

Laboratory Diagnosis and Surveillance of Thyroid Cancer

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12/21/22

Objectives

- After attending this presentation, the attendee should be able to:
 - Explain the presentation, workup, treatment, and prognosis of thyroid carcinomas.
 - Describe the laboratory tests used to monitor thyroid cancer treatment, recurrence, and the impact of certain autoantibodies on these assays.
 - Compare and contrast thyroid cancer testing strategies and their implications for healthcare resources.

Outline

- Introduction to our patients
- Thyroid anatomy, histology, and physiology
- Thyroid dysfunction and nodule presentation, workup and diagnosis
- Thyroid cancer types, epidemiology, etiology, prognosis, treatment, and surveillance
- Laboratory surveillance
- Patient Case

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Thyroid Cancer Survivor Stories

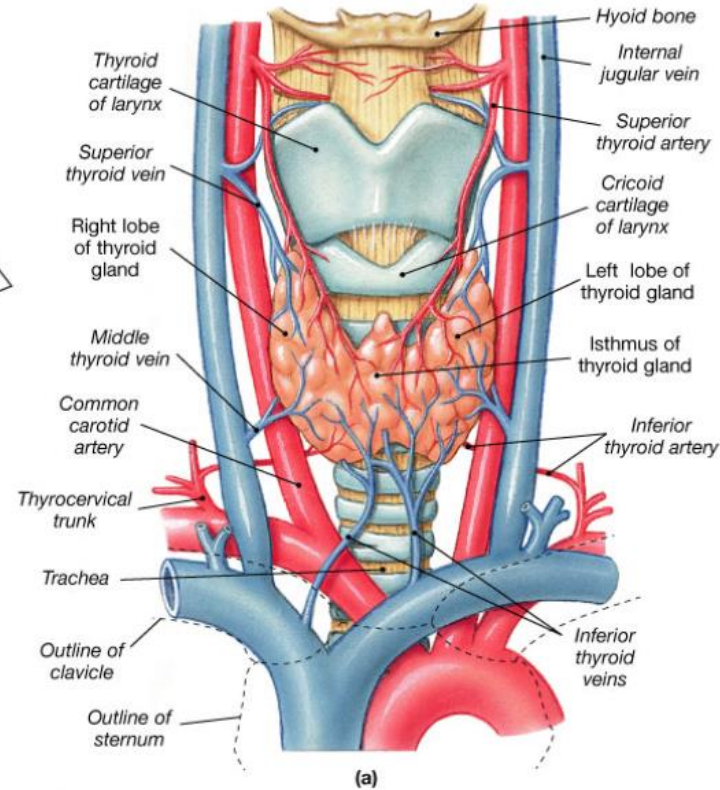
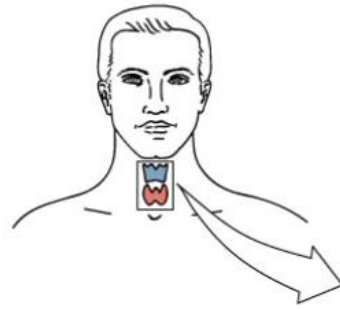
- Diagnosis
 - Fighting a battle with their illness
- Treatment
 - Comorbidity: physical, therapy related, and mental
 - Range of emotions when being faced with cancer
- Survivorship
 - New normal
 - Medications, lifestyle changes, uncertainty of recurrence
 - Surveillance for recurrence
 - Improving quality of life

Outline

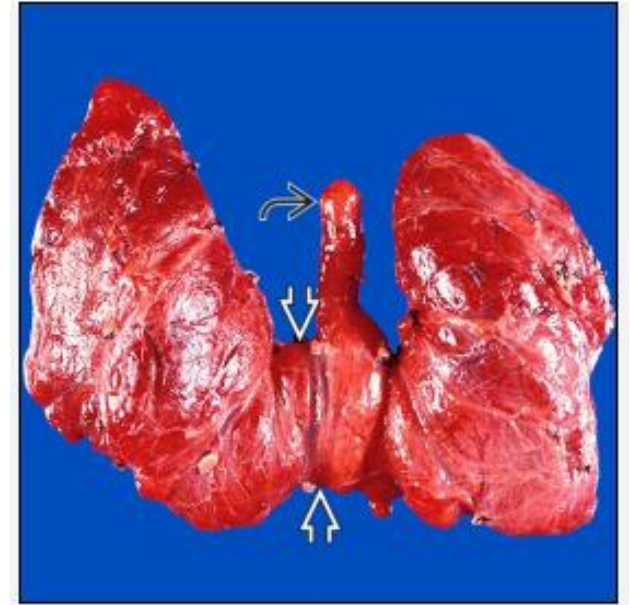
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Thyroid Anatomy

- Endocrine gland
 - Highly vascularized
- Anterior neck
 - Above clavicle
 - Below thyroid cartilage
- Butterfly-shaped
 - Lateral lobes
 - Central isthmus
 - Pyramidal lobe

