lobectomy, stasis must again be achieved but with less gland there will be changes. If the set point is some specific T3/T4 concentrations then one would expect TG to increase as TRH and TSH must increase since we have only half or less the number of follicles producing T3 and T4. Thus, looking at TG, after a lobectomy we expect TSH to double, TG to double and T3/T4 to remain the same. At least in theory. One must always be cautious of logical extensions in medicine.

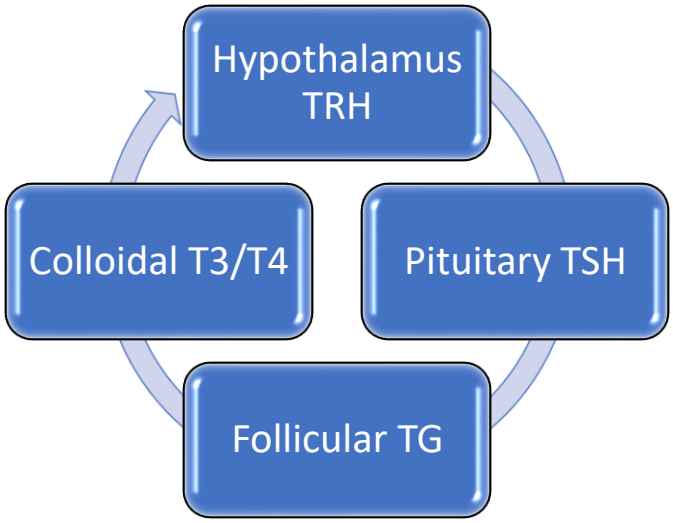
This then is the question we are addressing. Namely, in a thyroidectomy, the entire thyroid is removed and artificial T4/T3 is supplied and TSH produced but theoretically it is used nowhere. However, if the tumor has metastasized then the TSH stimulates the thyroid cells that have

metastasized and they produce TG which can be measured. Again, that is the logic. That means TG is a monitor or marker for post thyroidectomy metastasis.

But, again, what is it for a lobectomy? Clearly one would assume that to meet the same T3/T4 set point we must drive the half lobe at twice the rate of the original. Thus, TSH must double, and in turn TG must double. Thus, a greater TG is required and would then be measured. Yet TG is not measured before surgery so any doubling is at best suggestive.

1.2 Current Understanding

Our current understanding is Thus, simply reflected in the following chart.



However, the details are often much more complicated. Are there real markers that are universal. A comparison is PSA and prostate cancer, PCa. PSA increases with age. Why, because the prostate grows. More prostate then more PSA. Thus, as a patient ages we are generally not overly concerned about an increasing PSA unless it increases too quickly. If we know that PSA equals volume and if rate of change in volume is well controlled then we would anticipate PSA growth. Thus, after a lobectomy we may anticipate cell growth which is a common effect after cell injury. Thus, a TG increase. The issue then is; what are the other factors besides just TG that should be taken into account?

1.3 Recent Observations

Now several recent observations have been made regarding TG and its use in lobectomies and subsequent putative tracking of metastatic growth. These are important observations since more thyroid cancers are being diagnosed early or as the result of an incidental finding.

As Goldfarb notes:

The follow-up (measurement of thyroglobulin levels and documentation of cancer recurrence) of 208 patients at a single hospital in Korea were examined.

Most patients had a microcarcinoma (<1cm). A total of 15 patients with cancer recurrence were added to look for predictive factors.

Only patients that did not need thyroid hormone supplementation, had normal TSH levels, and no thyroglobulin antibodies, were included. They looked at the thyroglobulin:TSH ratio (since TSH changes can affect the value of thyroglobulin), and assessed if there was a >20% increase in thyroglobulin level in 2 consecutive thyroglobulin levels and in whom there was a biopsy proven cancer recurrence. Only 4 patients (2%) had a structural recurrence over an average follow-up of 7 years, with no deaths.

Of the 19 total patients with cancer recurrence, 13 recurred with cancer in the remaining thyroid lobe and 6 in the lymph nodes. In general, the serum thyroglobulin as well as the thyroglobulin:TSH ratio increased by about 10% per year for all patients. However, both patients with and without recurrence had increases, and decreases in their thyroglobulin levels – there was no pattern or association and changes in thyroglobulin were therefore not predictive of recurrence.

WHAT ARE THE IMPLICATIONS OF THIS STUDY? Following serum thyroglobulin after thyroid lobectomy may not be accurate in predicting recurrence. This may mean that patients either should only have ultrasound surveillance, or, due to the low rate of recurrence and nonexistent deaths, maybe no surveillance at all is needed, especially for microcarcinomas

In the paper by Park et al as commented by Goldfarb above the authors note:

The serum Tg levels increased gradually, and the proportion of patients with levels >10 ng/dL increased annually by 13.9%, 18.8%, 22.1%, 21.9%, 28.4%, and 28.9% during the six-year follow-up period ($\beta = 0.574$, $p = 0.027$). The relative serum Tg levels increased by 10% annually ($\beta = 0.105$, $p < 0.001$), and the levels of Tg and Tg/TSH ratios in 19 patients with recurrent disease did not differ significantly ($\beta = 0.150$, $p = 0.090$). Patients without recurrent disease were more likely to have serum Tg levels increased by >20% ($p = 0.022$).

There were no significant differences in the proportions of patients with serum Tg levels increased by ≥50% or ≥100% in terms of the disease recurrence. Conclusions: Serum Tg levels and the Tg/TSH ratio increased gradually after lobectomy in patients with and without recurrences, without any significant differences. Periodic measurements of serum Tg levels seem to have limited value in predicting recurrent PTCs after lobectomy. …

Thus, the conclusion which seems to have some currency indicates that TG measures after lobectomy may have little if any value. TG changes may also have limited value. The remaining portion of the thyroid may regrow, and Thus, may generate excess TG. Set points may change and likewise force changes in TG. Also, there may be benign nodules present that in themselves tend to generate excess TG.

The intent of this note is to examine this effect for microcarcinomas. These FVPTC are often overlapping with NIFTP, now considered a non-malignant condition. The key question to understand is; in quasi malignant states which have low probability of metastasis, does TG monitoring merit any use? Namely, are there normal physiological reasons for large and even increasing TG levels, which do not reflect an increase in morbidity or mortality. We will try to examine this issue herein.

Now in a recent abstract by Jeeyavudeen et al[3]:

The accuracy of thyroglobulin (Tg) as a tumour marker following lobectomy for differentiated thyroid cancer (DTC) remains controversial. A Tg (<10 µg/l) looked promising in identifying those without clinically apparent recurrence after median 51 months of follow-up. Longer term follow up allows assessment of the diagnostic utility of thyroglobulin in predicting relapse.

Methods: Ninety-nine patients who underwent lobectomy for DTC were retrospectively analyzed using hospital electronic records. Thyroid function and Tg levels were only available for the last ten years.

Results: The mean patient age was 65±12 years (2/3 were women). Median follow-up was 23 years (IQR 12–31 years). Seven died due to non-thyroid related issues. Four patients required further intervention, three had completion thyroidectomy (two for recurrence in contralateral lobe and one for benign nodule) and one had lymph node dissection (further clinical details unknown). Using a Tg cut off <10 ug/dl to predict long-term relapse gave a sensitivity 50%, specificity of 89.5%, positive predictive value 16. 6% and a negative predictive value 97.7%

	Case 1	***Case 2***	***Case 3***
Initial surgery	*Left lobectomy*	*Right lobectomy*	*Right lobectomy*
Initial pathology	*PTC*	*PTC*	*FTC*
Tg (µg/dl) at completion thyroidectomy	*34*	*5*	*21*
Thyroid enlargement	*Not present*	*Present*	*Not present*
Subsequent pathology	*Colloid degeneration*	*PTC*	*Micro-PTC*

Conclusion: Serum Tg was elevated in two patients who underwent completion thyroidectomy following lobectomy for DTC but a cut-off of 10 µg/l didn't differentiate recurrent PTC from benign nodularity. One case with recurrent PTC had Tg <10 µg/dl. The high negative predictive value (NPV) of Tg <10 µg/dl for recurrence suggests some benefit in long-term follow-up after lobectomy for DTC, but its low sensitivity limits its clinical utility. Clinical± radiological surveillance remains useful for these patients.

[3] https://www.endocrine-abstracts.org/ea/0065/ea0065p147.htm

Thus, the recent examinations indicate a problematic usefulness of post lobectomy TG monitoring.

1.4 OVERVIEW

The objective of this review is to examine the utility of monitoring thyroglobulin, TG, post lobectomy, in patients with microcarcinomas of anticipated minimal morbidity. Although this may be a somewhat limited study it does open the door on similar efforts which we have examined over time regarding similar examinations in prostate cancer. Namely, the PSA exams[4]. It also opens the question of when is cancer actually cancer[5]?

We present the following:

1. A summary of the key features of thyroid cancers

2. Details regarding TG and its functions and some metrics regarding what could be determined a reasonable level before and after a lobectomy.

3. We examine some of the issues regarding the measurement assays and the interference from TG Abs as well as for other factors such as use of melatonin.

4. The advantages of lobectomy versus thyroidectomy for microcarcinomas and their disadvantages.

5. We then discuss a clinical example which may present some interesting challenges to patient treatment and follow up.

[4] https://www.researchgate.net/publication/283356719_TRUST_BUT_VERIFY_THE_VALUE_OF_PSA

[5] https://www.researchgate.net/publication/334947163_What_is_Meant_by_Cancer

2 THYROID AND THYROID CANCER

Our intent here is not to present a pathologist's view but just to highlight some of the pathological histological features and they attempt a nexus to the underlying genetic cause. Examining the histology, we can ask such questions as:

1. Why do the cells lose their relational aspects?

2. Why do the cells proliferate?

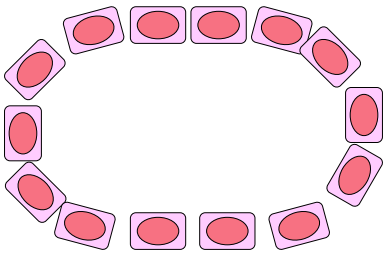
3. What drives vascularization?

4. What are the causes of the morphological changes such as notching and clear nuclei?

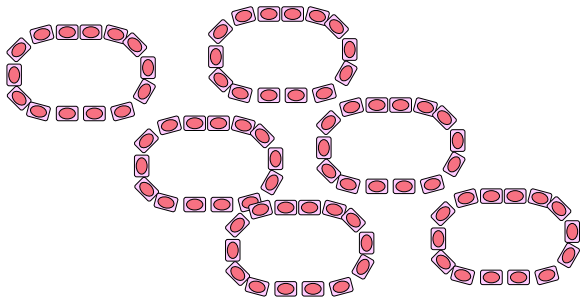
By examining the histology of the cell and especially of the cancerous variants it begs the question; what are the genetic factors which cause or facilitate these changes. Some of these are understood while others remain questionable. This section is in no way an attempt to present the histology of thyroid cancers. It is merely an attempt to raise the question of shape versus cause versus therapeutic.

2.1 THE THYROID CELL

Basically, the thyroid follicular cells are on the outer side of a thyroid follicle. It is the functional boundary of a gland. This is shown below for a simple thyroid boundary. The cells on the boundary are well behaved and connected. Cell interfaces such as E-cadherin stabilize these cells. Internally to this glandular structure is a collagen internal fill. From this colloid under the pituitary control the T3 and T4 hormones are released. From this simple construct we have the basis of the thyroid control path.

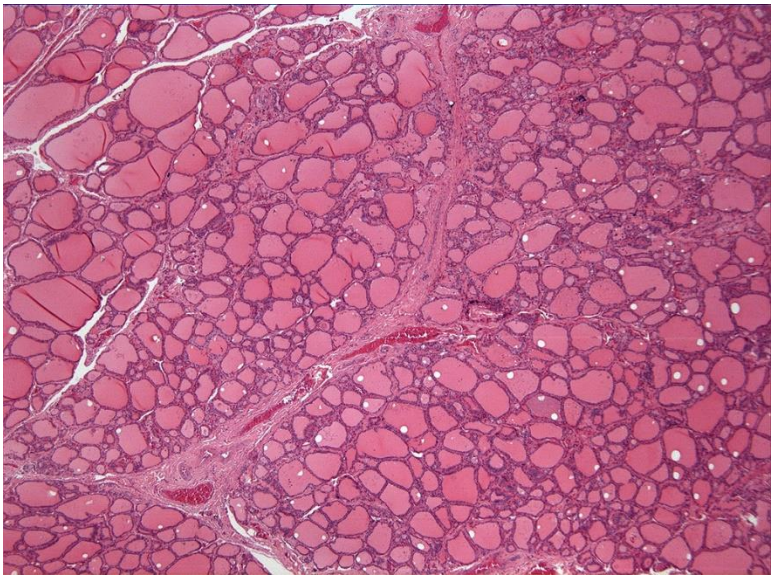


Now the thyroid gland is a compilation of these follicles as shown below. There are blood organs between the follicles and also C cells, cells separate from those that form the effective gland. The highly simplistic view is seen below.

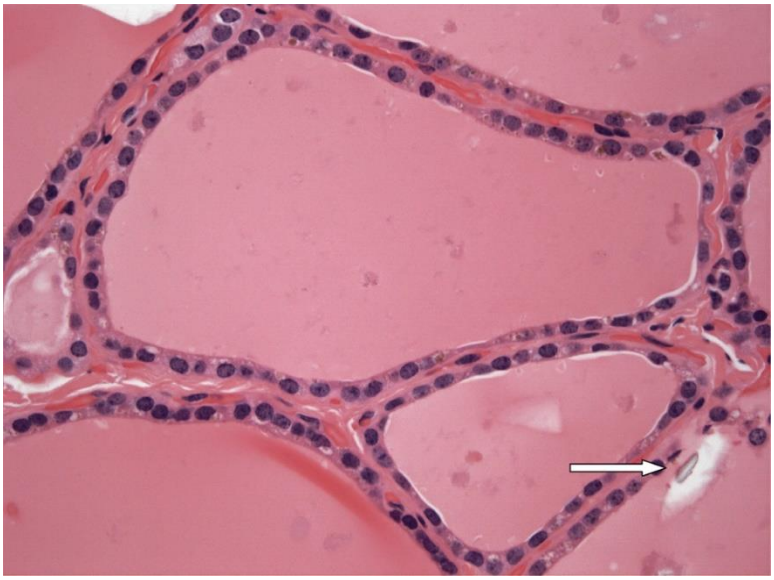


Inside is the collagen material used by the cells to produce T3 and T4.

The normal thyroid cells are shown below (From *Epstein, Biopsy Interpretation of the Thyroid*). The separate cells for enclosures which contain colloid and then it is processed and released by the cell. Surrounding the cell is and there are blood networks throughout the thyroid providing the cells with their requirements and transporting the cell products.



Further specific detail of a follicle is shown below (again from Epstein). Note the clarity and simplicity as well as structure of the cells in each small gland portion:

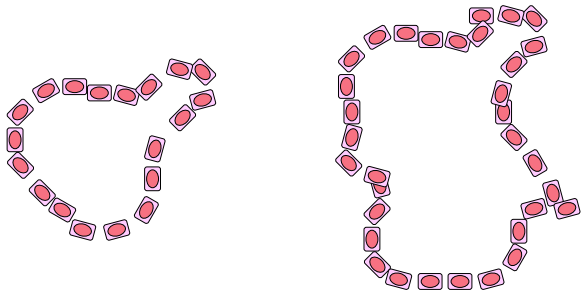


Note that the cells are well demarcated and organized. Now as we shall see, several variations occur as the cell becomes malignant. Growth results in a proliferation of cells, everywhere, loss of adhesion via EMT results in cell dislocation, and the morphology of individual cells change as well.

2.2 Papillary Growth

Now papilla are the small bumps or perturbances of the normal cells which generally are somewhat uniform as we have depicted.