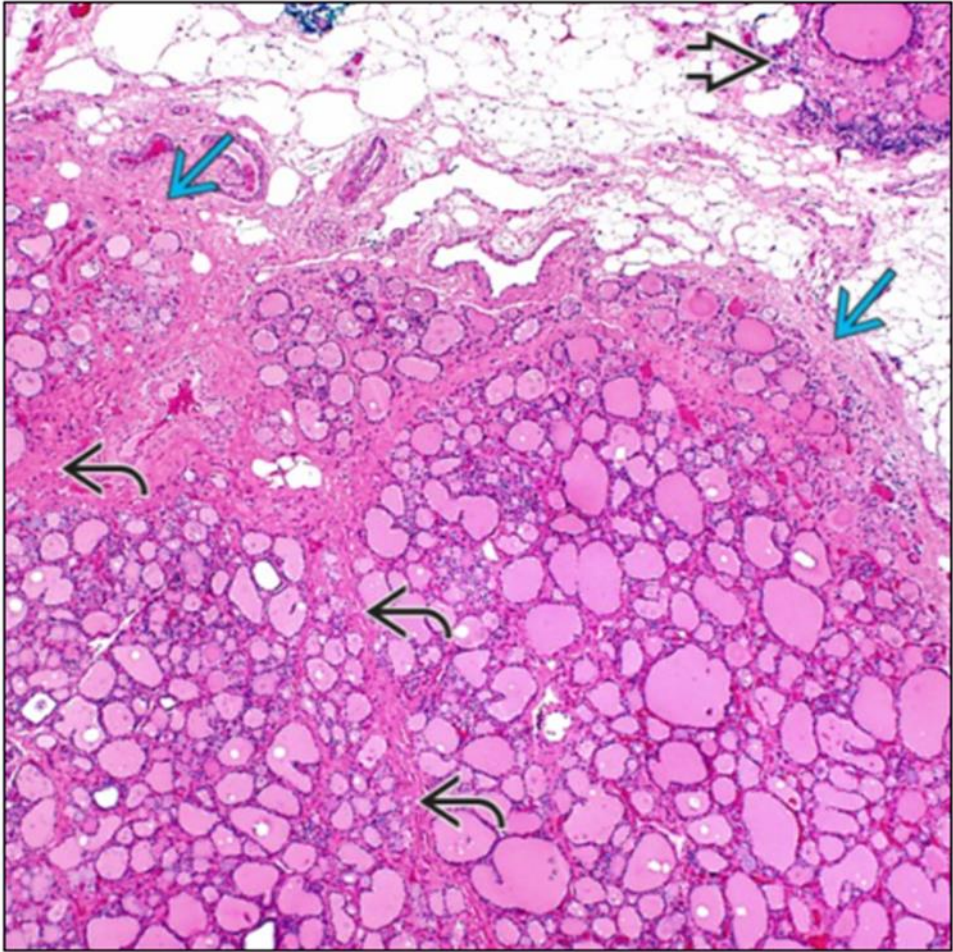


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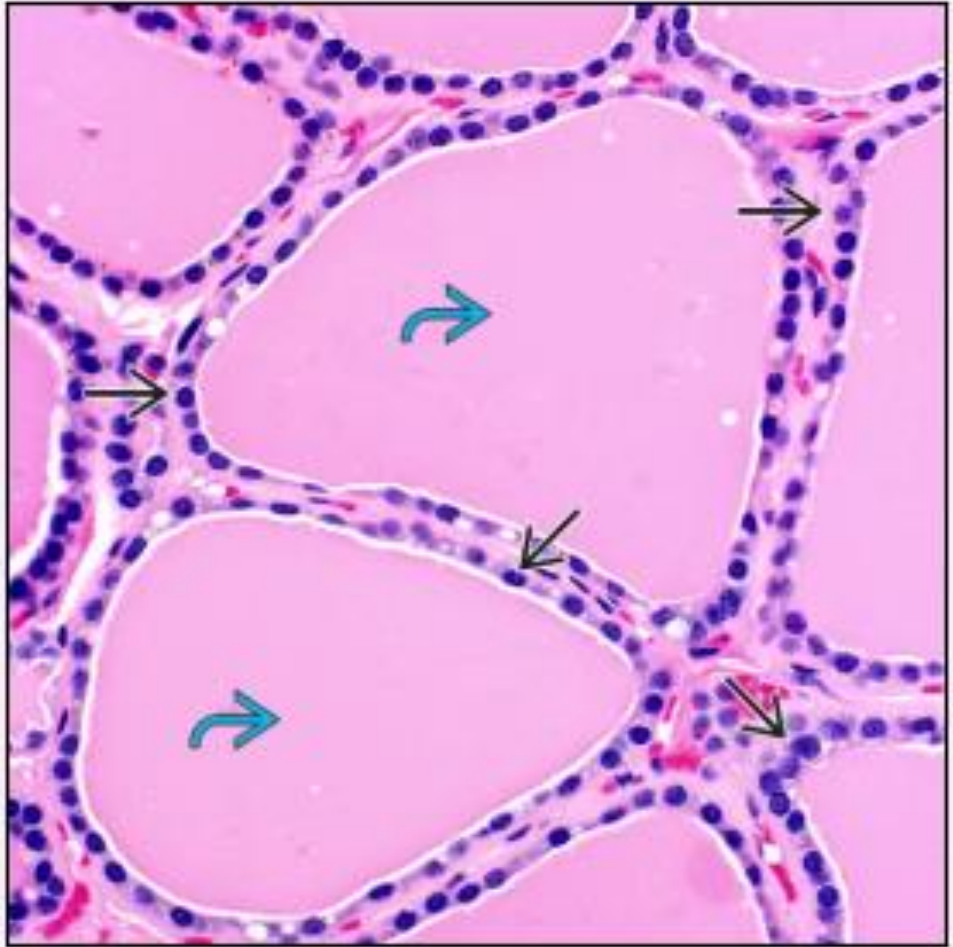


<https://app.expertpath.com/document/thyroid/>

Thyroid Histology



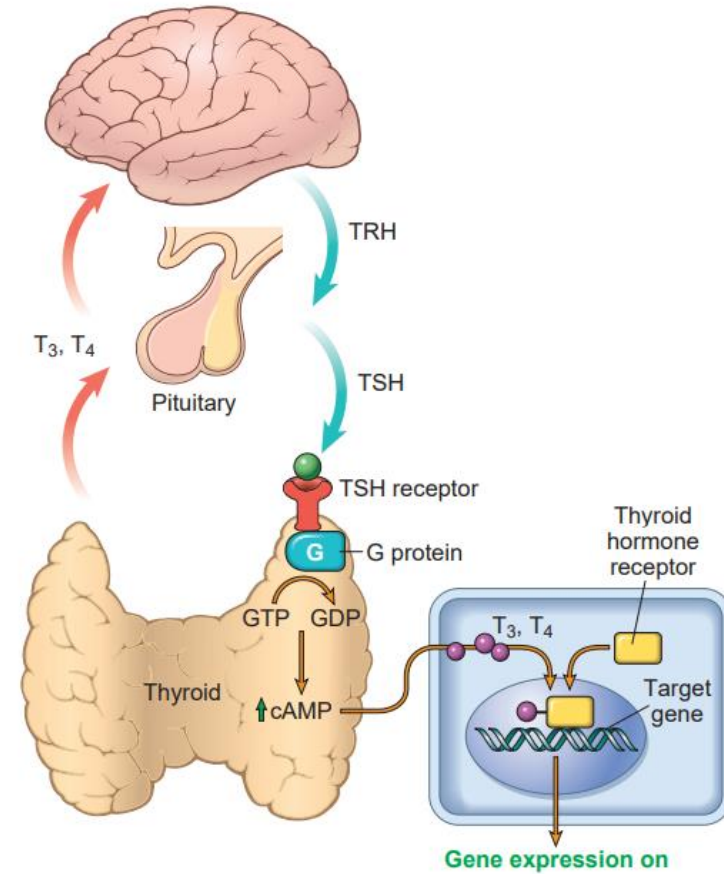
<https://app.expertpath.com/document/thyroid/>



<https://app.expertpath.com/document/thyroid/>

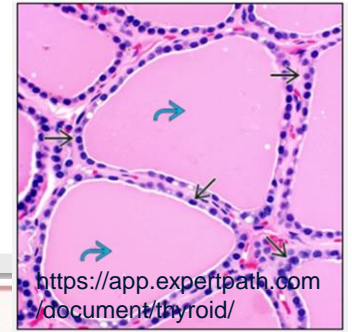
Hypothalamus-Pituitary-Thyroid Axis

- Hypothalamus
 - Thyrotropin releasing hormone (TRH)
- Pituitary Gland
 - Thyroid stimulating hormone (TSH)
- Thyroid Gland
 - Triiodothyronine (T3)
 - 15%, highly active
 - Thyroxine/tetraiodothyronine (T4)
 - 85%, converted to T3 in periphery
- Feedback mechanism

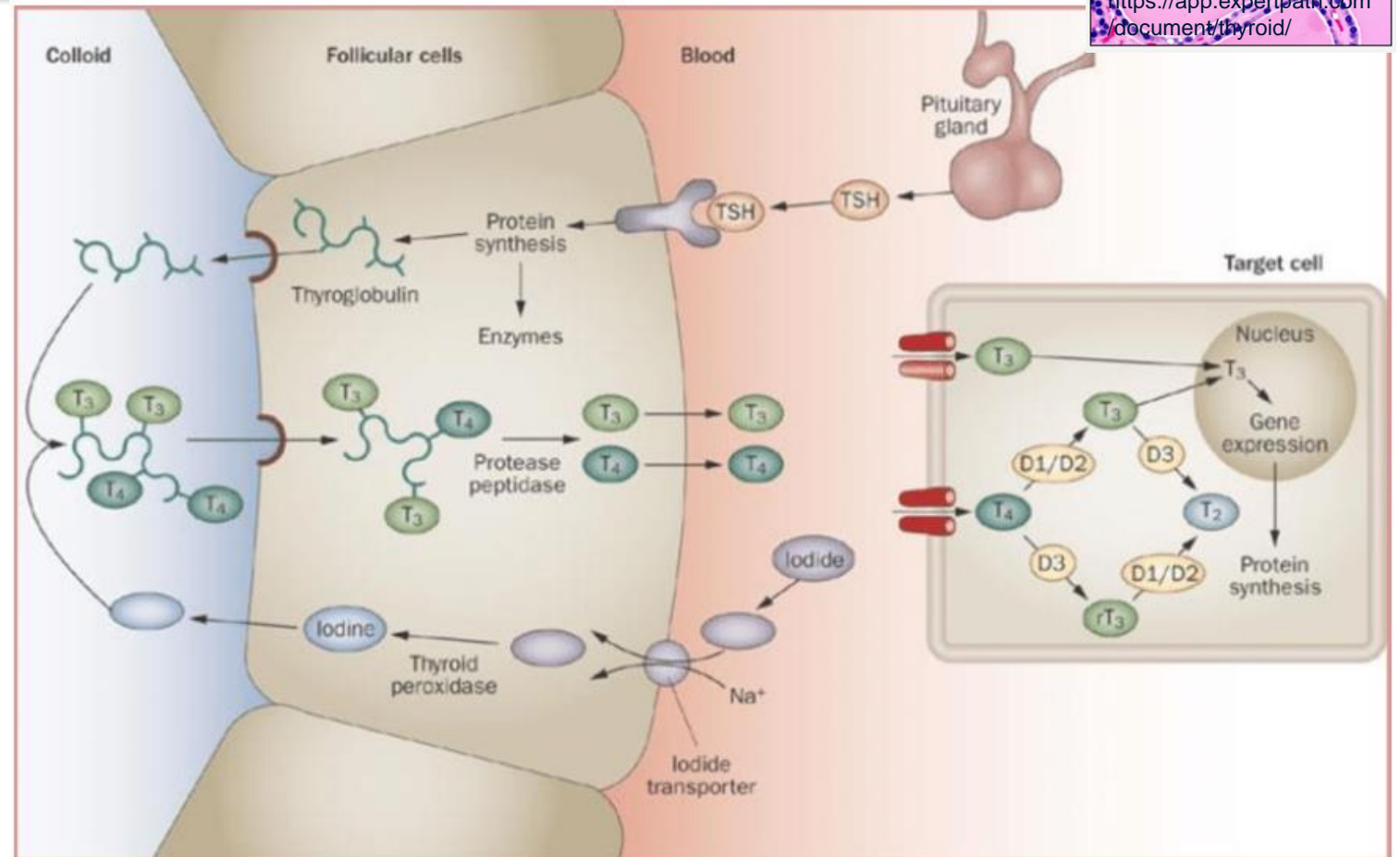


ROBBINS AND COTRAN, Ninth Edition. ISBN: 978-1-4557-2613-4

Thyroid Hormone Synthesis



- TSH acts on follicular cell
 - Synthesis of thyroglobulin (Tg)
 - Uptake of iodide → iodine
- Iodination and coupling in colloid
 - Iodination of tyrosine on Tg
 - Monoiodotyrosine (MIT)
 - Diiodotyrosine (DIT)
 - Coupling MIT and DIT
 - Formation of T₃ and T₄
- Resorption into follicular cell
 - Vesicles fuse with lysosomes
- Secretion of T₃ and T₄ in circulation
 - Target cell deiodination and action



Synthesis of thyroid hormones. Lehman et al. 2010

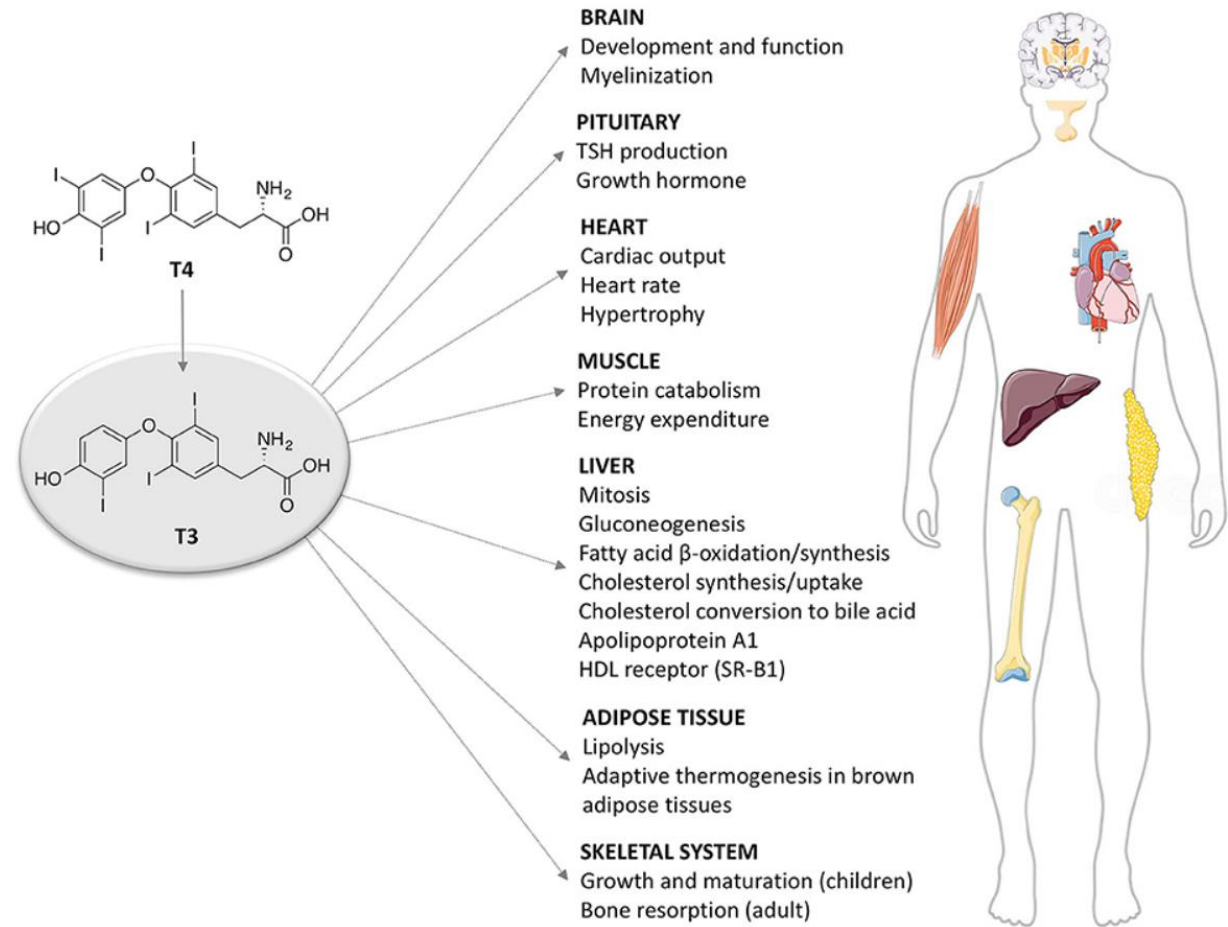
Physiology of T4 and T3

- T4 > T3, and bound to plasma proteins
 - Thyroxine binding globulin (TBG)
 - Transthyretin (TTR)
 - Albumin

TABLE 55-3 Thyroid Hormone Transport in Plasma

TBP	TBG	TTR	Albumin
Concentration	4-5.4 mg/dL	10-20 mg/dL	3.5-5 g/dL
Affinity for T ₄	High	Modest	Low
T ₄ capacity, mcg/dL	22	120	1000
Distribution			
T ₄	67%	20%	13%
T ₃	53%	1%	46%

TIETZ TEXTBOOK. ISBN: 978-1-4160-6164-9



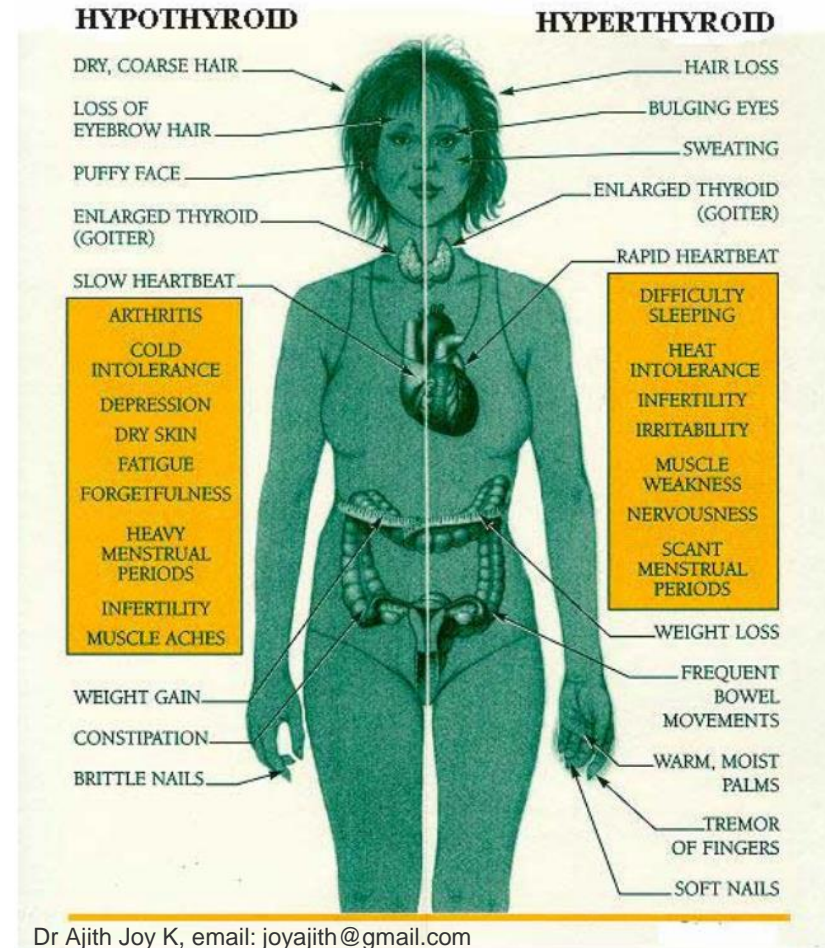
Saponaro et al. Frontiers in Medicine. 2020

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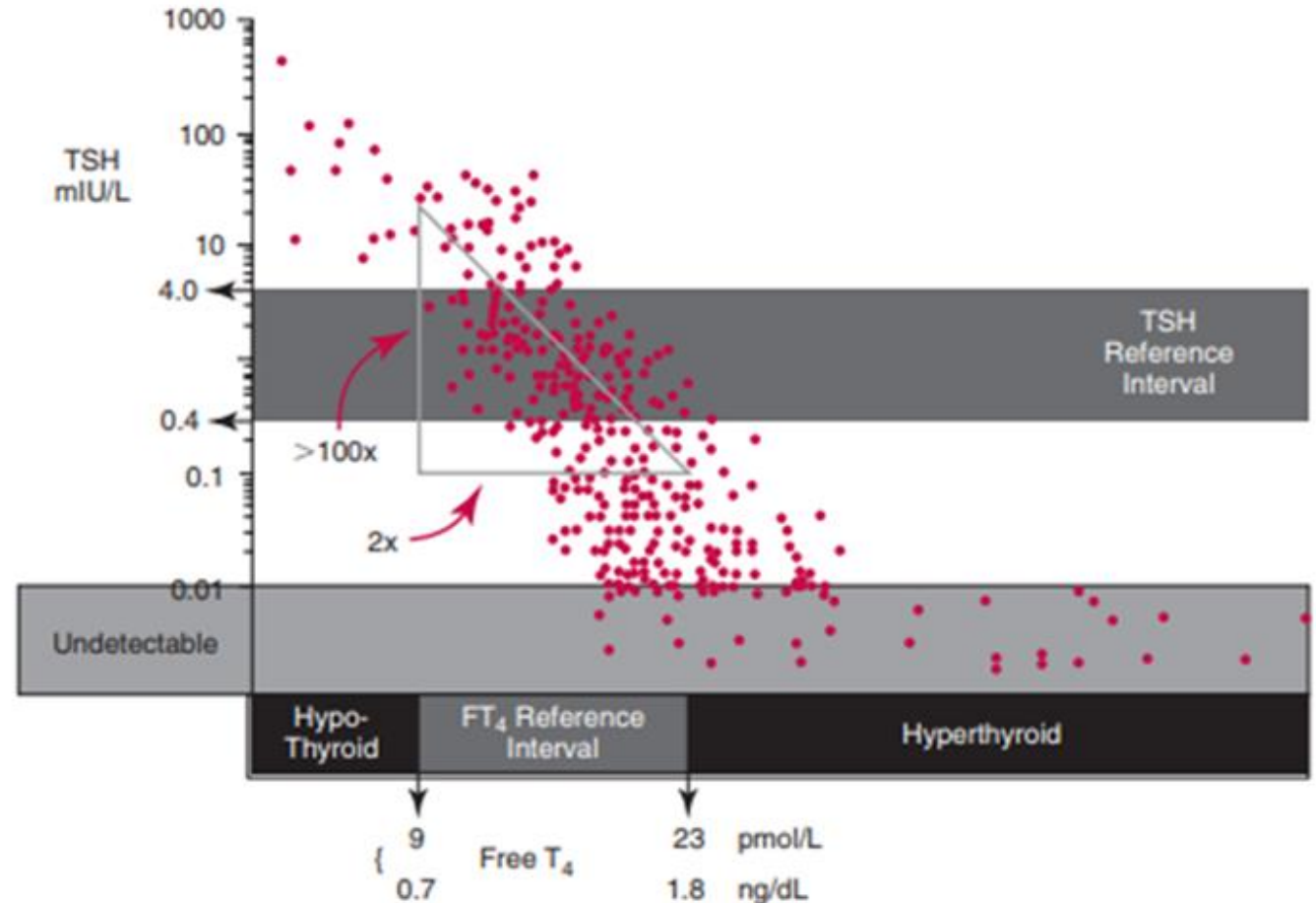
Thyroid Dysfunction