The papillary like cells are shown as below:



The above show the papilla, the bumps or offshoots. It can be argued that this papilla formation is a result of a quasi-EMT process where the E-cadherin bond structure is starting to deteriorate. Namely, the genetic control of this is breaking down because of the suppression of the pathways that control epithelial like structure[6]. Now as Nucera and Pontecorvi have noted:

[6] See McGarty, EMT and Cancers, January 2019, https://www.researchgate.net/publication/330222973_EMT_and_Cancers

Most human thyroid cancers are differentiated papillary carcinomas (PTC). Papillary thyroid microcarcinomas (PTMC) are tumors that measure 1 cm or less. This class of small tumors has proven to be a very common clinical entity in endocrine diseases. PTMC may be present in 30-40% of human autopsies and is often identified incidentally in a thyroid removed for benign clinical nodules.

Although PTMC usually has an excellent long-term prognosis, it can metastasize to neck lymph nodes; However, deaths related to this type of thyroid tumor are very rare. Few data exist on molecular pathways that play a role in PTMC development; however, two molecules have been shown to be associated with aggressive PTMC.

S100A4 (calcium-binding protein), which plays a role in angiogenesis, extracellular matrix remodeling, and tumor microenvironment, is over-expressed in metastatic PTMC. In addition, the BRAFV600E mutation, the most common genetic alteration in PTC, is present in many PTMC with extra thyroidal extension and lymph node metastasis.

The above observation is interesting. Namely, that almost 40% of people will be harboring small PTCs which unless sampled by a good ultrasound examiner would never be found. Furthermore they would never grow. They also note regarding the papillary growth above:

BRAFV600E triggers a cascade that leads to human papillary thyroid microcarcinoma (PTMC) proliferation. The constitutive kinase activity of BRAFV600E phosphorylates and activates MEK1/2. Phospho-MEK1/2 induces hyperphosphorylation of ERK1/2 which translocates into the nucleus, triggering cell cycle progression, and abnormal cell proliferation by up-regulating cyclins (e.g., Cyclin D1) crucial for the checkpoint machinery in G1-S phases and inhibiting anti-cell cycle cyclins. Up-regulation of cyclins (e.g., Cyclin D1) leads to hyper-proliferation of papillary thyroid microcarcinoma cells and increase in papillae size.

Now there are several additional and specific histological characteristics. For example, as Das notes[7]:

Psammoma bodies (PBs) are concentric lamellated calcified structures, observed most commonly in papillary thyroid carcinoma (PTC), meningioma, and papillary serous cystadenocarcinoma of ovary but have rarely been reported in other neoplasms and nonneoplastic lesions. PBs are said to represent a process of dystrophic calcification.

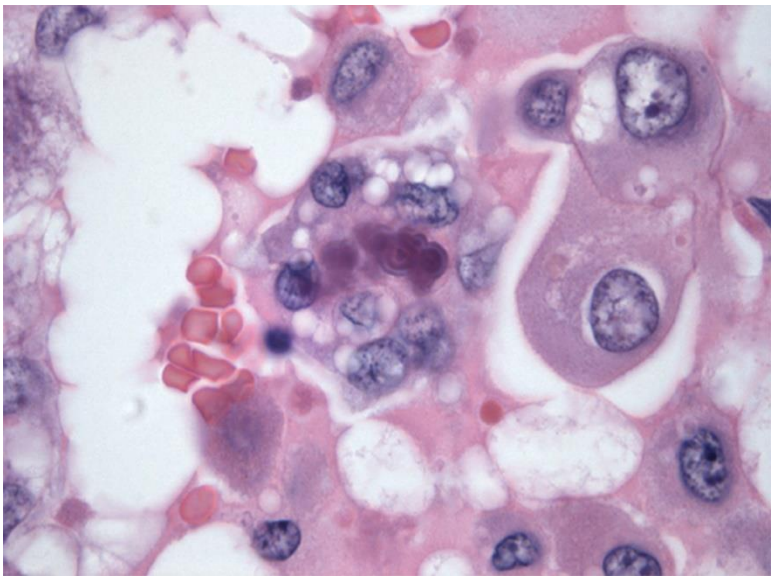
Despite numerous ancillary studies over a span of three and half decades, formation of PBs remains a poorly understood mechanism. Ultrastructural study of PTC has shown that thickening of the base lamina in vascular stalk of neoplastic papillae followed by thrombosis, calcification, and tumor cell necrosis leads to formation of PBs. Studies on serous cystadenocarcinoma of ovary and meningioma, however, revealed that collagen production by neoplastic cells and subsequent calcification was responsible for the formation of PBs.

[7] Note: A psammoma body is a round collection of calcium, seen microscopically. The term is derived from the Greek word ψάμμος (psámmos), meaning "sand".

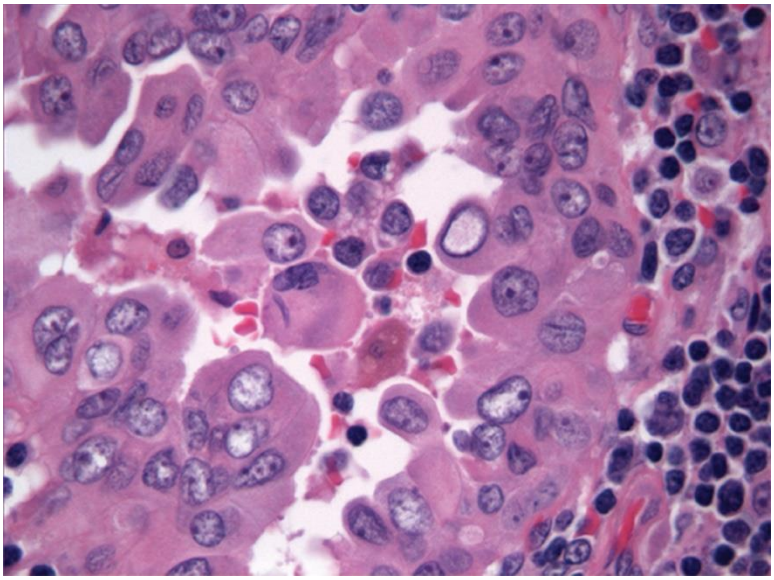
The existence of some precursor forms of PBs was reported in meningiomas and more recently in PTC, which were mostly in the form of extracellular hyaline globules surrounded by well-preserved neoplastic cells or in a smaller number of cases intracytoplasmic bodies liberated from intact tumor cells.

Cellular degeneration and necrosis, leading to the disappearance of neoplastic cells, were noticed by us only around PBs but not around the precursor forms. Based on the above findings, it is suggested that rather than being the outcome of dystrophic calcification of dead or dying tissue, PBs may indeed represent an active biologic process ultimately leading to degeneration/death of tumor cells and retardation of growth of the neoplasm. It may also serve as a barrier against the spread of neoplasm.

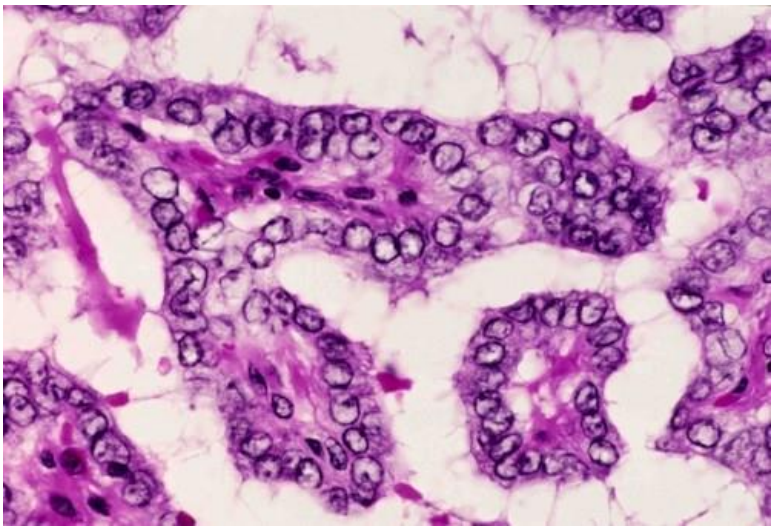
We show psammoma bodies below.



An additional example of specific histological characteristics is one with clear nuclei as shown below:

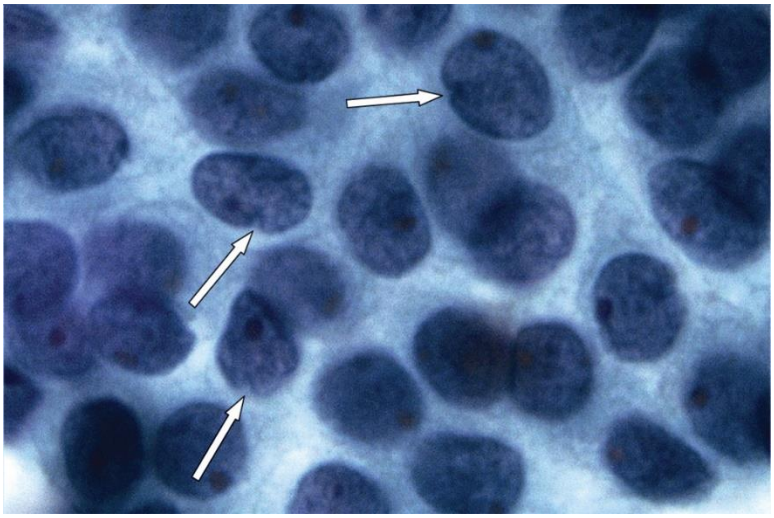


Another example of one type of papillary carcinoma is one with "Orphan Annie" eyes, the wide open white eyes of the nucleus in the cells below.



Note also the well demarcated papillary form with outstretches of the otherwise well-structured cell.

Finally an added one is cells with nuclear grooves is a characteristic that is part of this diagnosis as shown below:



These are small notches seen in the side of the nucleus.

Now these are what a trained histopathologist would be looking for. However, an underlying question is; why are they present and what causes these specific characteristics. We frequently see in medicine the answers to what but not why. Some of these answers are yet to be determined.

2.3 FOLLICULAR GROWTH

In contrast to follicular carcinoma, where the boundary patency gets deformed, follicular carcinoma is where there is a proliferation of the follicular cells.

Baloch and LiVolsi have noted:

Follicular carcinoma comprises about 5% of thyroid cancers; however, in iodide-deficient areas, this tumor is more prevalent making up 25-40% of thyroid cancers. The true incidence of follicular carcinoma is difficult to determine since the follicular variant of papillary carcinoma may still be placed into this category. Risk factors include iodine deficiency, older age, female gender, and radiation exposure (although the relationship of radiation to follicular carcinoma is far less strong than with papillary cancer).

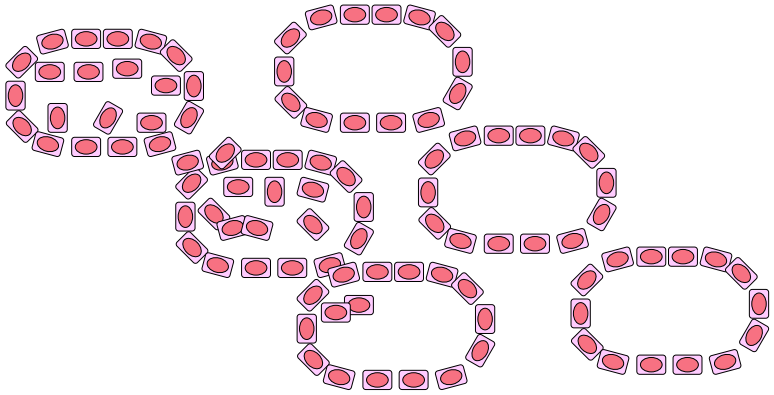
Clinically, follicular carcinoma usually presents as a solitary mass in the thyroid. Follicular carcinoma has a marked propensity for vascular invasion and avoids lymphatics; hence, true embolic lymph node metastases are exceedingly rare. Follicular carcinoma disseminates hematogenously and metastasizes to bone, lungs, brain, and liver …

What are the minimum criteria for making this diagnosis? Invasion of the capsule, invasion through the capsule, and invasion into veins in or beyond the capsule represent the diagnostic criteria for carcinoma in a follicular thyroid neoplasm. The criterion for vascular invasion applies solely and strictly to veins in or beyond the capsule, whereas, the definition of capsular invasion is controversial. Some authors require penetration of the capsule to diagnose a

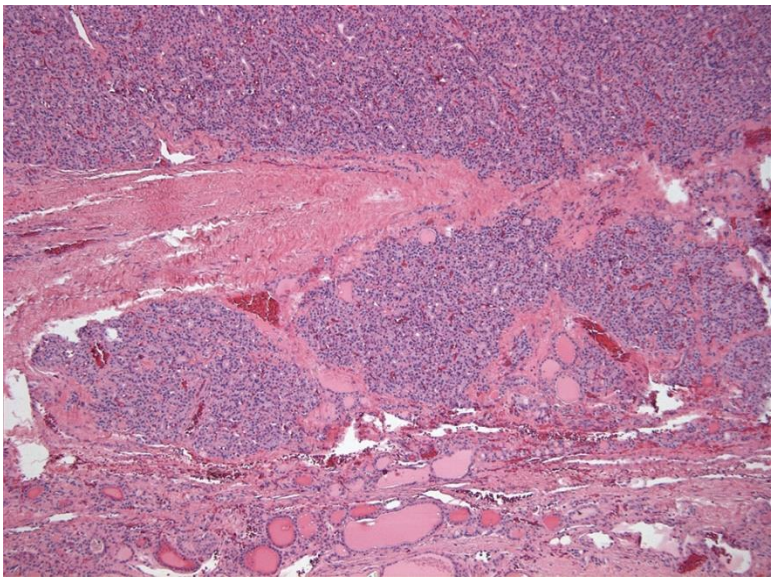
follicular tumor as carcinoma, while others need tumor invasion through the capsule into the surrounding normal thyroid.

Is capsular invasion insufficient for the diagnosis of follicular cancer? Distant metastases have been reported in follicular carcinoma diagnosed only on the basis of capsular and not vascular invasion, however, in some cases, metastases were already present at initial diagnosis. The presence of vascular invasion is also indicative of malignancy in a follicular tumor. Invasion of vessels within or beyond the lesional capsule is necessary for a definitive diagnosis of vascular invasion. The lesions with vascular invasion should be separated from the minimally invasive follicular carcinomas that show capsular invasion only, because angio-invasive lesions have a greater probability of recurrence and metastasis.

Thus, a simplistic view of a follicular cancer is shown below.



We depict a follicular cancer below:



This shows the multiplicity of cells in what was initially a well-ordered cell structure filled with collagen.

Note the extensive infiltration. Again, in simplistic terms, papillary is a form where we lose shape, Namely, a putative EMT transition and follicular is where we see extensive proliferation. Clearly both forms may occur.

2.4 Neuroendocrine Growth and Medullary Thyroid Cancer

Medullary thyroid cancer is basically a neuroendocrine cancer. Neuroendocrine cancers are an interesting subset of many cancers and it worth reviewing the overall paradigm of their growth.