- Hypothyroidism
 - Low circulating thyroid hormone
 - High thyroid hormone stimulation
 - Hashimoto's , many other causes
- Hyperthyroidism
 - High circulating thyroid hormone
 - Low thyroid hormone stimulation
 - Graves', many other causes
- Goiter
 - Enlarged thyroid



Circulating TSH Free T4 (FT4)

- Primary markers to workup disorder
- TSH and FT4 relationship
 - Hypothyroidism
 - Low FT4 and High TSH
 - Hyperthyroidism
 - High FT4 and Low TSH
 - Reference interval determination
 - Thyroid disease unlikely
 - FT4 and TSH normal

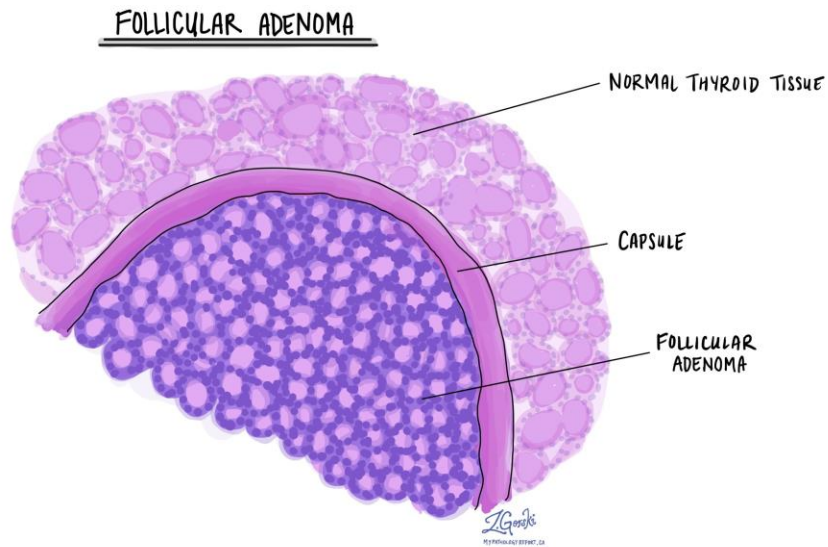


TIETZ TEXTBOOK. ISBN: 978-1-4160-6164-9

Thyroid Nodules

- Adenoma

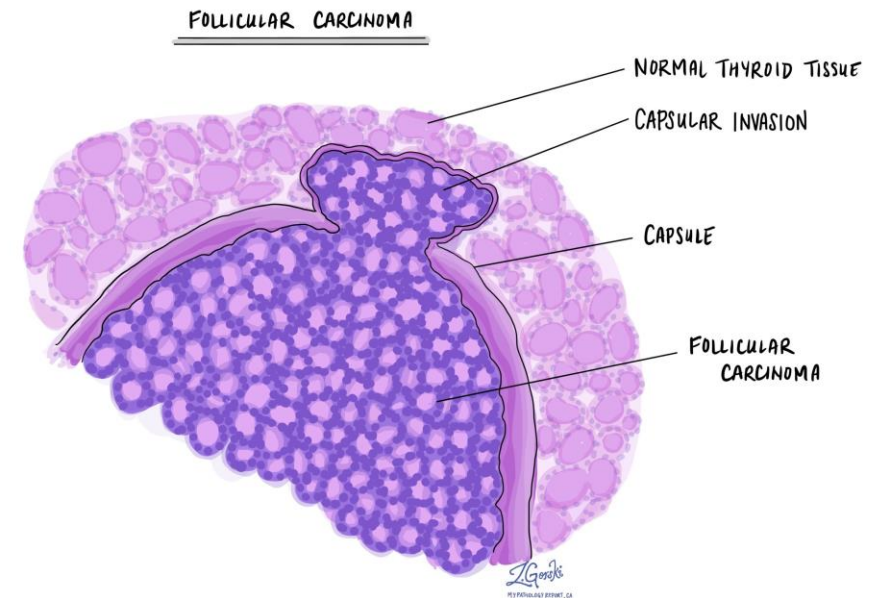
- Non-cancerous proliferation
- Encapsulated



Follicular adenoma of the thyroid gland. Jason Wasserman MD. 2022.

- Carcinoma

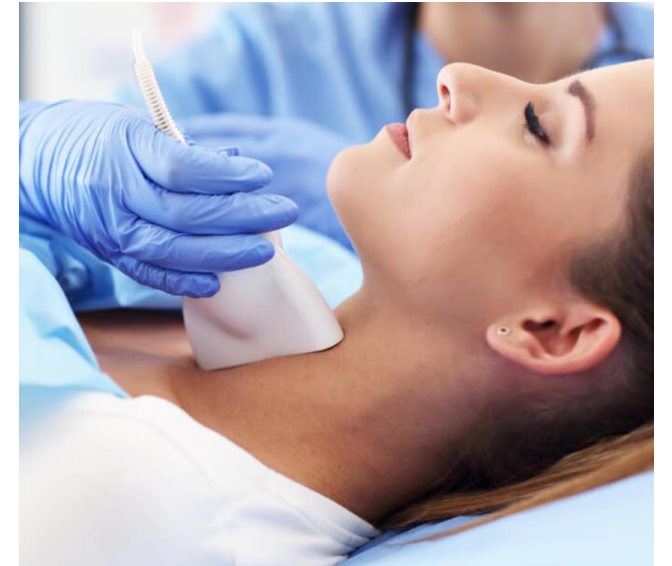
- Cancerous proliferation
- Invasive



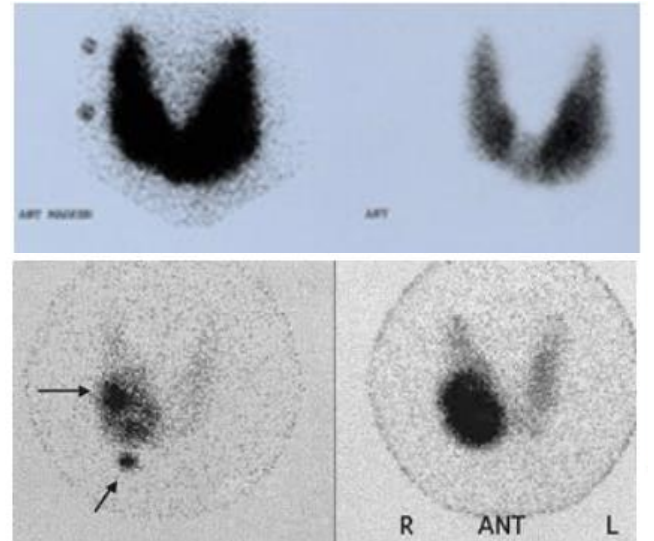
Follicular adenoma of the thyroid gland. Jason Wasserman MD. 2022.

Thyroid Nodule Testing

- TSH, Ultrasound (US) and Fine Needle Aspiration (FNA)
 - TSH Normal
 - US suspicious → FNA
 - Size >1cm, irregular borders, taller than wide, invasion
 - TSH Increased → work up hypothyroid
 - TSH Decreased → work up hyperthyroid
 - **Radionuclide scanning/radioiodine imaging**
 - Hot nodules (rarely malignant)
 - Not hyperfunctioning → FNA



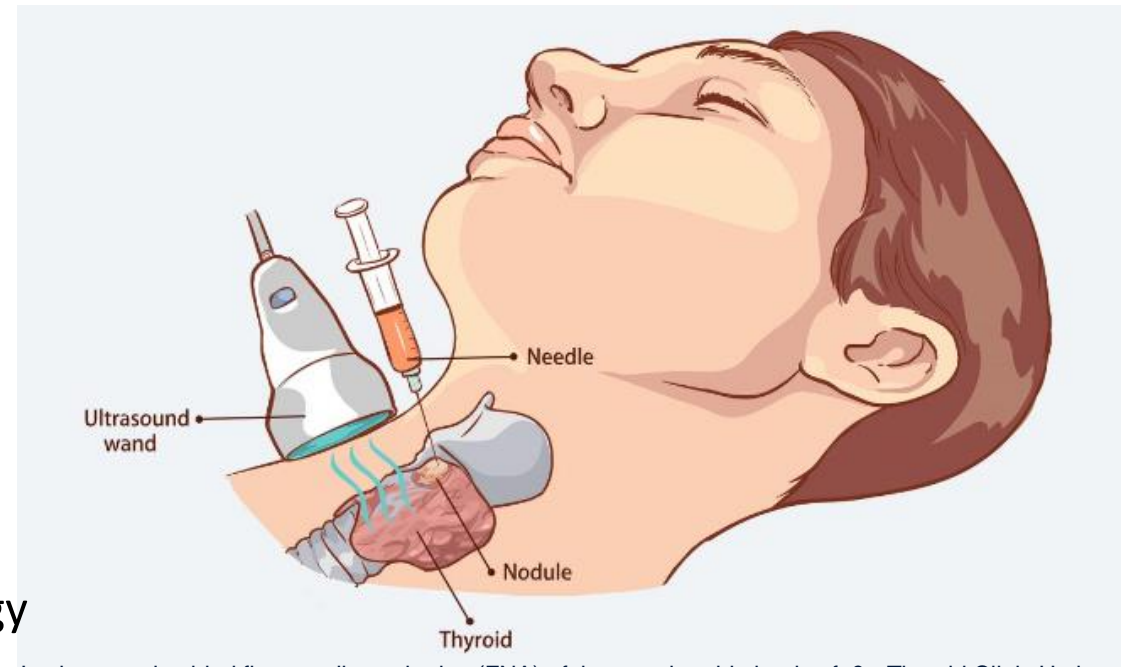
Thyroid Ultrasound | Carreras Medical Center (toplinemd.com)



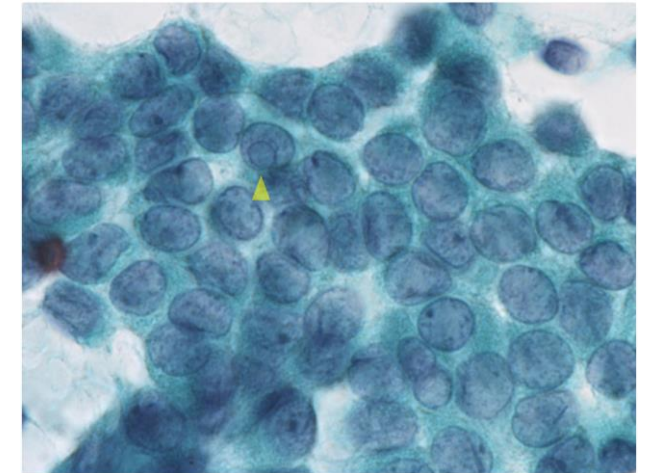
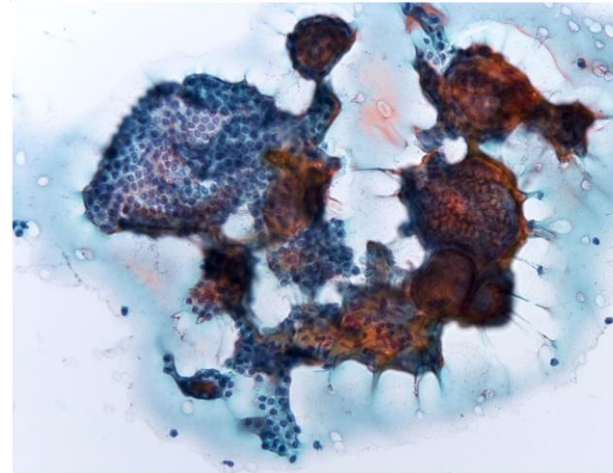
Radioactive Iodine | Columbia Surgery

US Guided FNA

- Minimally invasive
 - Targeted cell population
- Adequacy
 - ≥ 6 groups of follicular cells with ≥ 10 cells per group
- The Bethesda System for Reporting Thyroid Cytology
 - Nondiagnostic (ND)/Unsatisfactory
 - Benign
 - Atypia of undetermined significance (AUS)
 - Follicular neoplasm (FN)
 - Suspicious for malignancy (SM)
 - Malignant/positive for malignancy

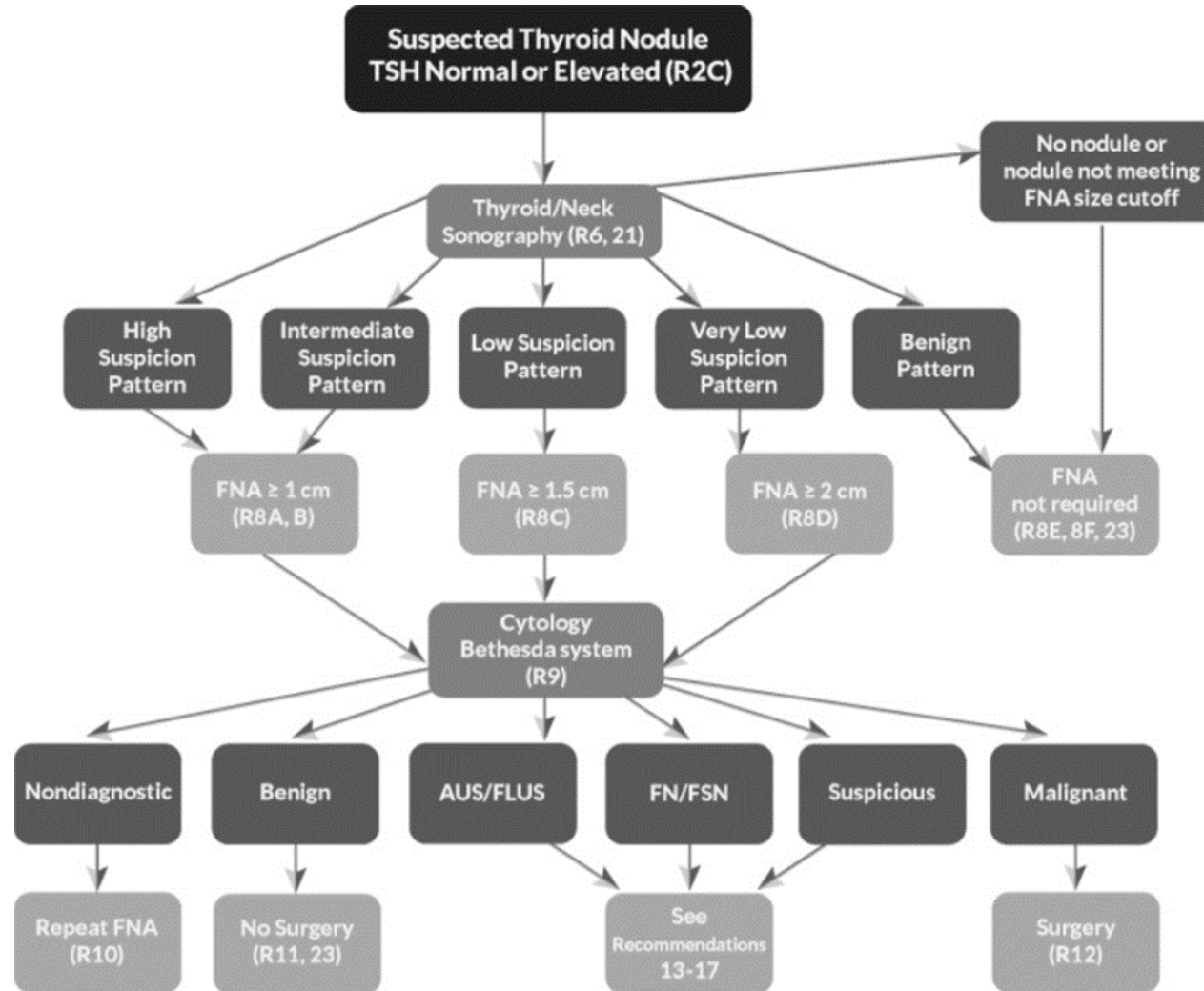


[Is ultrasound guided fine needle aspiration \(FNA\) of the parathyroid gland safe? - Thyroid Clinic Utah](#)



<https://www.pathologyoutlines.com/topic/thyroiddiagnostic.html>

Thyroid Nodule Diagnostic Algorithm

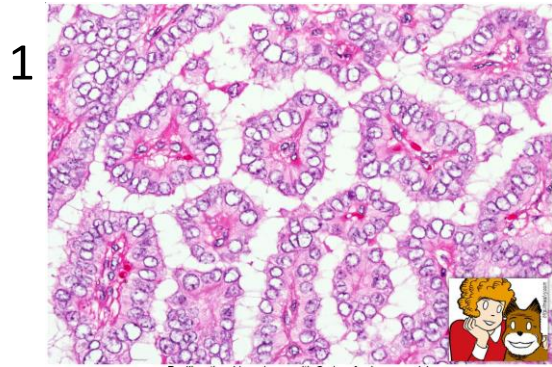


Haugen BR, et al. American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. 2015.