2.4.1 Neuroendocrine Paradigm

Namely, we look at neuroendocrine type effects and Thus, it requires a slightly more detailed understanding of the prostate[8]. As NCI notes[9]:

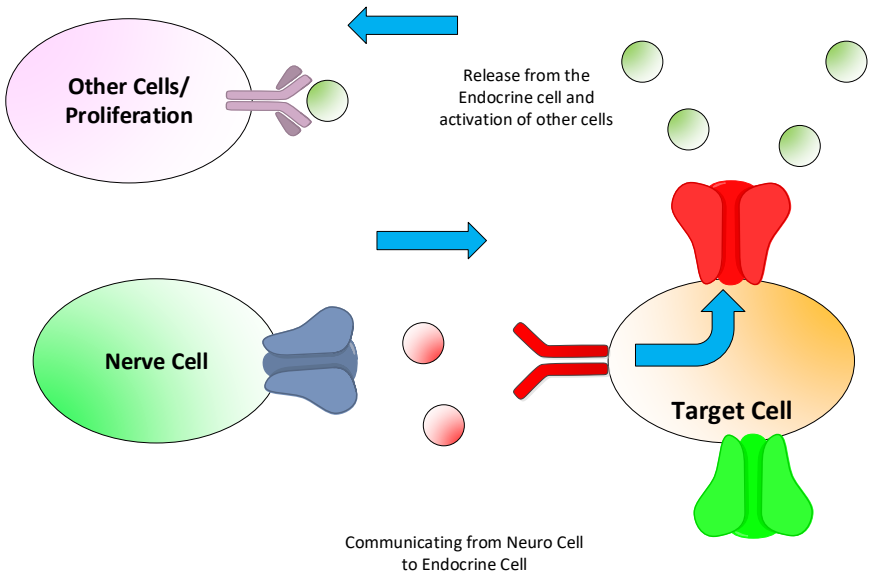
Neuroendocrine: Having to do with the interactions between the nervous system and the endocrine system. Neuroendocrine describes certain cells that release hormones into the blood in response to stimulation of the nervous system.

We then, in a rationalistic manner, can try and connect the other empirical facts and see if the initial observation can also be logically correct and from that logic ascertain a new therapeutic approach.

A simplistic view of a neuroendocrine system is shown below. Basically the neuro cell activates the endocrine cell which in turn sends out signals to other collections of cells to do whatever they are supposed to do.

[8] https://www.researchgate.net/publication/325497685_Neuroendocrine_PCa_Galen_Logic_and_Rationalism

[9] https://www.cancer.gov/publications/dictionaries/cancer-terms/def/neuroendocrine



The above is simplistic but based upon a substantial base of validated cellular signalling factors. Namely, these results are empirical in a broad sense. Now when examining various cancers, we often look at the cancer cell as being the driving factor. However, in a neuroendocrine environment, the cancer cell may be getting its signalling from a cancer initiating cell which in turn is being signaled by a neuro cell. The cancer initiating cell may be blocked by blocking the signalling between it and the causative neuro cell. That is the logical or rationalistic part of this exercise.

The questions now are;

(i) If the malignancy occurs in the neuroendocrine cell, then does it create an environment for proliferation of other cells?

(ii) If the malignancy occurs in the neuroendocrine cell does it send out signals that either block other homeostatic processes or does it accelerate angiogenesis in the new malignancy?

(iii) If the malignancy starts in a non-neuroendocrine cell, are there processes that effectively "turn on" the neuroendocrine cell to facilitate such effects as proliferation, angiogenesis, gene suppression or activation in other cells?

These are but a few of the questions which may be posed. Again we indicate that this is a bit simplistic but it does present the key issues related hereto.

We have examined neuroendocrine driven cancers when examining the prostate. They are simply cancers where a local neuroendocrine cell starts controlling the proliferation process.

As Franz notes:

Medullary thyroid cancer (MTC) is a tumor of the parafollicular C cells that accounts for approximately 10% of all thyroid malignancies. An estimated 75% of MTC cases are sporadic, and the remaining 25% are familial. Embryologically, these cells originate within the neural crest and function similarly to other neuroendocrine cells within the amine precursor uptake and decarboxylation system. C cells are distributed throughout the entire thyroid gland, although they tend to predominate in the upper poles.

Calcitonin, a hormone active in calcium metabolism, is synthesized and secreted by C cells and therefore serves as a useful serum marker for the presence of MTC. Calcitonin levels are most useful in screening individuals who are genetically predisposed to the disease and in following patients who already have been treated. The recent identification of the gene responsible for heritable forms of MTC has allowed earlier identification of individuals at risk for the disease

2.4.2 Medullary carcinoma

Kim and Kuo have noted:

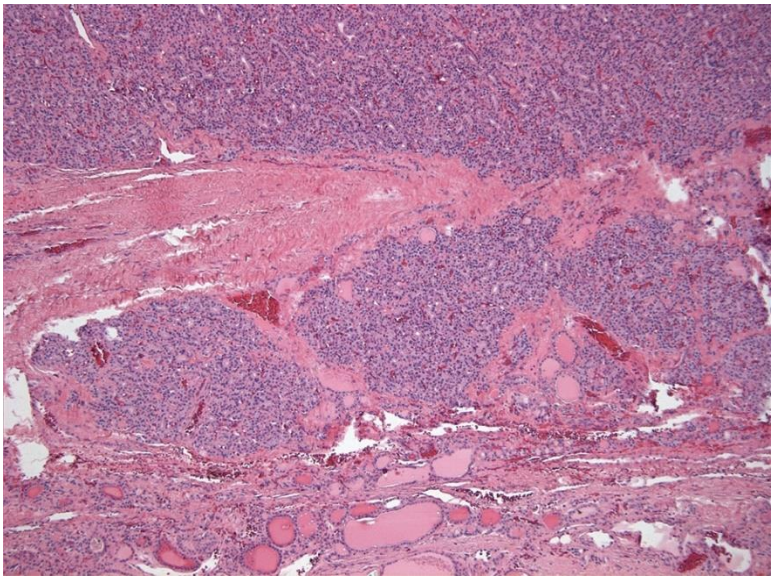
Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor derived from the thyroid C cells producing calcitonin. MTC accounts for 0.6% of all thyroid cancers and incidence of MTC increased steadily between 1997 and 2011 in Korea. It occurs either sporadically or in a hereditary form based on germline rearranged during transfection (RET) mutations. MTC can be cured only by complete resection of the thyroid tumor and any loco-regional metastases.

The most appropriate treatment is still less clear in patients with residual or recurrent disease after initial surgery or those with distant metastases because most patients even with metastatic disease have indolent courses with slow progression for several years and MTC is not responsive to either radioactive iodine therapy or thyroid-stimulating hormone suppression. Recently, two tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib, are approved for use in patients with advanced, metastatic or progressive MTC.

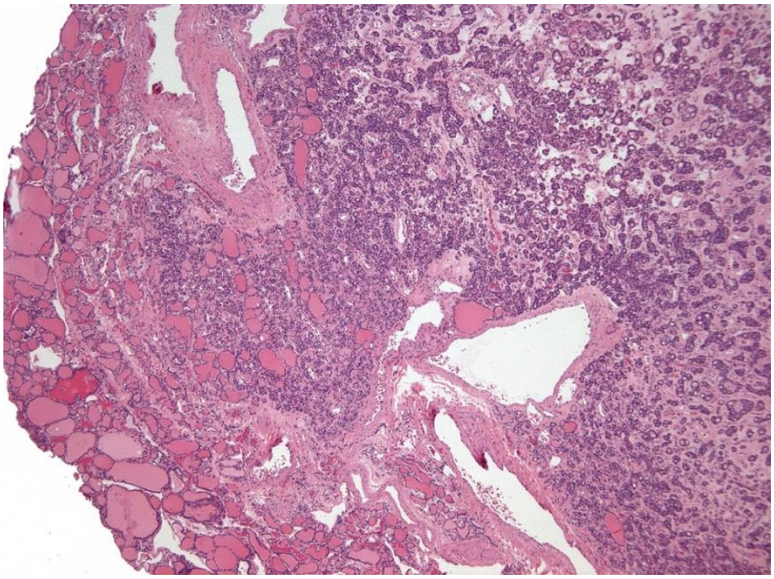
Baloch and LiVolsi note:

Medullary thyroid carcinoma comprises less than 10% of all thyroid malignancies. This tumor is of great diagnostic importance because of its aggressiveness, its close association with multiple endocrine neoplasia syndromes (MEN2A and 2B), and a relationship to a C cell hyperplasia, a probable pre cursor lesion.

While the majority of medullary carcinomas are sporadic, about 10-20% are familial. Since these familial cases have been identified, a gene associated with medullary carcinoma has been identified on chromosome 10 and involves mutations in the RET oncogene.

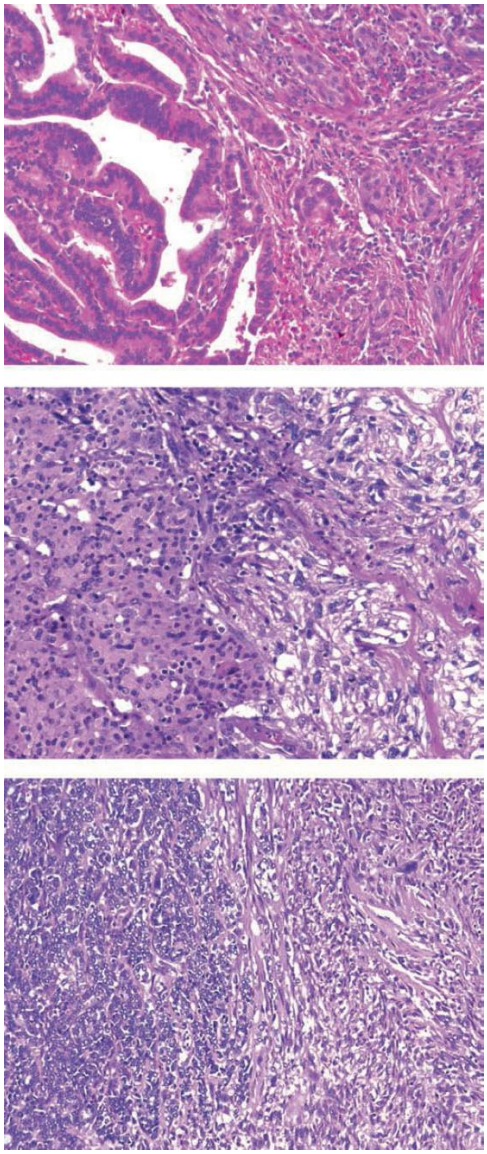


and below:



2.5 ANAPLASTIC

This is a highly aggressive cancer with nearly 100% mortality in 6 to 12 months. It is also quite rare but seems to be a sequella to a Graves diseased thyroid. Given its rarity and complexity it is worth just a mention.



2.6 MICROCARCINOMAS

Microcarcinomas are generally small and relatively indolent lesions. There is no construct of carcinoma in situ in thyroid lesions and perhaps microcarcinomas may at some time fit that description. As Nikiforov et al have noted[10]:

Papillary microcarcinoma is defined as a papillary carcinoma which is 1 cm or less in size. Previous definitions were more restrictive, and in addition to size also required that the tumor was not suspected clinically and found incidentally on pathologic examination. However, the current, 2017 World Health Organization (WHO) definition is based solely on the tumor size criterion.

[10] Nikiforov et al p 250

Papillary microcarcinomas are very common and represent the fastest growing group of papillary carcinomas. In surgical thyroidectomies performed for benign thyroid lesions, papillary microcarcinomas are found in 5% to 17% of cases. ***Based on the SEER data, papillary carcinomas 1 cm or less constituted about 30% of all papillary carcinomas in 1988 and about 40% at present, making it the most common variant of papillary carcinoma in the United States.*** *Similar trends have been observed in France and many other countries.*

This is probably due to the increased use of ultrasonography and other imaging techniques, which can detect 0.2- to 0.3-cm-sized tumors. Based on autopsy series, papillary carcinomas are found in 6% to 9% of thyroids in individuals with no known thyroid disease in many regions of North America, Europe, and South America… in 28% to 36% of thyroid glands in Japan and Finland

A particular variant is the FVPTC, or follicular variant papillary thyroid carcinoma[11]:

Encapsulated Follicular Variant The prevalence of this variant of papillary carcinoma is likely to be 5% to 7% of all currently diagnosed papillary carcinomas,22 which is lower than observed before26,319 because many tumors diagnosed as encapsulated follicular variant papillary carcinoma prior to 2016 are likely to be classified now as NIFTP.

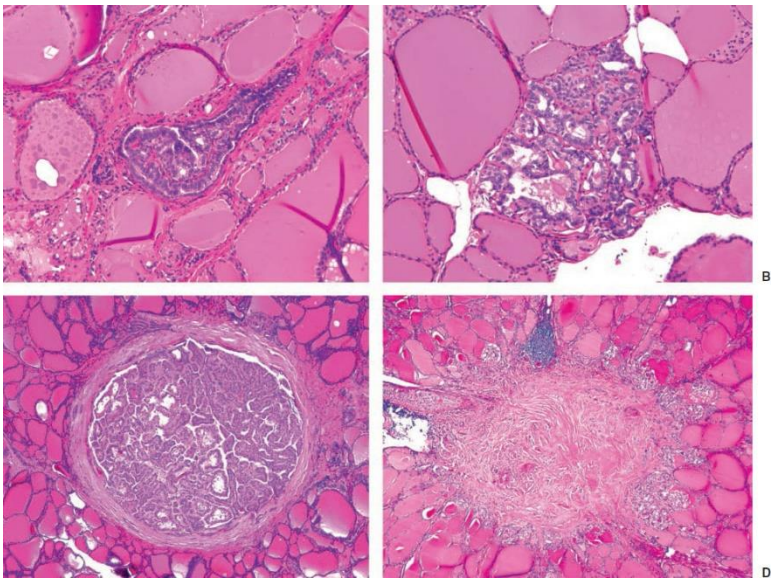
This subtype of the follicular variant is characterized by total encapsulation or clear demarcation from the surrounding benign thyroid tissue. On ultrasound and gross examination, the tumors show clear demarcation from the adjacent thyroid parenchyma.

Microscopically, the main differential diagnosis is NIFTP. The diagnosis of the encapsulated follicular variant of papillary carcinoma requires these three initial criteria:

Nuclear features of papillary carcinoma Follicular growth pattern with no papillae Tumor encapsulation Plus the presence of one of the exclusion criteria for NIFTP Either (a) invasion, (b) significant solid/trabecular/insular growth pattern (30% to 50%), or (c) high-grade features, that is, tumor necrosis or high mitotic activity.

Nuclear features of papillary carcinoma are moderately or well-developed, with a nuclear score of 2 to 3. Nuclear features are present either diffusely or multifocally, intermixed with the areas showing flattened cells with the bland nuclei resembling those of benign thyroid cells. In some cases, on low-power view, multiple distinct easily recognizable groups of follicles with nuclear features of papillary carcinoma are seen in an overall hyperplastic-looking nodule, giving rise to a so-called sprinkling sign. Clefting of follicles in these areas is often seen. As discussed, the entire lesion containing multifocal areas diagnostic for papillary carcinoma should be considered as a single papillary carcinoma. This should be distinguished from a single, discrete, well-delineated focus of papillary carcinoma developed within a hyperplastic nodule or a follicular adenoma.

[11] Nikiforov Chapter 13.


B
D

3 THYROGLOBULIN

Thyroglobulin, TG, is a critical protein for interfacing with the signals demanding more T3 and T4 and the production of T3 and T4. It is produced and used primarily in the follicular cells where it moves out to the follicle and activates the generation of T3 and T4. It is produced exclusively by thyroid cells no matter where the cell is. Thus, if a thyroid cancer metastasizes then the mets being thyroid cells continue to produce TG. After a thyroidectomy therefore TG becomes a somewhat reliable marker for the presence of thyroid mets.

However, in the case of a lobectomy, the residual thyroid still functions and produce TG. Thus, a marker for a met is ambiguous. We examine TG and its functions and then proceed to examine the implications in the cases of lobectomies as we indicated in the introduction.

Cintia et al have recently written a superb paper on thyroglobulin. Coscia et al also have presented a recent comprehensive update as well. Both papers provide a clear understanding of our current knowledge.

We shall not attempt to reiterate it in detail but provide some necessary highlights. Thyroglobulin is a protein generated in thyroid follicular cells and as we shall indicate it assists in the production of T3 and T4. Now fundamentally TG is driven by TSH. If we can override TSH by say levothyroxine, we may shut down TSH production and in turn shut down TG. Understanding this dynamic is essential in an analysis of TG as a market in micro carcinoma in lobectomy.