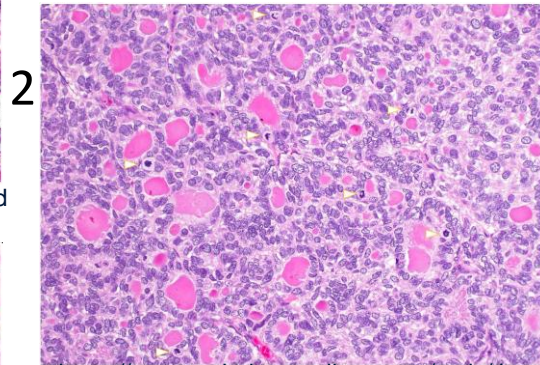
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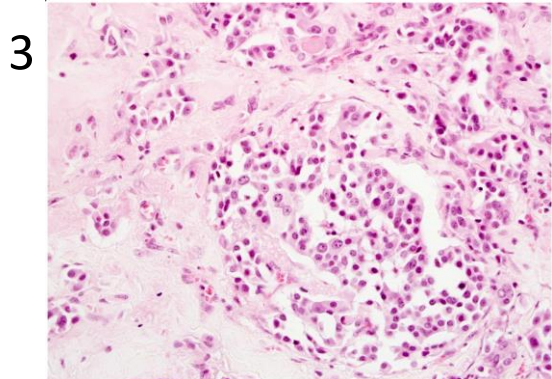
Types of Thyroid Carcinoma



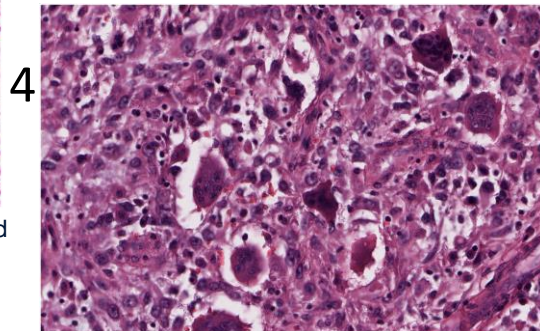
<https://www.pathologyoutlines.com/topic/thyroidpapillary.html>



<https://www.pathologyoutlines.com/topic/thyroidfollicular.html>



<https://www.pathologyoutlines.com/topic/thyroidmedullary.html>



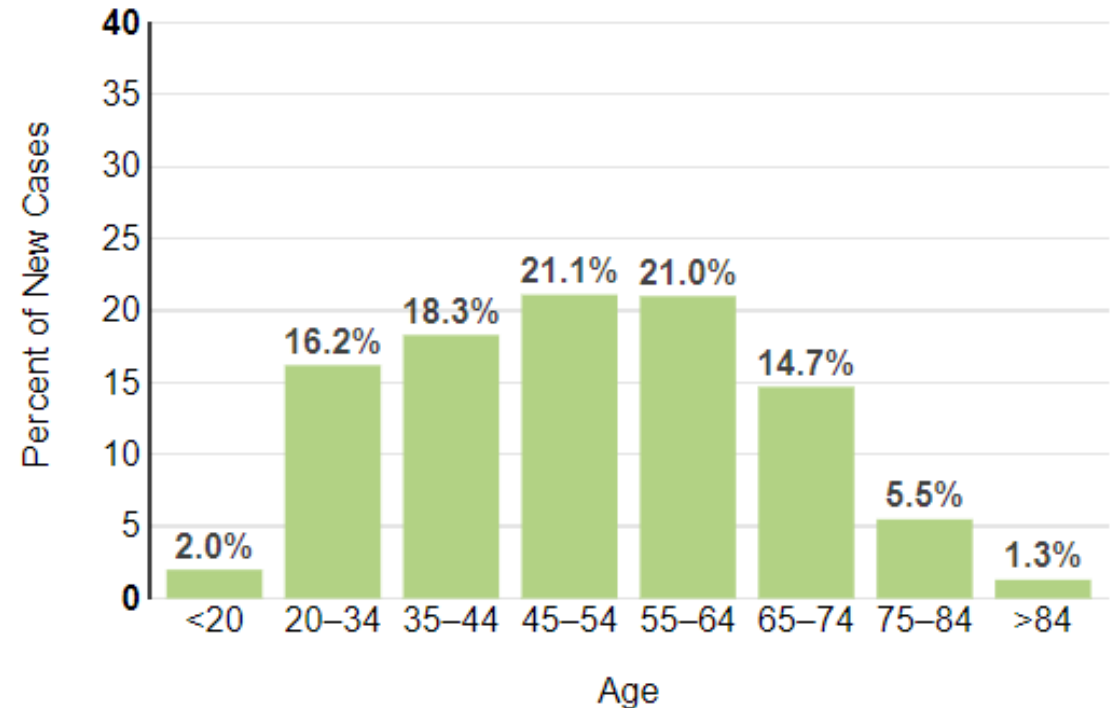
<https://www.pathologyoutlines.com/topic/thyroidUndiff.html>

	Carcinoma	Frequency*	Pathology and Spread
1	Papillary carcinoma	Approximately 80%	Solitary or multifocal lesions with papillary structures and ground glass "empty" nuclei; preferentially lymphatic spread
2	Follicular carcinoma	Approximately 15%	Infiltrative follicular structures without ground glass nuclei; preferentially hematogenous spread
3	Medullary carcinoma originate from C cells	Up to 5%	Solitary or multifocal lesions with pale round or spindle cells and stromal amyloid deposits; hematogenous and lymphatic spread
4	Anaplastic carcinoma	Rare	Highly anaplastic pleomorphic with giant cells or spindle cells, sarcomatous appearance; rapid hematogenous metastases

Netter's Illustrated Human Pathology Second Edition ISBN: 978-0-323-22089-7

Thyroid Cancer Statistics

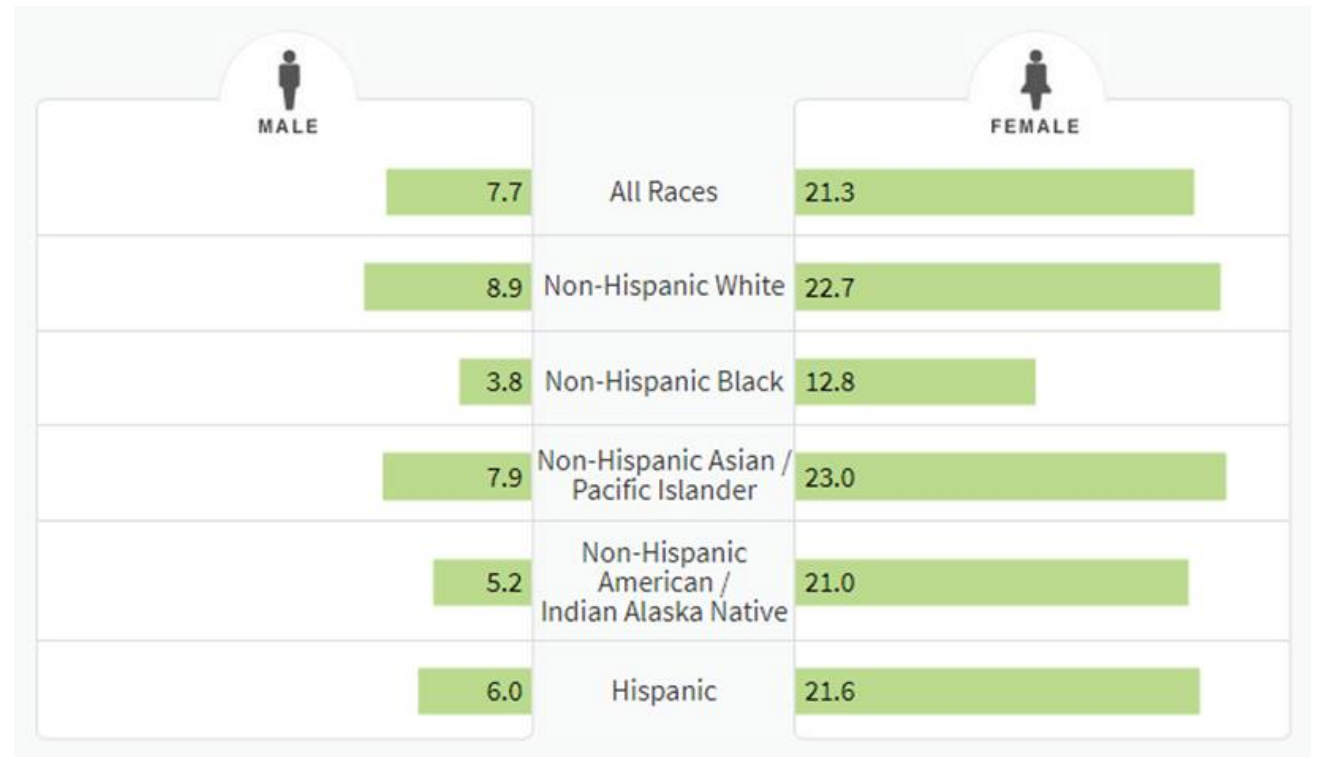
- Endocrine organ most commonly affected by primary malignancy
- Relatively uncommon compared to other cancers ranking 12th most common for 2022
 - 43,800 people with new diagnosis (11,860 men and 31,940 women)
 - 2.3% of all new cancer cases
 - 2,230 deaths (1,070 men and 1,160 women)
 - 0.4% of all cancer deaths
 - 915,664 patients living with thyroid cancer in the United States (2019)
 - Median age of diagnosis is 51 (2015-2019)