Here are some interesting facts concerning the thyroid and TG:

i. Patel et al have noted that generally we have 1 ng/mL of TG per 1 g of thyroid mass. This if the thyroid does grow, the mass increases and the TG concentration also increases.

ii. Burkenshaw in a classic article performed a significant amount of work on thyroid masses. The ranges he determined were from 40.9 to 157 g. These measurements were for diseased thyroids.

iii. Wilson et al[12] noted that the average adult thyroid was approximately 15-20 gm. Thus, dramatically lower than the previous which were all diseased in some form.

iv. Wilson et al continue and state that the two lobes are 2.0 cm and 2.5 cm in thickness and width and 4.0 cm in height. Added is the isthmus of 2.0 cm by 2.5 cm and 0.5 in thickness. This yields a total volume of 42.5 cu cm. This is a density of about 0.5 gm/cc. It also is a TG level of about 20 ng/mL.

[12] See Wilson et al p 391

It is useful to have these metrics when examining the thyroid post-lobectomy.

From NCBI[13]:

Thyroglobulin (Tg) is a glycoprotein homodimer produced predominantly by the thyroid gland. It acts as a substrate for the synthesis of thyroxine and triiodothyronine as well as the storage of the inactive forms of thyroid hormone and iodine. Thyroglobulin is secreted from the endoplasmic reticulum to its site of iodination, and subsequent thyroxine biosynthesis, in the follicular lumen. Mutations in this gene cause thyroid dyshormonogenesis, manifested as goiter, and are associated with moderate to severe congenital hypothyroidism. Polymorphisms in this gene are associated with susceptibility to autoimmune thyroid diseases (AITD) such as Graves disease and Hashimoto thryoiditis.

As Rinaldi et al have noted, TG has been observed to be a pre-detection marker if measured over time. They state:

High Tg levels precede by up to 8 years the detection of TC, pointing to a long sojourn time of the disease. Low TSH levels may predispose to TC onset. Neither marker has sufficient accuracy to be a screening test….

This is an interesting observation. Low TSH is a weakly defined measure. High TG likewise may be problematic since it depends greatly on thyroid volume. They continue:

Although our findings can inform on the etiology of TC, they do not support the use of either Tg or TSH level for screening or early detection of TC. Even for Tg, which performed better than TSH, the trade-off between sensitivity and specificity is too poor, notably in the light of the high prevalence of subclinical thyroid diseases and the aggressive treatment required, despite very good prognosis. Likewise, the American Thyroid Association recommends periodic measurement of Tg levels only in the follow-up of TC patients to monitor for residual or recurrent disease after thyroidectomy.

The measurement of TSH is recommended in TC diagnosis to distinguish nonfunctional nodules from hyperfunctioning nodules, which are associated with TSH suppression and are rarely malignant.

In conclusion, our study does not support the involvement of TSH overstimulation in the etiology of TC, but it leaves to clinical trials the assessment of the indications for TSH suppressive treatment in TC patients. Improving the management of TC and subclinical thyroid diseases is a public health priority on account of the increasing frequency and cost of these conditions and the uncertainties over their biology

[13] https://www.ncbi.nlm.nih.gov/gene/7038

The interplay between over and under active thyroids has been an active area of investigation regarding markers for subsequent thyroid cancers. Indrasena notes:

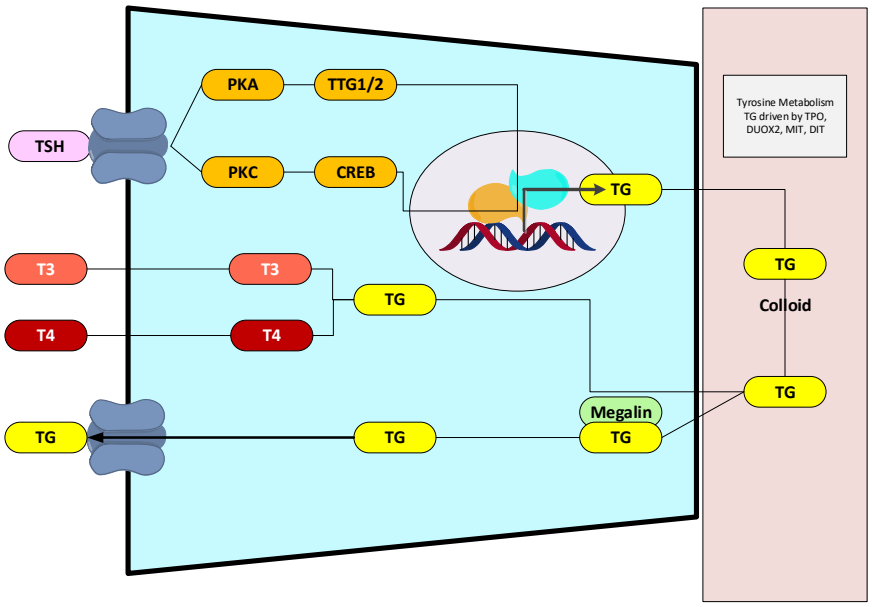
Those who have high serum TG before surgery will show up recurrence as rising serum TG during the postoperative period. Those who do not have high serum TG before surgery will not show up rising serum TG in the presence of recurrent disease. In the latter situation, normal TG level gives only a false reassurance regarding recurrence of disease.

Nevertheless, rising serum TG during the postoperative period must be interpreted cautiously because this could be due to the enlargement of noncancerous residual thyroid tissue inadvertently left behind during surgery

The last remark above is critical. Indeed, enlargement of residual tissue compounds the TG level. Namely, the TG is produced but the tissue will grow and, in that stage, may even produce significant TG.

3.1 PATHWAYS

Let us now examine some of the details of TG generation and the internal pathways. We consider a follicular cell with TSHR on one side and the internal follicle on the other. From the database at KEGG[14]:



[14] https://www.kegg.jp/pathway/hsa04918

Note that TG is produced, it is then used and it also then moves outward into the circulatory system. Several proteins assist in the TG generation as shown. Note that PKA and PKC are transmembrane proteins[15]. CREB is a transcription factor in TG generation[16]. TTG1/2 are transcription regulators also involve in producing TG[17].

From NCBI[18]:

Thyroid hormones triiodothyronine (T3) and thyroxine (T4) are essential for normal development, growth and metabolic homeostasis in all vertebrates, and synthesized in the thyroid gland. The functional unit of the thyroid gland is the follicle, delimited by a monolayer of thyrocytes. Polarized thyrocytes surround the follicular lumen; with their basal and apical surfaces facing the bloodstream and the lumen, respectively.

To synthesize thyroid hormones, thyrocytes take up iodide at their basal side and concentrate it into the lumen. They also secrete in this lumen the specialized protein thyroglobulin (TG) which serves as a store for the hormones. In the follicular lumen oxidation of iodine, iodination of tyrosines (MIT, 3-monoiodotyrosine; DIT, 3,5-diiodotyrosine) and coupling of iodotyrosines takes place on tyrosine residues in TG, resulting in T3 and T4 synthesis. Iodinated TG is resorbed through the apical membrane and degraded to form T3/T4 in lysosomes; the T3/T4 is then secreted through the basal membrane.

The production of T3 and T4 are essential and are well described in the literature. The feedback flow and release of TG is a means to measure its presence. Various methods are available for measurement but care must be taken in using the same method between measurements since measurement biases may exist.

From Coscia et al[19]:

Thyroglobulin (TG) is the protein precursor of thyroid hormones, which are essential for growth, development and the control of metabolism in vertebrates. Hormone synthesis from TG occurs in the thyroid gland via the iodination and coupling of pairs of tyrosines, and is completed by TG proteolysis. Tyrosine proximity within TG is thought to enable the coupling reaction but hormonogenic tyrosines have not been clearly identified, and the lack of a three-dimensional structure of TG has prevented mechanistic understanding. Here we present the structure of full-length human thyroglobulin at a resolution of approximately 3.5 Å, determined by cryo-electron microscopy.

[15] PKC see https://www.ncbi.nlm.nih.gov/gene/112476 and PKA https://www.ncbi.nlm.nih.gov/gene/5566

[16] https://www.ncbi.nlm.nih.gov/gene/1385

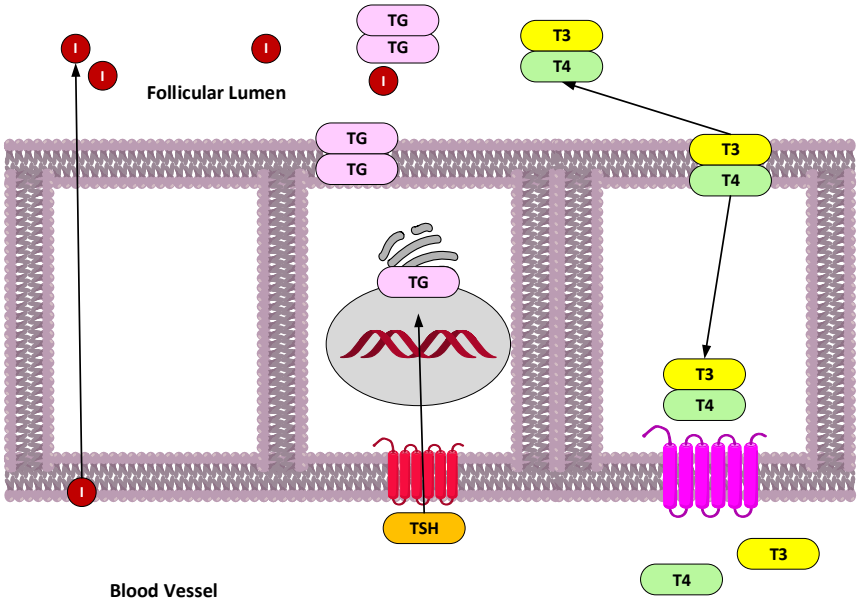
[17] https://www.ncbi.nlm.nih.gov/gene/4004

[18] https://www.ncbi.nlm.nih.gov/biosystems/835410?Sel=geneid:7038#show=genes

[19] https://www.nature.com/articles/s41586-020-1995-4

We identified all of the hormonogenic tyrosine pairs in the structure, and verified them using site-directed mutagenesis and in vitro hormone-production assays using human TG expressed in HEK293T cells. Our analysis revealed that the proximity, flexibility and solvent exposure of the tyrosines are the key characteristics of hormonogenic sites. We transferred the reaction sites from TG to an engineered tyrosine donor–acceptor pair in the unrelated bacterial maltose-binding protein (MBP), which yielded hormone production with an efficiency comparable to that of TG. Our study provides a framework to further understand the production and regulation of thyroid hormones.

We depict this process in a varied form as below;

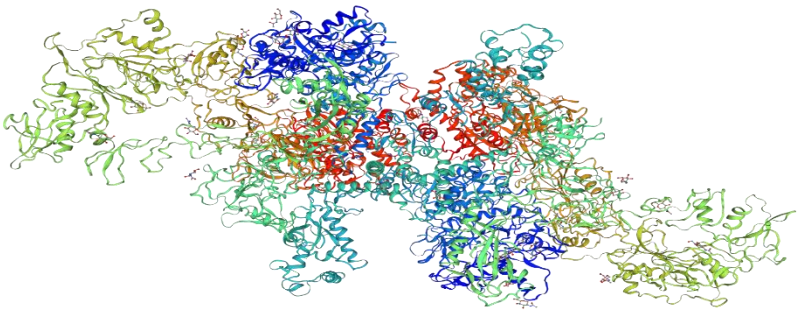


This simplified description is adequate for the general understanding of TG dynamics.

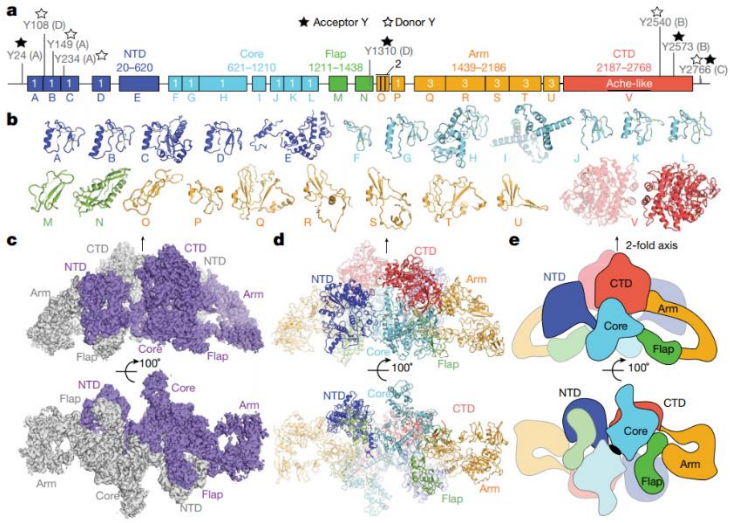
3.2 STRUCTURE

The structure of TG protein has been studied extensively. We provide a brief summary and references regarding it. From Swissmodel[20]:

[20] https://swissmodel.expasy.org/repository/md5/ef0ede8f025be4dc8b407c6d030e82e7 also https://www.rcsb.org/3d-view/6SCJ/1



From Coscia et al we have a more detailed summary of the TG protein configurations:



3.3 ASSAYS

Measurement of TG is often antibody facilitated. Thus, one assay may not be consistent with another. It has been seen that substantial variability may occur in this measurement. As Giovanella et al have noted:

Differentiated thyroid cancer (DTC) is the most common endocrine cancer and its incidence has increased in recent decades. Initial treatment usually consists of total thyroidectomy followed by ablation of thyroid remnants by iodine-131. As thyroid cells are assumed to be the only source of thyroglobulin (Tg) in the human body, circulating Tg serves as a biochemical marker of persistent or recurrent disease in DTC follow-up.

Currently, standard follow-up for DTC comprises Tg measurement and neck ultrasound combined, when indicated, with an additional radioiodine scan. Measurement of Tg after stimulation by endogenous or exogenous TSH is recommended by current clinical guidelines to detect occult disease with a maximum sensitivity due to the suboptimal sensitivity of older Tg assays.

However, the development of new highly sensitive Tg assays with improved analytical sensitivity and precision at low concentrations now allows detection of very low Tg concentrations reflecting minimal amounts of thyroid tissue without the need for TSH stimulation.

Use of these highly sensitive Tg assays has not yet been incorporated into clinical guidelines but they will, we believe, be used by physicians caring for patients with DTC. The aim of this clinical position paper is, therefore, to offer advice on the various aspects and implications of using these highly sensitive Tg assays in the clinical care of patients with DTC

As Spencer et al (2014) note that the methods for TG monitoring do indeed have significant impact:

Reliable detection of interfering TgAbs is method and cutoff dependent. No cutoff eliminated both false-negative and false-positive TgAb misclassifications. Functional sensitivity cutoffs were optimal for minimizing false negatives but have inherent imprecision (20% coefficient of variation) that, exacerbated by TgAb biologic variability during DTC monitoring, could cause TgAb status to fluctuate for patients with low TgAb concentrations, prompting unnecessary Tg method changes and disrupting Tg monitoring.

Laboratories using reflexing should limit Tg method changes by considering a patient's Tg plus TgAb testing history in addition to current TgAb status before Tg method selection.

With regard to TG testing, Soh and Aw have recently noted:

Tg is a 660-kDa homodimeric glycoprotein that is produced by thyroid follicular cells. It is an important tumor marker for DTC following total thyroidectomy and radioiodine remnant ablation. Most clinical laboratories use immunometric assays to measure serum Tg. These assays should be calibrated according to the Certified Reference Materials and Methods-457 international standard to minimize variations.

An exemplary reference study quotes a serum Tg of 29 ng/mL for men and 38 ng/ mL for women on the Beckmann-Coulter DXI 800 immunoassay system[21] …. All subjects (M=209, F=229) had no personal or familial history of thyroid disease, normal TSH (0.5–2.0 mIU/L), negative thyroid antibodies, and normal thyroid ultrasound.