<https://seer.cancer.gov/statfacts/html/thyro.html>

Thyroid Cancer Risk Factors

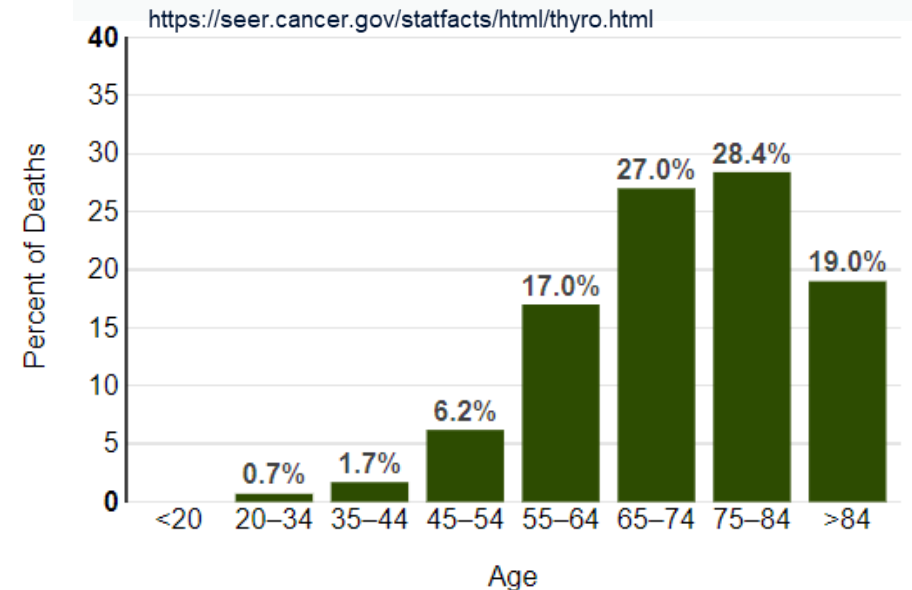
- Radiation exposure
 - Head or neck during childhood
 - Treating other cancers
 - Fallout
 - Chernobyl in 1986 or Fukushima in 2011
- Family history
- Age 25-65
- Female gender
- History of goiter or thyroid disease
- Genetic conditions



<https://seer.cancer.gov/statfacts/html/thyro.html>

Thyroid Cancer Prognosis

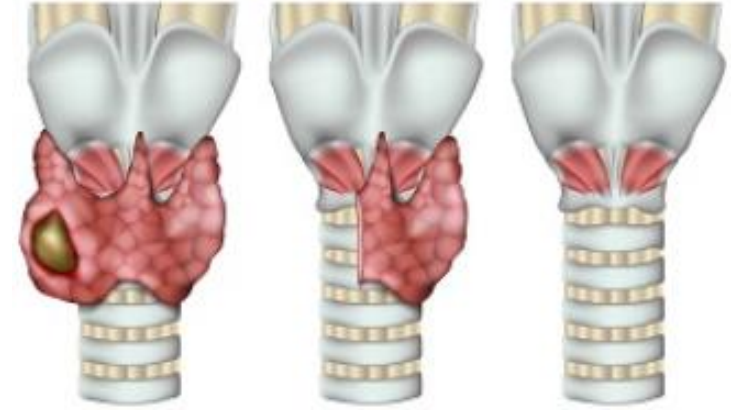
- Dependent on several factors:
 - Age
 - Type of thyroid cancer
 - Stage of cancer
 - Complete removal by surgery
 - Genetic conditions
 - General health
 - Recurrence



<https://seer.cancer.gov/statfacts/html/thyro.html>

Thyroid Cancer Treatment

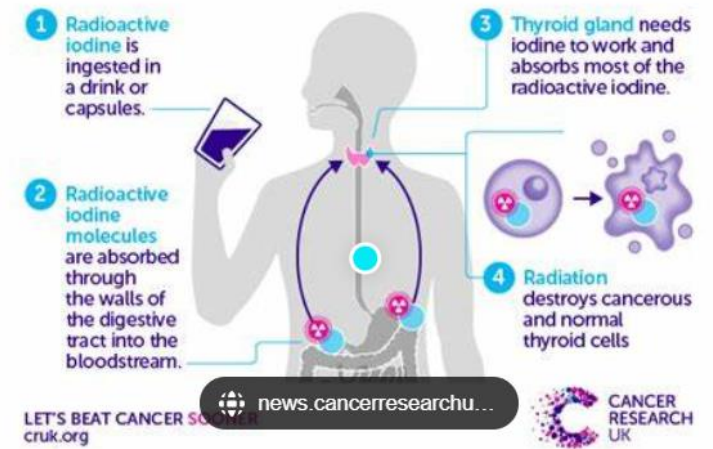
- Standard Treatment
 - Surgery
 - Radiation therapy, including radioactive iodine (RAI) ablation
 - Chemotherapy
 - Thyroid hormone therapy
 - Targeted therapy
 - Watchful waiting or active surveillance
- Clinical trials
- Side effects



<https://www.nursingce.com/ceu-courses/thyroid-dysfunction>

USING RADIOACTIVE LIQUID THERAPY TO TREAT THYROID CANCER

Iodine therapy specifically targets the thyroid and has very little effect on other parts of the body.



<https://news.cancerresearchuk.org/2017/10/24/internal-radiotherapy-tackling-cancer-from-within/>

Thyroid Cancer Follow-up

- Clinical surveillance
 - History and physical exam
 - Neck ultrasound
 - Whole-body radioiodine (I^{131}) scan
 - High risk patients
- Laboratory surveillance
 - Hormone therapy suppresses TSH
 - TSH levels monitored to determine if hormone treatment dose is correct
 - Routine thyroglobulin (Tg) testing
 - Produced by normal thyroid and **cancer cells**



<https://www.windsor.edu/biomedical-sciences/index.php/2018/04/04/becoming-primary-care-physician/>



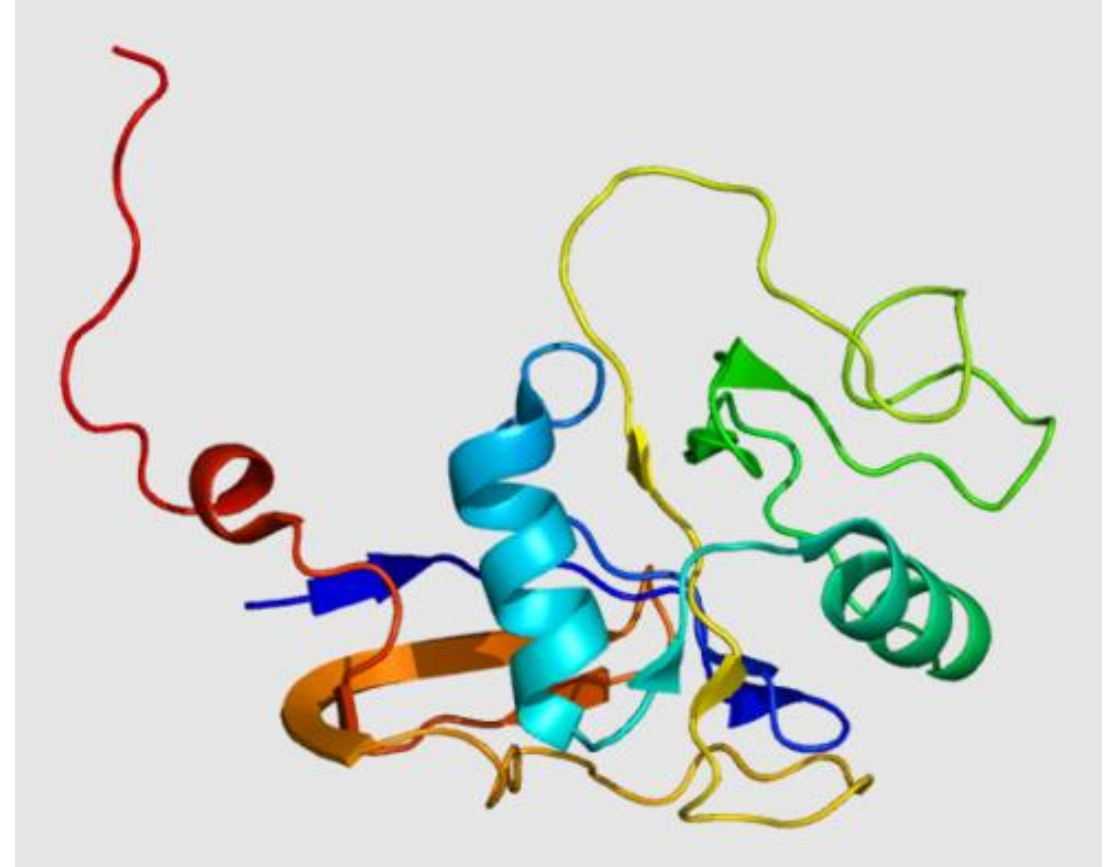
<https://time.com/tag/medication/>

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Thyroglobulin (Tg)