[21] https://www.beckmancoulter.com/products/immunoassay/dxi-800

One of the caveats of using immunometric Tg assays is potential interference by thyroglobulin antibodies (Tg-Ab), which are present in up to 25% of patients with DTC.

Elevated Tg-Ab can lead to falsely low levels of serum Tg.

Hence, serum Tg should always be measured together with Tg-Ab during follow-up for DTC. Greater emphasis should be placed on imaging (thyroid ultrasound, I-131 whole body scan), or even fluorodeoxyglucose-positron emission tomography-computerized tomography (FDG-PET CT) scan in patients with elevated Tg-Ab levels. Even though there is less interference from Tg-Ab when radioimmunoassays are used, these assays are not widely employed because of their lower sensitivity and longer assay times

Also Soh and Aw remark on TGAb tests:

Serum Tg-Ab is a marker of thyroid autoimmunity. Since serum Tg-Ab is elevated in 10% of the general population (especially in women), it is not as sensitive or specific as a thyroid biomarker compared with thyroid peroxidase antibodies (TPO-Ab) or TSH receptor antibodies (TRAb). In the absence of TPO-Ab, TgAb is not significantly associated with thyroid disease. The main clinical utility of the Tg-Ab test is to ensure the reliability of the serum Tg test in the follow-up of patients with DTC. For patients with elevated Tg-Ab (which renders serum Tg unreliable as a tumor marker), Tg-Ab itself can serve as a surrogate tumor marker for DTC…

3.4 ANTIBODIES

Antibodies can be generated against self and if they have a significant impact they result in a variety of autoimmune diseases. In a similar fashion AG often is a driver of AG antibodies, AGAb. These are often found in many thyroid inflammatory disorders and can interfere with AG measurements as we have been discussing. Now as Harish notes:

The most serious technical problem that limits the clinical value of Tg determinations is interference that is caused by endogenous TgAb. These are seen in autoimmune thyroid diseases including Grave's and Hashimoto's thyroiditis.

TgAb is more common in patients with sporadic goiter, multinodular goiter and cancer than in the general population. TgAb is 330 kd molecule which is often undetectable using older techniques.

The TgAb is polyclonal, belongs to IgG class not restricted to a particular subclass, although IgG2 is the predominant class in DTC. TgAb are detected in a higher percentage of DTC patients than the general population (25 % versus 10 %, respectively) (SPENCER et al. 1998). The extent and type of interference that is caused by these autoantibodies depends on the specific Tg method that is used by the clinical laboratory. In IMA, reported Tg concentrations can be falsely lowered by autoantibodies that bind Tg and prevent antigen interaction with assay's antibodies.

Underestimation of the total Tg concentration is characteristic of noncompetitive IMA assays, presumably because these assays measure free Tg and are unable to quantify Tg that is complexed with TgAb. Overestimation of Tg is typical of most competitive immunoassays that are capable of measuring free and TgAb-bound Tg, although underestimation may also be observed. Hence, in TgAb positive sera while IMA methods generally underestimate the Tg value, RIA methods could yield either a false high or false low values.

The clinician must be aware of such interference by TgAb resulting in false values. A false negative result may cause a delay in detecting and treating recurrent or metastatic disease. A false positive result can lead to further clinical studies or therapy and unnecessary patient anxiety. Thus, presence of TgAb invalidates the negative predictive value of Tg*…*

The last remark above is often of singular merit. As Reverter et al have noted regarding the Ab increase and decrease:

Our results suggest that not only the appearance of a significant increase in TgAb but also stable concentrations of TgAb should be regarded as a sufficient risk condition for an active search for recurrent or persistent disease. Conversely, a significant decrease in TgAb levels can represent a good prognostic sign.

As Frolich and Wahl note:

Thyroglobulin is a large (600 kDa) glycoprotein consisting of dimers and containing on average 2–3 molecules of T4 and 0.3 molecules T3. The molecule is heterogeneous regarding hormone content, glycosylation, and size. The production of antibodies against Tg can be induced by massive destruction of the thyroid gland, but high Tg levels in blood do not per se induce antibody production. Out of the 40 epitopes that have been identified, 6 according to some authors and 1–2 according to others are immunogenic.

Antibodies against Tg differ between healthy subjects and AITD patients in that polyclonal antibodies are seen in normal subjects and oligoclonal antibodies in AITD patients. Antibodies in healthy subjects and AITD patients differentially recognize mainly two conformational epitopes of the molecule.

Pattern of anti-Tg antibodies are similar in GD and HT patients and similar in healthy individuals and patients with TC. In general, low levels of self-antigens induce tolerance. It has been hypothesized that normal blood levels of Tg induce self-tolerance in T cells but not in B cells. B cells that recognize Tg arrest their migration in the T cell zone of peripheral lymphoid tissues but do not interact with CD4 helper cells.

The lack of interaction prevents the B cells from migrating out of the T cell zones into the follicles, and they undergo apoptosis. As a consequence of the B cell activity, healthy individuals have very low, usually below detection threshold levels of anti-Tg antibodies. In the presence of higher Tg levels after tissue damage, changed conformation of the Tg molecule due to high I2 levels, and supernormal TSH levels, the anti-Tg antibody titers become abnormal.

Administration of I2 induced antibody production in 8–20% of subjects, together with intra-thyroidal lymphocyte infiltration in some of the patients.

The proposed mechanisms are either antibody formation due to massive release of antigens following thyrocyte destruction or generation of new epitopes by a changed and more immunogenic conformation of the Tg molecule with high I2 content. The effects of I2 on immune responses of Tg and TPO antigens in thyroid autoimmunity might not be completely the same. On the basis that salt intake is the main source of I2, universal salt iodization has been introduced as protective measure against goiter. Excessive I2 intake, defined as table salt I2 concentrations of 40–100 mg/kg for 5 years, increased thyroid autoimmunity.

Anti-Tg antibodies do not fix complement because the epitopes are too widely spaced to allow cross-linking. Furthermore, antiTg antibodies in GD belong mainly to the IgG4 class, which is not complement binding. Low levels of IgA antibodies have also been reported. IgM antibodies against Tg have been reported to 1% in healthy individuals. The functional consequence of anti-Tg antibodies is not clear as they do not cause thyroid cell destruction. Circulating antibodies could be detected in about 10% of healthy young subjects and 15% of people >60 years of age. Among HT patients, antibody prevalence was 60–80% and in 50–60% in GD patients.

Another study identified anti-Tg antibodies in 70–80% of AITD patients, 30–40% of GD patients, and 10–15% of patients with non-thyroid immune disorders Anti-Tg antibodies can cross the placenta barrier, but the effect on the neonate is unclear. The distribution among the classes of antibodies against Tg has been reported differently. IgG1 and IgG4 were the most important classes in GD and HT patients according to one study, while other authors reported distribution between IgG1, IgG2, and IgG4 classes. Interestingly, distribution differed between GD and HT patients; IgG4 class was dominant in patients with GD and IgG2 class in HT patients. This different distribution may reflect the different type of immune action taking place in the thyroid.

The authors present the following Table representing the characteristics of the three key antigens; TPO or thyroid peroxidase, TSH-R the TSH receptor, and TG thyroglobulin.

actor	*Anti TSH-R*	*Anti TPO*	*Anti TG*
Antigen location	Extracellular	Intracellular	Intrafollicular, low levels in blood circulation
Access of immune cells to antigen	Without tissue destruction	After thyrocyte destruction	With and without tissue destruction
Duration of antigen exposure	Short, low levels (normalization upon treatment)	Prolonged time, intermediate levels (pathologic levels also upon treatment)	Prolonged time, high levels (pathologic levels also upon treatment)
Type of antibody	Oligoclonal, different epitopes	Polyclonal, one domain immunodominant	Polyclonal, different epitopes
Class of antibody	Mainly IgG 1, other subclasses to low extent	lgG1, lgG4 > lgG2, lgG3; low levels of IgA	IgGI, lgG4 > lgG2 ‧lgG3; low levels of IgA and IgM (healthy individuals)
Action on neonate	Transplacental passage; transient hyperthyroidism or hypothyroidism with delayed development of thyroid gland	Transplacental passage; potential effects on cognitive development	Transplacental passage; potential effects on cognitive development
Prevalence in AITD	‧‧90% GD; -10% HT	>80% in GD and HT	>50% in GD and HT
Prevalence in other AD	Usually no expression, one study 18% in T1DM	16-37% RA; 40% T1DM; 12-30% CD	12-23% RA; 30% Tl DM; 11-32% CD
Action of antibodies	Stimulating, blocking, apoptosis	Little action *per se*	No defined action
Extra-thyroidal targets	Few, defined effects (GO, GDP), partly known mechanism	Several, ill-defined actions (HE, breast cancer), mechanism of action not known	No specific targets identified
Action in breast cancer progression	No protective effect	Potential protective effects	Potential protective effects

Now Spencer et al (2005) in an earlier paper had noted:

Serum Thyroglobulin (Tg) measurement is primarily used as a tumor marker for managing patients with differentiated thyroid carcinomas (DTC). Currently, most laboratories use immunometric assay (IMA) methods in preference to RIAs, because IMA offers the practical advantages of shorter incubation times and automation. Technical problems compromise the clinical utility of current Tg assays.

Indeed, these technical factors still remain. It is critical that in monitoring patients that TG me measured with the same assay and more likely co-monitor TG Ab. They continue:

For example, between-method biases exceed the within-person biological variability of Tg, such that a change in Tg method can disrupt the serial monitoring of patients. The between-method variability that persists despite CRM-457 standardization probably reflects differences in assay specificity for circulating Tg isoforms. Suboptimal Tg assay functional sensitivity compromises the detection of recurrent DTC in the absence of recombinant human TSH (rhTSH) stimulation.

This problem is exacerbated by the wide methodological biases that preclude a comparison of assay sensitivities in absolute terms [nanograms per milliliter (micrograms per liter)] as is customary for analytes such as TSH. It follows that the methods with the highest sensitivity for detecting recurrent DTC would be those displaying the greatest discrimination between their functional sensitivity and the lower reference limit for euthyroid subjects with intact thyroid glands.

At the upper end of the measurement range, Tg methods can suffer from hook problems, by which the very high antigen concentrations sometimes seen in patients with metastatic disease exceed the binding capacity of the capture antibody and cause inappropriately low values.

Assay interferences are particularly problematic. Heterophilic antibody or Tg autoantibody (TgAb) interferences can cause over- or underestimation of serum Tg concentrations. Although manufacturers have reduced the risk of heterophilic antibody interference by adding blockers to assay reagents, TgAb interference is more difficult to detect and overcome. The prevalence of TgAb in DTC patients (20%) is approximately twice that of the general population. There has been growing recognition that serial TgAb measurements per se provide a clinically valuable surrogate tumor marker, because TgAb concentrations respond to changes in Tg antigen.

However, TgAb methods are highly variable and cannot be used interchangeably, and the use of exogenous Tg recovery tests to detect interfering TgAb is widely considered unreliable. Circulating TgAb interferes with serum Tg measurements in a qualitative, quantitative, and method-dependent manner.

IMA methods are prone to underestimate serum Tg in the presence of TgAb, whereas RIA methods have the potential to either under- or overestimate Tg depending on the affinity and specificity of the antibody reagents. The technical issues that currently compromise the reliability of Tg measurements have prompted the American and European Thyroid Associations to sanction a need to assess the impact of Tg and TgAb method differences on the management of patients with DTC.

Thus, in conclusion, TG measurements are at best suggestive, and may not be dispositive. As with any measurements like these, temporal changes tend to dominate any prognostic assessment.

4 LOBECTOMY VS THYROIDECTOMY

Treatment of thyroid cancers generally involves surgical resection of the total thyroid or if diagnosed as a more indolent and localized lesion a lobectomy. Lobectomy generally involves the removal of the lob and the isthmus and may allow retention of the parathyroid. Depending upon observation lymph nodes may or may not be removed and examined during a surgical procedure.

As Park and Yoon had noted:

Surgical treatment for a differentiated thyroid carcinoma (DTC) derived from follicular cells is a starting point of a multifaceted treatment approach. Even though DTC is generally non-aggressive and has good prognosis, choosing the extent of thyroid surgery for DTC remains controversial.

The 2009 American Thyroid Association (ATA) guidelines for patients with DTC recommended total thyroidectomy as the initial surgical option for nearly all DTCs >1cm regardless of other risk factors, based on retrospective data suggesting benefits to survival, recurrence rate, routine use of radioactive iodine (RAI) remnant ablation and detection of recurrent disease using thyroglobulin (Tg) level.

However, recent large-scale studies found no significant survival difference between total thyroidectomy and thyroid lobectomy when adjusting for variables related to mortality risk or disease complexity and severity, besides tumour size.

Recent data also show very similar clinical outcomes for thyroid lobectomy and total thyroidectomy, and the extent of initial thyroid surgery has little impact on disease-specific survival in properly selected low-to-intermediate-risk patients …

they continue:

The NCCN guidelines recommend measuring Tg and anti-Tg antibody 6–12 weeks after initial surgery and assessing trend patterns in thyroid lobectomy patients. Physical examination, TSH and Tg/anti-Tg antibody measurements are recommended 6 and 12 months after the initial operation, and annually thereafter if patients are disease free. Periodic neck ultrasound is recommended only for patients with reasonable suspicion of structural recurrence. TSH suppression therapy with levothyroxine is theoretically recommended to maintain low TSH levels, although data to allow for precise specification of the appropriate target TSH levels is lacking.

In general, target TSH levels are either slightly below or slightly above the lower limit of the reference range for disease-free patients who are at low risk of structural recurrence. However, maintaining TSH levels at 0.1–0.5mU/L is recommended for low-risk patients with biochemical evidence but no structural evidence of disease.

Patients who remain disease free for several years can maintain TSH levels within the reference range.

The 2015 ATA guidelines recommend deciding postoperative management during the early postoperative period (within 2 years of initial treatment) based on the ATA initial risk estimates, which specify initial target TSH levels as 0.1–0.5mU/L for ATA intermediate-risk patients and 0.5–2.0mU/ for ATA low risk patients, assuming patients who have undergone thyroid lobectomy while monitoring for structural recurrence by neck ultrasonography and measuring serum Tg/anti-Tg antibody and TSH.

However, a retrospective multivariate analysis of 1047 PTC patients with a median follow-up duration of 107 months (range: 13–216 months) demonstrated that structural recurrence only occurred in 42 patients (4.0%); additionally, TSH levels at 1 year after initial surgery (HR 1.15 (95% CI 1.03–1.28), P=0.013) were an independent risk factor for structural recurrence and significantly associated with DFS (P=0.012). The cut-off of TSH levels affecting structural recurrence was 1.85mU/L, calculated by receiver-operating curve. Interestingly, this study found that ATA risk category (low- or intermediate-risk) was not associated with structural recurrence by either univariate or multivariate analysis.

Target TSH values of <1.85mU/L during the early postoperative period are within the target TSH levels (0.5–2.0mU/L) recommended for ATA low-risk patients after thyroid lobectomy, but higher than that (0.1–0.5mU/L) recommended for ATA intermediate-risk patients. Although further studies are needed to confirm target TSH levels after thyroid lobectomy, these results suggest that ATA intermediate-risk patients, like ATA low risk patients, can be monitored using TSH levels at a low-normal range during the early postoperative period prior to evaluating initial therapy responses.

5 CLINICAL EXAMPLES

We now want to consider some real examples of how TG may or may not be helpful. Unlike a large cohort we will use an interesting specific patient and examine the complexities of TG as a tracker. We first review some measurement issues and then detail the patient example.

5.1 THYROGLOBULIN MEASUREMENTS

Let us begin with issues regarding TG measurement. From Spencer and LoPresti regarding the processing of TG measurements we have:

Measurement of serum thyroglobulin is primarily used as a tumor marker in the postoperative management of patients with differentiated thyroid cancer. Unfortunately, the technical quality of current thyroglobulin assay methods varies and influences the clinical utility of this test. Two different methodologic approaches are used to measure serum thyroglobulin: the original competitive radioimmunoassay methodology and noncompetitive immunometric assay methods.

Although the newer immunometric assays offer the technical benefits of eliminating the use of isotopes, using smaller specimen volumes, and having higher sensitivity potential, shorter turnaround times and the convenience of automation, immunometric assays also have a higher propensity for interference from both thyroglobulin autoantibodies and heterophilic antibodies, if present in the specimen. It is critical that physicians understand the technical limitations inherent in thyroglobulin measurement in order to effectively use this test for the postoperative management of patients with differentiated thyroid cancers.

Serum thyroglobulin concentrations must be interpreted relative to the mass of thyroid tissue present, any injury effects, the degree of TSH-receptor stimulation and the thyroglobulin assay used ….Between-assay variability necessitates the use of the same thyroglobulin and thyroglobulin antibody assays for the serial monitoring of patients

Because there is a strong relationship between basal thyroglobulin and recombinant human TSH (rhTSH)-stimulated thyroglobulin measurements, sensitive thyroglobulin assays (functional sensitivities ≤0.1 μg/l), when used in conjunction with ultrasound, can greatly reduce the need for rhTSH-stimulated thyroglobulin measurements

Immunometric thyroglobulin methods are more prone to interference from thyroglobulin autoantibodies and/or heterophilic antibodies than are radioimmunoassay methods

Serial thyroglobulin antibody concentrations can be used as a surrogate tumor marker test

Interfering thyroglobulin antibodies may not always be detected using current methods; recovery tests are an unreliable means to detect interference

Again, we note the potential impact of TGAb. We will examine this in the example to follow. Now Lamartina et al note regarding testing:

The treatment paradigm for thyroid cancer has shifted from a one-size-fits-all approach to more personalized protocols that range from active surveillance to total thyroidectomy followed by radioiodine remnant ablation. Accurate surveillance tools are available, but follow-up protocols vary widely between centres and clinicians, owing to the lack of clear, straightforward recommendations on the instruments and assessment schedule that health-care professionals should adopt.

For most patients (that is, those who have had an excellent response to the initial treatment and have a low or intermediate risk of tumour recurrence), an infrequent assessment schedule is sufficient (such as a yearly determination of serum levels of TSH and thyroglobulin). Select patients will benefit from second-line imaging and more frequent assessments. This Review discusses the strengths and weaknesses of the surveillance tools and follow-up strategies that clinicians use as a function of the initial treatment and each patient's risk of recurrence.
Key points

Thyroid cancer follow-up varies according to the histotype, the initial treatment, the initial risk of recurrence and the response to treatment.

An excellent response to the initial treatment is defined by an undetectable serum thyroglobulin in the absence of thyroglobulin antibody and the absence of abnormal findings on neck ultrasonography.

Patients with a low or intermediate risk of disease recurrence who have an excellent response to treatment can be followed up with yearly serum TSH, thyroglobulin and anti-thyroglobulin antibody determination.

When serum levels of thyroglobulin (or anti-thyroglobulin antibody titre) have a rising trend with time, ultrasonographic, cross-sectional or functional imaging should be considered according to the patient's risk and local resources.

Patients with a high risk of disease recurrence who do not respond excellently to treatment should be followed up with serum TSH, thyroglobulin and anti-thyroglobulin antibody determination and neck ultrasonography every 6–12 months.

When untreated, structural disease should be followed up with periodic imaging, with the frequency and the imaging tools depending on disease burden, location and pace of disease progression.

5.2 PATIENT EXAMPLE

We now want to examine a sample patient case. We progress through initial presentation to follow up. It raises several issues. First the actual diagnosis[22]. Second, the importance of TG measurements in what appears to be a substantially low risk patient.

5.2.1 Initial US

The patient presents with an ENT concern regarding sinusitis. Upon examination the ENT observes thyroid nodules and recommends an ultrasound. The initial US report is as noted below:

Longitudinal and transverse imaging of the thyroid was obtained.

The right lobe measures 5 cm in length by 1.8 cm in depth by 1.9 cm in width. The volume is eight. There are multiple nodules detected.

There is a heterogeneous solid nodule which may contain some microcalcification. This also contain cystic areas. This nodule is identified in the upper pole and measures 1.6 cm x 1 cm x 1.1 cm.

There is another heterogeneous nodule which may contain some microcalcification. This is identified in the mid-pole laterally and measures 1.4 cm x 7 mm x 1.1 cm.

There is another heterogeneous solid nodule at the level of the lower pole measuring 1.4 cm x 1 cm x 1 cm. This also contains an internal cystic area. There are multiple other smaller nodules within the right lobe.

The left lobe measures 6 cm in length by 2.7 cm in depth by 2.5 cm in width. The volume is 19. There is a large lobular complex nodule occupying the mid to lower pole. This is a largely fluid containing nodule However, there are areas of nodularity and there are thick septations. This extends towards the isthmus. The nodule measures 3.7 cm in length by 2 cm in depth by 3.3 cm in width. The soft tissue appearing nodular component measures 1.3 cm x 8 mm x 1.4 cm. There was no internal vascularity detected within this nodular component.

IMPRESSION: Large complex nodule on the left side measuring up to 3.4 cm in size.

Multiple nodules on the right side, some of which appear to contain microcalcification. This is considered a suspicious feature.

It will be seen that there are multiple deficiencies in this report. First it lacks and TIRADS staging. In fact, the information presented is grossly inadequate to do so. Second, as we shall see, the focus on the right lesions was a distraction because the single large left lesion harbored a

[22] This is a patient anonymized report. Also permission was granted by the patient for use in this example. This is meant to be demonstrative and not dispositive. This is to be used for discussion only and is not meant for any medical or diagnostic purpose.

significant albeit micro lesion. The patient, upon self-referral to a specialist, proceeded to have a second US and a concomitant FNA.

5.2.2 FNA

After initial US a separate US and FNA were performed. The second notes:

Now an FNA and US was subsequently performed where the right side was unremarkable but the left side was suspicious and the FNA results were as follows:

> *Thyroid, left lower lobe,4.2cm, ultrasound-guided fine needle aspiration biopsy:*
>
> *SUSPICIOUSFOR NEOPLASM Bethesda Category IV.*
>
> *Hypercellular specimen consists of abundant microfollicles and syncytial clusters of overlapping follicular cells showing slightly enlarged nuclei, pale chromatin, scattered grooves and rare nuclear pseudo-inclusions. The background consists of scanty, Inspissated colloid, hemosiderin-laden macrophages and blood.*
>
> *These findings are suspicious for a follicular neoplasm. Comment: Although the architectural features suggest a follicular neoplasm, some nuclear features raise the possibility of a follicular variant of papillary carcinoma or its recently described indolent counterpart NIFTP; definitive distinction among these entities is not possible on cytologic material.*

As a result of the above, the decision was made to perform a left lobectomy.

5.2.3 Biopsy

The biopsy after surgery, left lobectomy, noted:

Note 1 (Surgical):

Papillary thyroid microcarcinoma (4 mm), growing as follicular variant, circumscribed.

No extrathyroid extension identified.
No carcinoma identified on surgical margins.
The tumors greatest dimension is 0.4 cm.
Extrathyroid invasion is not identified.
Vascular invasion is not identified.
No tumor is identified on surgical margins.

Note 2 (FNA):

Hypercellular specimen consists of abundant microfollicles and syncytial clusters of overlapping follicular cells showing

1. slightly enlarged nuclei, pale chromatin,
2. scattered grooves and
3. rare nuclear pseudoinclusions.

The background consists of scanty, inspissated colloid, hemosiderin-laden macrophages, and blood syncytial clusters of overlapping follicular cells slightly enlarged nuclei, pale chromatin, scattered grooves rare nuclear pseudoinclusions.

Now the above does raise the question of a NIFTP. We consider that in the following Table. Here we list first the NIFTP criteria. The we consider compliance with that, followed by the Path report. Finally we list the FNA results. As one can see this may actually be an NIFTP lesion although characterize as micro FVPTC.

NIFTP[23]	***NIFTP Compliance***	***Surgical Path***	***FNA***
Encapsulation or clear demarcation	Yes	Papillary thyroid microcarcinoma (4 mm), growing as follicular variant, circumscribed.	
Nuclear score 2–3 Sum of three nuclear features: 1. Size and shape: Enlarged, elongated, overlapping 2. Membrane irregularities: irregular contour, groove, pseudo-insertions 3. Chromatin: clearing, margination, glassy nuclei	Score 1 Enlarged (Yes) 2. membrane irregularities, grooved (Yes) 3. Chromatin clearing		1. slightly enlarged nuclei, pale chromatin, 2. scattered grooves and 3. rare nuclear pseudoinclusions.
No vascular or capsular invasion	No invasion	Vascular invasion is not identified.	
No tumor necrosis	No necrosis		
No high mitotic activity (<3/HPF)	X		
Follicular growth pattern with <1% Papillae (criteria modified in 2018 to "no well-formed papillae")	Yes	growing as follicular variant	
No psammoma bodies (round collection of calcium)	No psamomma	NA	NA
<30% solid/trabecular/insular growth pattern	X	NA	NA

5.2.4 Ultrasound

Now, an ultrasound at three months after the lobectomy the results were as follows. Note the volume of the right love is 5.8 x 2.3 x 2.7. This was four months after the initial US where the volume was 5.0 x 1.8 x 1.9. Namely, the right lobe had substantial growth post-surgery. Namely, it went from 17.1 cu cm to 36.0, a more than two-fold increase. We believe that this growth is material to TG measures. The US results read as follows:

[23] Shrestha et al, Cytomorphology of Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features and the Impact of New Nomenclature on Molecular Testing, Med. Sci. 2019, 7, 15

Right lobe: The right lobe measures 5.8 x 2.3 x 2.7 cm. It is heterogeneous in echogenicity.

1.7 x 0.7 x 1.3 cm upper pole nodule.

Composition: 1 point, Mixed cystic and solid
Echogenicity: 2 points, Hypoechoic
Shape: 0 points, Wider-than-tall
Margin: 0 points, Smooth
Echogenic foci (can select multiple): 0 points, None or large comet-tail artifacts

ACR TI-RADS: TR3, 3 points, Mildly Suspicious, FNA if 2.5 cm or greater or follow-up at 1, 3, and 5 years if 1.5 cm or greater.

1.5 x 1.0 x 1.0 cm midpole nodule

likely represents 2 smaller nodules in conglomerate.
Composition: 1 point, Mixed cystic and solid
Echogenicity: 1 point, Hyperechoic or isoechoic
Shape: 0 points, Wider-than-tall
Margin: 0 points, Smooth
Echogenic foci (can select multiple): 0 points, None or large comet-tail artifacts

ACR TI-RADS: TR3, 3 points, Mildly Suspicious, FNA if 2.5 cm or greater or follow-up at 1, 3, and 5 years if 1.5 cm or greater.

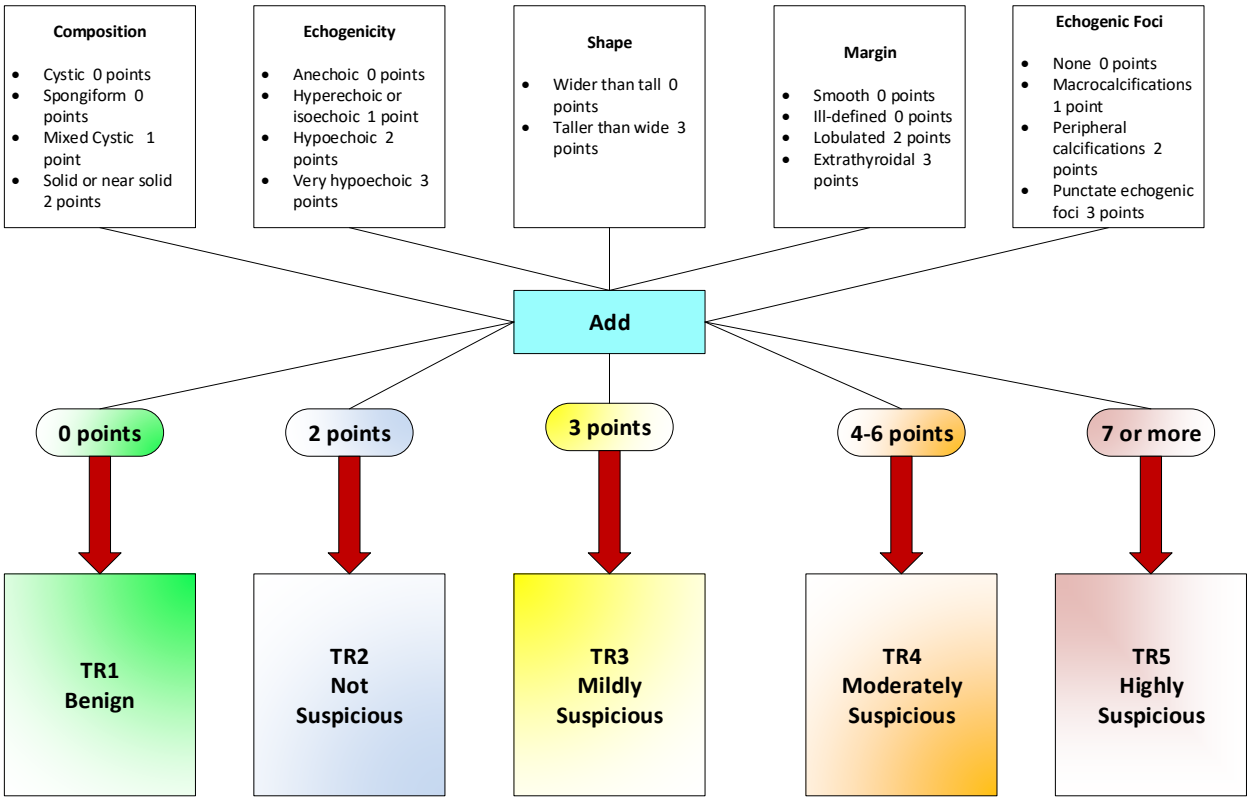
0.8 x 0.8 x 1.1 cm lower pole nodule.

Composition: 1 point, Mixed cystic and solid
Echogenicity: 1 point, Hyperechoic or isoechoic
Shape: 0 points, Wider-than-tall
Margin: 0 points, Smooth
Echogenic foci (can select multiple): 0 points, None or large comet-tail artifacts

ACR TI-RADS: TR2, 2 points, Not suspicious, no FNA.

Left lobe: Status post left thyroidectomy.
Additional observations: There are benign-appearing cervical lymph nodes with echogenic centers.
IMPRESSION:
Status post left hemithyroidectomy with benign-appearing cervical lymph nodes. No cervical lymphadenopathy.
Heterogeneous right thyroid lobe with multiple nodules as described.
No FNA is recommended based on imaging criteria.

Now it is worth reviewing the TIRADS formulation in some detail (see Sanchez for details). We depict this process below:



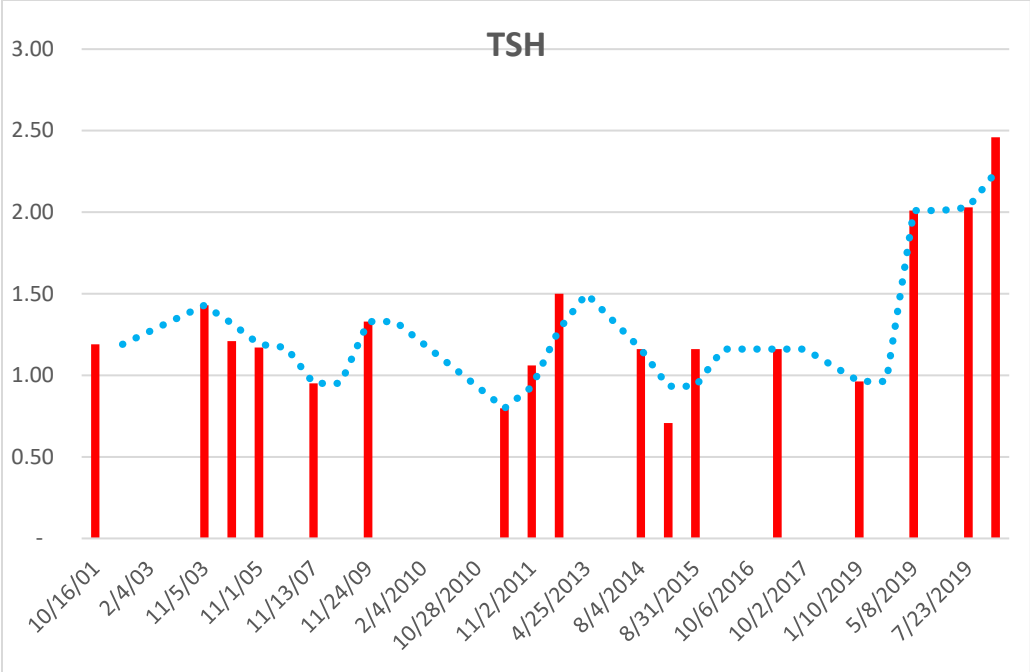
Thus, in the above US analysis it is a TIRADS 3 and worthy of follow up but unlikely malignant. One should note that the first US was lacking in any of this analysis.

5.2.5 Subsequent Lab Work

We now present a collection of key lab metrics both before and after lobectomy. These present some light on the TG issues.

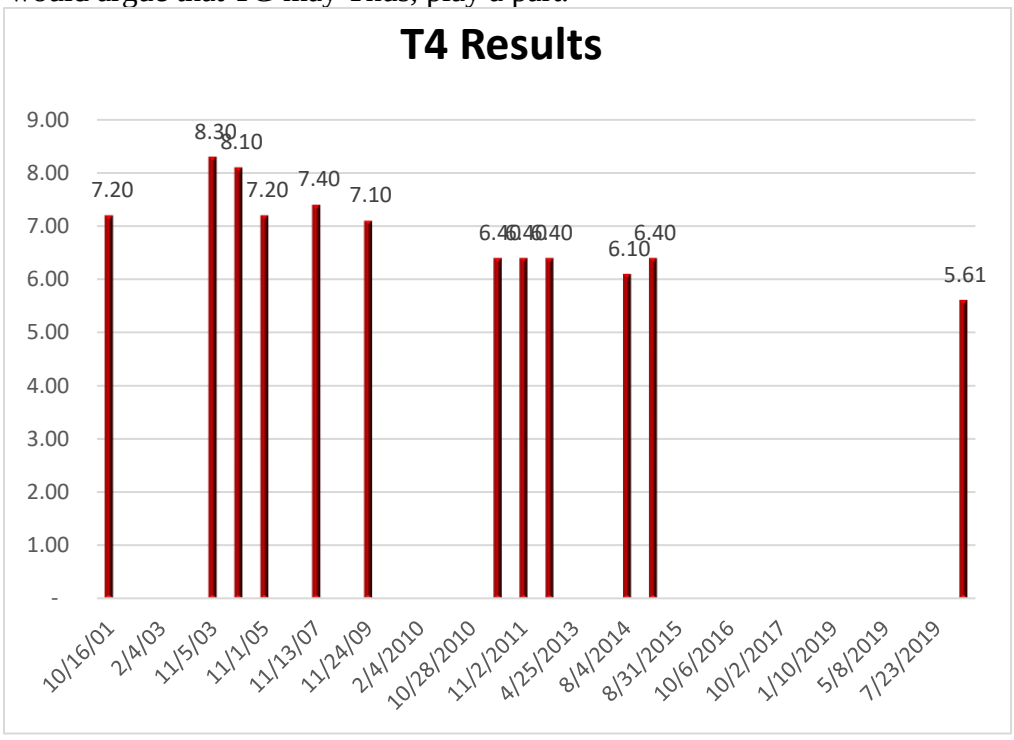
5.2.5.1 TSH

We first present the changes in TSH for this patient over a long period. Notice it has doubled as expected post lobectomy and continues to increase but well withing normal ranges. TSH is the driver to generate TG and in turn T3/T4. Recall that T4 and T3 are controlled by some internal set point. In this patient we have a long record of what that set point was as well as the TSH history driving to that set point. TSH is below:



5.2.5.2 T4

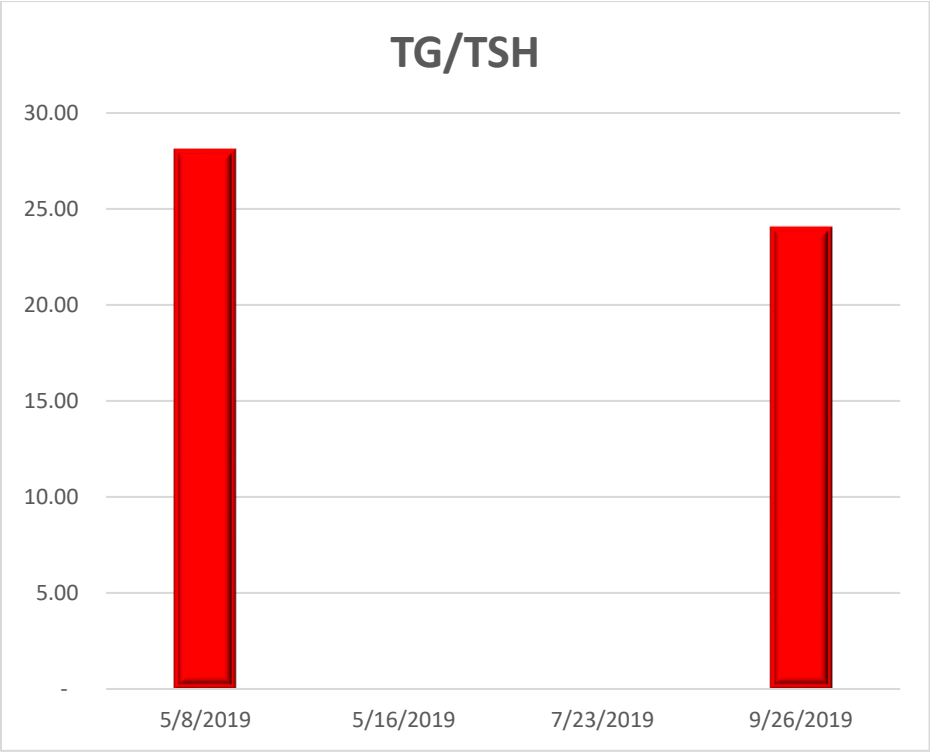
Now we have T4 also for a long period as shown below. We see that even with doubling of TSH the T4 is lower than normal. Namely, we would expect an attempt to drive this a bit higher. Thus, we would argue that TG may Thus, play a part.



There clearly is not change. The patient has a stable T4 level even after adjusting for the lobectomy.

5.2.5.3 TG/TSH Levels

Now we examine the TG levels. There were 56.9 at two months and 59.2 at eight months. This is high and could be grounds for concern despite everything else. However, the volume of the remaining lobe was 36 at two months and no measurement at eight months. Thus, using the 1 per 1 cc metric we saw before we can state that it should be 36. But the multiple nodules could be producing a higher number. However, we examined the TG/TSH levels.



Clearly these are improving and despite the high TG, most likely due to the lobectomy we have limited concern. The reasons are as follows:

1. The patient always had a low TSH, yet a stable T4. Thus, the low TSH must have activated a higher than normal TG to achieve the T4 set point.

2. The patient saw a doubling of the volume of the residual right side of the thyroid if the measurements are correct. Thus, TG is being driven by two factors. First the higher TSH and second the increase thyroid volume.

We may then ask if we would expect TG to decrease.

3. Finally the patient admitted that an excess of melatonin was taken for poor sleep conditions. This we Thus, we examined melatonin usage as another driver of increased TG.

5.3 OBSERVATION

As Schlumberger et al note:

The postoperative administration of radioiodine can be avoided in low-risk patients with undetectable TSH-stimulated serum thyroglobulin and no lymph-node metastases detected at surgery. Sensitive methods for serum thyroglobulin determination can be used to avoid TSH stimulation 9–12 months after surgery in low-risk patients who have an undetectable serum thyroglobulin on levothyroxine treatment; the role of these sensitive assays in the period immediately after surgery needs to be established by further studies. Finally, a low activity of radioiodine (1.1 GBq) should be administered selectively in low-risk patients receiving levothyroxine treatment following injections of recombinant human TSH. These modifications of current protocols will improve the quality of life of patients, potentially decrease morbidity and considerably reduce the cost of treatment and follow-up.

Now Harish had noted:

The presence of a thyroid remnant, defined as surgically treated patients without subsequent radioiodine ablation, decreases the specificity and the clinical utility of the Tg level.

After lobectomy, the Tg level is undetectable during thyroxine treatment in only half of the patients. …In the majority of patients with detectable Tg levels, ultrasound examination of the remnant lobe could show clinically unsuspected micronodules. Due to their small size, fine needle biopsy may be impossible and in case of progression, surgery may be warranted. Citing these data, some suggest total thyroidectomy for all patients with DTC.

Following thyroid hormone withdrawal, the Tg level is poorly informative in these patients, because it can be produced both by normal and by neoplastic thyroid tissue. One study reported 84 patients on follow up after hemithyroidectomy and found 3 of them with recurrence to have Tg levels above 10 ng/ml. however, the number of patients having this level without recurrence was not indicated. Levels above 10 ng/ml carries a 5.5 fold increased risk of recurrence …

In conclusion, Tg estimation in patients undergoing hemithyroidectomy or lobectomy is not very useful and has a low predictive value. Long term follow-up A precondition for use of Tg in the follow up of DTC includes total ablation the thyroid. This includes surgical ablation by total thyroidectomy, followed by a radioiodine ablation of the remnant to a dose of at least 300 Gy. to ensure that any measurable Tg values represent residual malignant disease …

Although some controversy exists about routine radioiodine ablation for thyroid remnants, this procedure has been adopted by various organizations because of beneficial effects on recurrence and mortality in patients with higher tumor stages and in those with residual tumor. A higher specificity for Tg measurements have been found after thyroid remnant ablation than after surgery alone in a recent meta-analysis.

For patients who were treated with a total thyroidectomy and adequate radioiodine ablation, there is no ‘‘normal’’ range of serum Tg values; any measurable Tg is considered sufficient evidence for persistent thyroid carcinoma. A lower cut off value has high sensitivity but low specificity. However, further action is recommended when serum Tg exceeds 5 – 10 ng/ml.

Conversely, in patients with absence of TgAb, low or undetectable serum Tg levels in the presence of known residual disease documents the insensitivity of this method for assessment in these patients.

We would have to take exception with this result. Namely, if the TG production is total mass dependent, then it is clear that a level of 10 would be exceptionally small depending on how the lobectomy is performed. More recent reporting as we have done in the Introduction counters his opinion.

6 OBSERVATIONS

We can now make several observations regarding the use of TG as a marker post lobectomy. What makes this an interesting study is that in dealing with similar issues in a prostate we use PSA as a marker. However, resection of the total prostate is always done since there is no critical hormonal reliance on residual prostate tissue. Thus, the thyroid lobectomy is performed to spar T3/T4 production residuals.

6.1 PRE AND POST MEASURES

Generally pre-operative measurement of TG is not performed. One can argue that it would be of significant use as a benchmark especially if we look for TG/volume measures and then post lobectomy want to measure TG/volume remaining. There have a few studies wherein they did make measurements. However, no formal recommendation has been proposed at this time.

6.2 MELATONIN INTERFERENCE OF TG LEVELS

There is an interesting relationship between the use of melatonin and the thyroid. As Lewinski and Karbownik note:

All the above mentioned results, while proving the inhibition of thyroid growth and/or thyroid function by the pineal, as well as the reports on the stimulation of the pineal gland activity and growth processes by the thyroid hormones, have prompted us to formulate a hypothesis on the existence of a reciprocal relationship between the thyroid and the pineal.

In agreement with this hypothesis, melatonin could act directly on thyroid follicular cells, inhibiting their proliferation. Accordingly, it is possible that plasma concentrations of thyroid hormones are direct modulators of the pineal function and growth. This review is the fourth one after previous three published before.

Because numerous studies are expected to be performed in a near future on melatonin and thyroid gland, especially with respect to oxidative stress and molecular mechanisms of interactions in question, a subsequent survey will be necessary to update the issue of melatonin-thyroid relationship. The experimental and clinical evidence, as presented in our survey, indicates an undoubtable role of melatonin in physiological and pathological processes of the thyroid gland, providing "a green light" for the use of this indoleamine under certain clinical conditions in humans.

A more recent paper by Garcia-Marin et al (2015) have noted:

Melatonin is an indoleamine with multiple functions in both plant and animal species. In addition to data in literature describing many other important roles for melatonin, such as antioxidant, circadian rhythm controlling, anti-aging, antiproliferative or immunomodulatory activities, our group recently reported that thyroid C-cells synthesize melatonin and suggested a paracrine role for this molecule in the regulation of thyroid activity. To discern the role played

by melatonin at thyroid level and its involvement in the hypothalamic-pituitary-thyroid axis, in the present study we have analyzed the effect of thyrotropin in the regulation of the enzymatic machinery for melatonin biosynthesis in C cells as well as the effect of melatonin in the regulation of thyroid hormone biosynthesis in thyrocytes.

Our results show that the key enzymes for melatonin biosynthesis (AANAT and ASMT) are regulated by thyroid-stimulating hormone.

Furthermore, exogenous melatonin increases thyroglobulin expression at mRNA and protein levels on cultured thyrocytes and this effect is not strictly mediated by the upregulation of TTF1 or, noteworthy, PAX8 transcription factors. The present data show that thyroid C-cells synthesize melatonin under thyroid-stimulating hormone control and, consistently with previous data, support the hypothesis of a paracrine role for C-cell-synthesised melatonin within the thyroid gland. Additionally, in the present study we show evidence for the involvement of melatonin in thyroid function by directly-regulating thyroglobulin gene expression in follicular cells….

Melatonin increases thyroglobulin expression: To investigate the role of melatonin in the biosynthesis of thyroglobulin by follicular cells, rat thyroid PC-Cl3 cells were cultured for 72 hours in 0.5% FBS medium containing 5H or 6H with or without 100 µM melatonin.

The effect of melatonin the expression of thyroglobulin was then analyzed at mRNA level by RT-qPCR, and at protein level by semiquantitative Western-blot and immunofluorescence…. significant increases in thyroglobulin mRNA expression were detected when cells were treated with 100 µM melatonin for 48 and 72 h and were maximum at 72 h.

Furthermore, the effect of melatonin was present under both 5H and 6H culture conditions (in the absence or presence of TSH).

Melatonin effect was maximum in the presence of TSH (6H conditions) at which a 6-fold increase in thyroglobulin mRNA levels was observed.

The upregulation of thyroglobulin expression was also observable at protein level as revealed by Western blot, and finally, in good agreement with mRNA and Western-blot data, the intensity of immunofluorescence staining in PC-Cl3 cells for thyroglobulin was higher after melatonin treatment when compared to control cells.

Data indicate that melatonin regulates the expression of thyroglobulin in cultured rat thyroid follicular cells.

Thus, we can ask whether a patient has been taking melatonin and if such will that distort the TG readings. Initially an unlikely scenario but we have begun to take this under consideration. We have seen a similar effect with diphenhydramine and PSA increases. It is known that diphenhydramine cause BPH and Thus, an increase in PSA. Perhaps there is also a similar effect with melatonin and TG in lobectomy patients.

6.3 TG Ab Interference of Levels

We have examined TG Ab interference with TG measurements. This can be a significant suppressive factor and it may result from a thyroid co-morbidity. Thus, having the remaining lobe with benign nodules may raise this risk. Data seems to be lacking at this time.

6.4 Risks and Age

Age seems always to be a co-factor. As noted in a review of the article by Koshkina et al[24], in older patient's active surveillance may be preferable to surgery. Many older patient present with an incidental finding and a generally indolent lesion. The report indicates the results of a study indicating the use of active surveillance:

Advancing age may be associated with reduced risk for papillary thyroid tumor growth under active surveillance,… "Older individuals undergoing active surveillance of small, low risk papillary thyroid cancer — particularly papillary microcarcinoma — may have a reduced risk for primary tumor growth that prompts surgery within a time frame of several years of follow-up, compared with younger patients," … In a systematic review and meta-analysis, … analyzed data from five studies consisting of adults undergoing active surveillance as primary management for low-risk papillary thyroid cancer (PTC), …. Studies were conducted in Japan (n = 3), South Korea (n = 1) and the United States (n = 1).

… "Active surveillance was defined as a structured program of clinical follow up, including clinical assessments and neck imaging, in lieu of immediate surgery, in which there are prespecified disease progression criteria [that] would prompt a recommendation for surgery with curative intent,"…

"In active surveillance studies, patients are also given an option to have surgery at any time point, even if the disease does not progress, if they prefer to do so. The patients in our review were all adults with PTC less than 2 cm in maximal diameter, where clinical disease was confined to the thyroid, meaning no metastatic disease nor extrathyroidal extension at diagnosis. Most of the patients included in the review had papillary microcarcinoma."

[24] https://www.healio.com/endocrinology/thyroid/news/online/%7B3c445618-05b2-4963-aaef-b7493ac61293%7D/risk-for-thyroid-tumor-growth-declines-with-advancing-age?page=2

7 REFERENCES

1. Ali and Cibas, The Bethesda System for Reporting Thyroid Cytopathology, 2nd Ed, Springer (New York) 2018
2. Baloch and LiVolsi, Surgical Pathology of the Thyroid Gland, in Randolph, Surgery of the Thyroid and Parathyroid Glands, 2nd Ed, Elsevier, (2013): 413-427.
3. Burkinshaw, A method of measuring the mass of the thyroid gland in vivo, Acta Radiologica, 1958
4. Cha and Koo, Next-generation sequencing in thyroid cancer, Transl Med (2016) 14:322
5. Cibas and Ali, The Bethesda System for Reporting Thyroid Cytopathology, THYROID, Volume 19, Number 11, 2009
6. Citterio et al, The role of thyroglobulin in thyroid hormonogenesis, Nature Review Endo, 15, pages323–338 (2019)
7. Coscia et al, The structure of thyroglobulin, Nature, 27 Feb 2020
8. Czarnywojtek et al, A Study of Thyroglobulin Concentration in the Thyroid and Serum of Patients with Different Thyroid Disorders, Archivum Immunologiae et Therapiae Experimentalis, 2002, 50,143–148
9. Das, D., Psammoma body: a product of dystrophic calcification or of a biologically active process that aims at limiting the growth and spread of tumor?, Diagn Cytopathol. 2009 Jul;37(7):534-41.
10. Delerba et al, Cancer Stem Cells: Models and Concepts, Annual Review of Medicine, 2007
11. Dubrovska, A Et Al, The Role Of PTEN/Akt/PI3K Signaling In The Maintenance And Viability Of Prostate Cancer Stem-Like Cell Populations, PNAS, Jan 2009 V 106 N 1
12. Duick et al, Thyroid and Parathyroid Ultrasound and Ultrasound-Guided FNA, 4th Ed, Springer (New York) 2018
13. Epstein, J., Biopsy Interpretation of the Thyroid, 2nd Ed, Walters Kluwer (New York) 2017
14. Franz, Medullary Thyroid Cancer, Department of Surgery at the University of South Florida College of Medicine, Tampa, Fla.
15. Frolich and Wahl, Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and extra-Thyroidal Diseases, Frontiers in Immunology, May 2017 | Volume 8 | Article 521
16. Gao et al, Papillary Thyroid Micro Carcinoma: The Incidence of High-Risk Features and Its Prognostic Implications, Front in Endo, 15 Feb 2019
17. Garcia Marin et al, Melatonin-synthesizing enzymes and melatonin receptor in rat thyroid cells, Histology and Histopathology, 2012
18. Garcia-Marin et al, Melatonin in The Thyroid Gland: Regulation By Thyroid-Stimulating Hormone And Role In Thyroglobulin Gene Expression, Journal Of Physiology And Pharmacology 2015, 66, 5, 643-652

19. Giovanella et al, Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer, European Journal of Endocrinology (2014) 171
20. Giza et al, Key principles of miRNA involvement in human disease, Discoveries, 2014, V2 No 4.
21. Goldfarb, Thyroglobulin levels do not predict recurrence after lobectomy for low risk papillary thyroid cancer, Clinical Thyroidology for the Public, Volume 11 | Issue 11 | November 2018
22. Guo et al, Cancer stem-like cells and thyroid cancer, Thematic Review, 21:5, 2014 Society for Endocrinology
23. Harish, Thyroglobulin: Current Status In Differentiated Thyroid Carcinoma, Endocrine Regulations Vol. 40, 53-67, 2006
24. Hsiao and Nikiforov, Molecular approaches to thyroid cancer diagnosis, Thematic Review, 2014 Society for Endocrinology
25. Indrasena, Use of thyroglobulin as a tumour marker, World J Biol Chem 2017 February 26; 8(1): 81-85
26. Isola et al, Dynamics Of The Melatonin Mt1 Receptor In The Rat Parotid Gland Upon Melatonin Administration, Journal Of Physiology And Pharmacology 2016, 67, 1, 111-119
27. Jeeyavudeen et al, Lobectomy for thyroid cancer and thyroglobulin as a tumor marker for long term follow up: current controversies and clarification, Endocrine Abstracts (2019) 65 P147
28. Johnson et al, Noninvasive Follicular Thyroid Neoplasms With Papillary-like Nuclear Features Are Genetically and Biologically Similar to Adenomatous Nodules and Distinct From Papillary Thyroid Carcinomas With Extensive Follicular Growth, Arch Path Lab Med Vol 142 July 2018
29. Khatami et al, Liquid Biopsy in Thyroid Cancer: New Insight, IJHOSCR, July 2018
30. Kim and Kuo, Recent Updates on the Management of Medullary Thyroid Carcinoma, Endocrinol Metab 2016;31:392-399
31. Kim et al, Utility of including BRAF mutation analysis with ultrasonographic and cytological diagnoses in ultrasonography-guided fine needle aspiration of thyroid nodules, PLOS One Aug 2018
32. Kimura et al, High Prevalence of BRAF Mutations in Thyroid Cancer: Genetic Evidence for Constitutive Activation of the RET/PTC-RAS-BRAF Signaling Pathway in Papillary Thyroid Carcinoma, CANCER RESEARCH 63, 1454–1457, April 1, 2003
33. Koshkina et al, Association of Patient Age With Progression of Low-Risk Papillary Thyroid Carcinoma Under Active Surveillance, JAMA OTO etc, April 16, 2020.
34. Koshkina, Risk for thyroid tumor growth declines with advancing age, JAMA Oto Head Neck, 2020 (see https://www.healio.com/endocrinology/thyroid/news/online/%7B3c445618-05b2-4963-aaef-b7493ac61293%7D/risk-for-thyroid-tumor-growth-declines-with-advancing-age?page=2)

35. Kumar and Rani, Metastasis of Thyroid Carcinoma to the Mandible, Journal of Chalmeda Anand Rao Institute of Medical Sciences Vol 6 Issue 1 November 2013
36. Lamartina and Handkiewicz-Junak, Follow-up of low risk thyroid cancer patients: can we stop follow-up after 5 years of complete remission?, European Society of Endocrinology, 2020.
37. Lamartina et al, Follow-up of differentiated thyroid cancer – what should (and what should not) be done, Nature Reviews Endocrinology, Aug 1, 2018
38. Lawson, D., O. Witte, Stem Cells In Prostate Cancer Initiation And Progression, Jrl Clin Inv, 2007 Pp 2044-2050.
39. Lewinski and Karbownik, Melatonin and the thyroid gland, Neuroendocrinology Letters, 2002
40. Lim et al, Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013, JAMA, 2017;317(13):1338-1348
41. Lu et al, Targeted next generation sequencing identifies somatic mutations and gene fusions in papillary thyroid carcinoma, Oncotarget, 2017, Vol. 8, (No. 28), pp: 45784-45792
42. Luo et al, The Emerging Roles of Thyroglobulin, Advances in Endocrinology, Volume 2014, Article ID 189194
43. Maas, et al, Extracellular vesicles: unique intercellular delivery vehicles, Trends Cell Biol. 2017 March ; 27(3): 172–188
44. Malaguarnera, et al, p53 family proteins in thyroid cancer, Endocrine-Related Cancer (2007) 14 43–60
45. Martins et al, CD8+ TIL Recruitment May Revert the Association of MAGE A3 with Aggressive Features in Thyroid Tumors, Journal of Immunology Research, Volume 2014,
46. Mehrzad et al, Invasive follicular variant of papillary thyroid cancer harboring the NRAS mutation Q61K and presenting with bone metastasis—A case report, International Journal of Surgery Case Reports 38 (2017) 180–184
47. Moeller and Fuhrer, Thyroid hormone, thyroid hormone receptors, and cancer: a clinical perspective, Review, 2013 Society for Endocrinology
48. Mould et al, Immune responses in the thyroid cancer microenvironment: making immunotherapy a possible mission, Soc for Endo 2017
49. Muller et al, Thyroid Hormone Regulation of Metabolism, Physiol Rev 94: 355–382, 2014
50. Murugan and Xing, Anaplastic Thyroid Cancers Harbor Novel Oncogenic Mutations of the ALK Gene, Cancer Res; 71(13) July 1, 2011
51. Na and Choi, Immune landscape of papillary thyroid cancer and immunotherapeutic implications, Endocrine Related Cancers. 2018 Society for Endocrinology
52. Nelkin, Recent advances in the biology and therapy of medullary thyroid carcinoma, F1000Research, 2018

53. Nikiforov and Nikiforova, Molecular Genetics and diagnosis of thyroid cancer, Nature Reviews of Endocrinology, 2011.

54. Nikiforov et al, Diagnostic Pathology and Molecular Genetics of the Thyroid, Wolters Kluwer 2020

55. Nikiforov et al, Diagnostic Pathology and Molecular Genetics of the Thyroid: A Comprehensive Guide for Practicing Thyroid Pathology, 3rd Ed, LWW (New York) 2019

56. Nikiforov et al, Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma, JAMA Oncol. 2016 August 01; 2(8): 1023–1029.

57. Nucera and Pontecorvi, Clinical outcome, role of BRAF V600E, and molecular pathways in papillary thyroid micro carcinoma: is it an indolent cancer or an early stage of papillary thyroid cancer? Front. in Endo, Cancer Endo, Feb 2012, Vol 3, Art 33

58. Park and Yoon, Lobectomy in patients with differentiated thyroid cancer: indications and follow-up, Endocrine-Related Cancer, (2019) 26, R381–R393

59. Park S et al, Changes in serum thyroglobulin levels after lobectomy in patients with low-risk papillary thyroid cancer. Thyroid. Epub 2018 May 30. PMID: 29845894

60. Patel et al, Serum Thyroglobulin: Preoperative Levels and Factors Affecting Postoperative Optimal Timing following Total Thyroidectomy, International Journal of Endocrinology, Volume 2019, Article ID 1384651

61. Randolph, Surgery of the Thyroid and Parathyroid Glands, 2nd Ed, Sanders (New York) 2013

62. Reverter et al, Prognostic Significance of Thyroglobulin Antibodies in Differentiated Thyroid Cancer, Journal of Thyroid Research Volume 2020, Article ID 8312628

63. Rinaldi et al, thyroid-Stimulating Hormone, thyroglobulin, and thyroid Hormones and risk of Differentiated thyroid carcinoma: the EPIC Study, JNCI, June 2014

64. Ruscica et al, Role of neuropeptide Y and its receptors in the progression of endocrine-related cancer, Peptides 28 (2007) 426 – 434

65. Ryder et al, Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer , Endocrine-Related Cancer (2008) 15 1069–1074

66. Sanchez, TI-RADS classification of thyroid nodules based on a score modified according to ultrasound criteria for malignancy, Rev. Argent. Radiol. 2014;78(3): 138-148

67. Sclumberger et al, Treatment and follow-up of low-risk patients with thyroid cancer, Nature Reviews Endocrinology, Aug 9, 2011

68. Sheahan et al, Follicular Variant of Papillary Thyroid Carcinoma Differences From Conventional Disease in Cytologic Findings and High-Risk Features, JAMA Otolaryngol Head Neck Surg. 2014;140(12):1117-1123

69. Shrestha et al, Cytomorphology of Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features and the Impact of New Nomenclature on Molecular Testing, Med. Sci. 2019, 7, 15

70. Soh and Aw, Laboratory Testing in Thyroid Conditions – Pitfalls and Clinical Utility, Ann Lab Med 2019; 39:3-14

71. Spencer et al, Clinical Impact of Thyroglobulin (Tg) and Tg Autoantibody Method Differences on the Management of Patients with Differentiated Thyroid Carcinomas, The Journal of Clinical Endocrinology & Metabolism 90(10):5566–5575, 2005

72. Spencer and LoPresti, Technology Insight: measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer, Nature Reviews Endocrinology, 12 Feb 2008

73. Spencer et al, Serum Thyroglobulin (Tg) Monitoring of Patients with Differentiated Thyroid Cancer Using Sensitive (Second-Generation) Immunometric Assays Can Be Disrupted by False-Negative and False-Positive Serum Thyroglobulin Autoantibody Misclassifications, J Clin Endocrinol Metab, December 2014, 99(12):4589–4599

74. Subbiah et al, Selective RET kinase inhibition for patients with RET-altered cancers, Annals of Oncology 0: 1–8, 2018

75. Tallini et al, The History of the Follicular Variant of Papillary Thyroid Carcinoma, J Clin Endocrinol Metab 102: 15–22, 2017

76. Todoro et al, Tumorigenic and Metastatic Activity of Human Thyroid Cancer Stem Cells, Cancer Res; 70(21) November 1, 2010

77. van der Graaf et al, Up to date with human thyroglobulin, Jrl of Endo, 2001.

78. Wartofsky and Van Nostrand, Thyroid Cancer, Springer (New York) 2016

79. Welch, H., G. Doherty, Saving Thyroids — Overtreatment of Small Papillary Cancers, NEJM, 379"4 July 2018

80. Wells and Santoro, Update: The Status of Clinical Trials With Kinase Inhibitors in Thyroid Cancer, J Clin Endocrinol Metab, May 2014, 99(5):1543–1555

81. Wells et al, Multiple Endocrine Neoplasia Type 2 and Familial Medullary Thyroid Carcinoma: An Update, J Clin Endocrinol Metab. 2013 Aug; 98(8): 3149–3164.

82. Wilson et al, Endocrinology 9th Ed, Saunders, 1998

83. Wondirsford and Radovick, Clinical Management of Thyroid Disease, Saunders (New York) 2009

84. Yoruker et al, MicroRNA Expression Profiles in Papillary Thyroid Carcinoma, Benign Thyroid Nodules and Healthy Controls, Journal of Cancer. 7(7) 2016

85. Zhu et al, Natural Killer Cell Based Therapy for Pulmonary Metastasis of Anaplastic Thyroid Cancer in a Nude Mouse Model, Frontiers in Immunology, July 2017

8 INDEX