- 660 kD glycoprotein dimer prohormone produced by the follicular cells
 - Specific to thyroid follicular cells
 - Normally low level in serum
 - Increased in thyroid carcinoma
- Levels used as a primary tumor marker
 - Differentiated thyroid carcinomas
 - Every 6-12 months
 - Should be undetectable following treatment
- Several testing methodologies



<https://go.drugbank.com/drugs/DB01584>

Access Thyroglobulin Assay

- Chemiluminescent assay (CIA)
- One-step immunoenzymatic sandwich assay
 - Sample (serum or plasma) incubated with
 - Biotinylated mixture of anti-Tg antibodies
 - Streptavidin coated paramagnetic particles
 - Monoclonal anti-Tg antibody alkaline phosphatase conjugate
 - Wash and Chemiluminescent substrate added
 - Light generated proportional [Tg]
 - Known interference
 - Heterophile antibodies
 - Serum autoantibodies to thyroglobulin (TgAb)

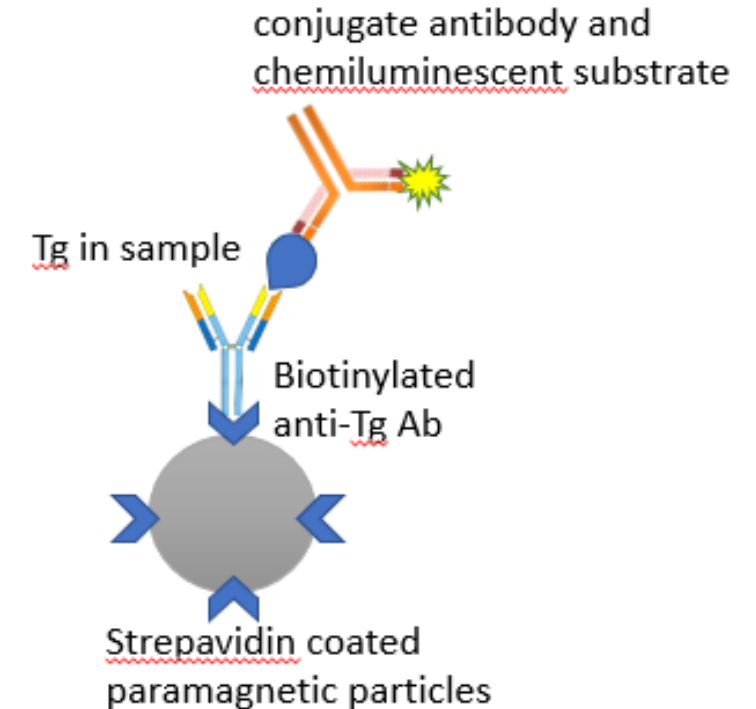


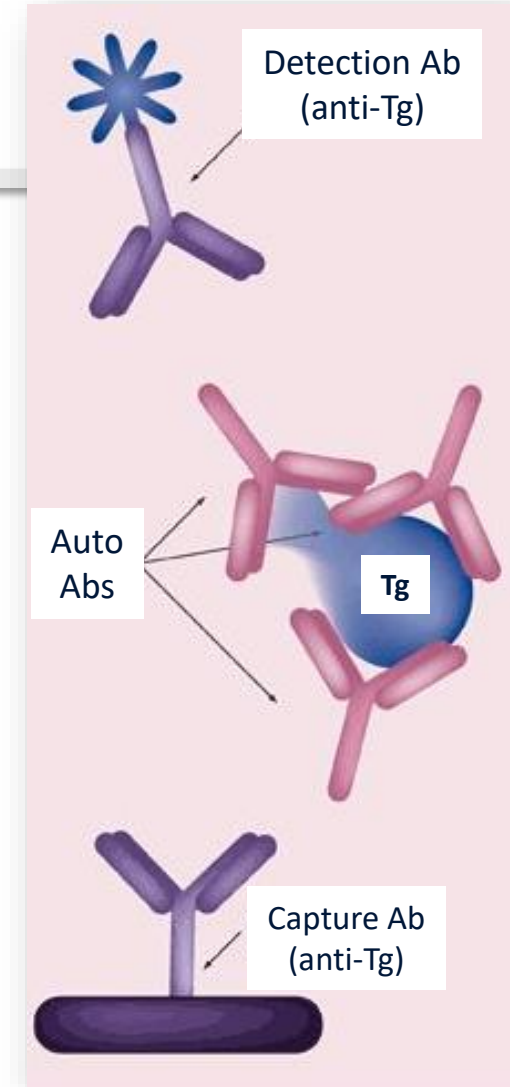
Image courtesy Clinical Chemistry Fellow Presentations

Antithyroglobulin autoantibodies (TgAb)

- Often present in patients with:
 - Thyroid Cancer
 - 20%¹
 - Autoimmune thyroid disease
 - 29-65% of Graves' patients¹
 - 50-86% of Hashimoto's patients¹
 - 10% of healthy individuals¹
- Simultaneous measurement of TgAb with Tg
 - Standard practice
 - Aids interpretation
- TgAb alone can increase with cancer recurrence
 - Lack standardization

TgAb CIA Interference

- Approximately 25% of thyroid cancer patients have antibodies against Tg (TgAb)¹
- TgAb may interfere with Tg immunoassays
 - Falsely low Tg

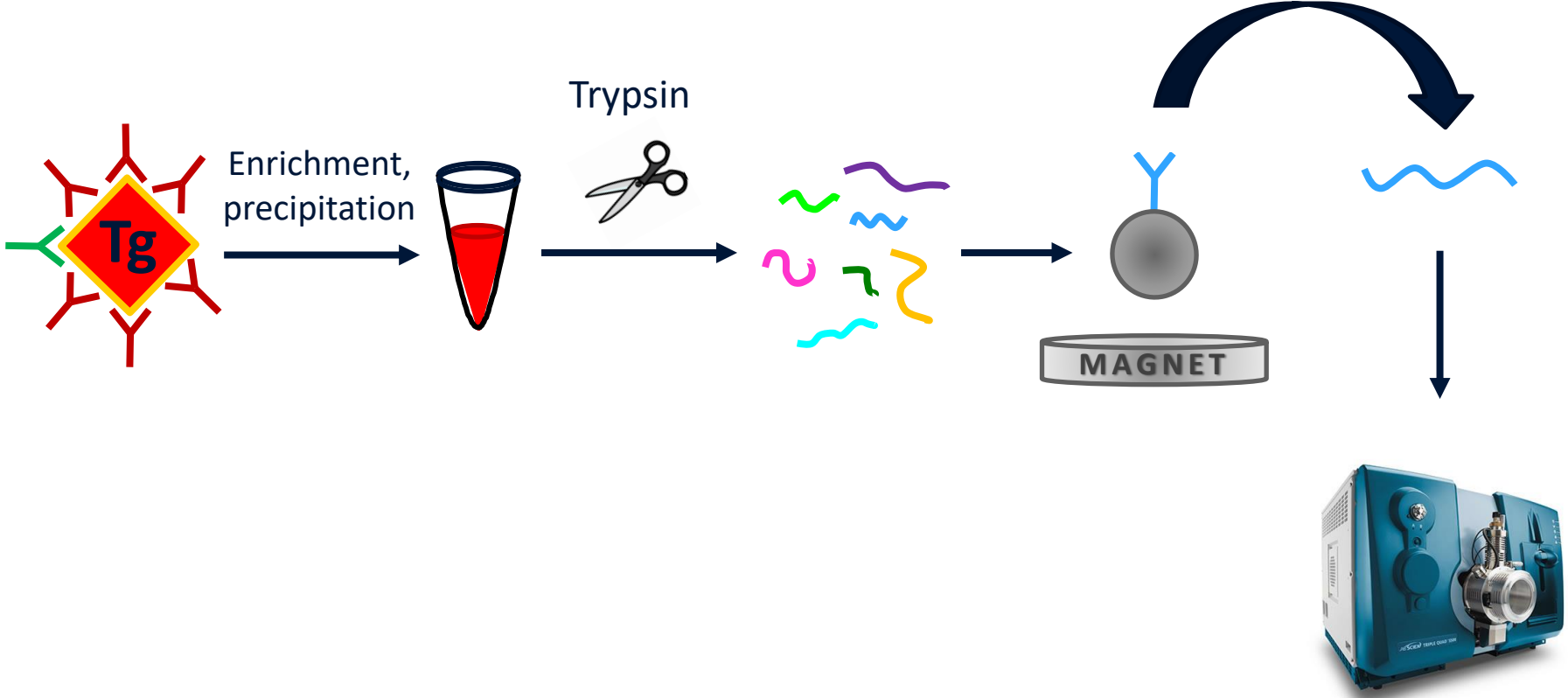


Source: Expert Rev Endocrinol Metab © 2009 Expert Reviews Ltd

Access Thyroglobulin Antibody II Assay

- Two-step immunoenzymatic sandwich assay
- Step 1:
 - Specimen in reaction vessel
 - Paramagnetic particles coated with Tg
 - Sample TgAb binds Tg
 - Incubation
 - Bound to solid phase magnetic field
 - Unbound material washed away
- Step 2:
 - Thyroglobulin-alkaline phosphatase conjugate added
 - Binds TgAb
 - Incubation and wash
 - Chemiluminescent substrate added
 - Lumi-Phos* 530
 - Light generated
 - Directly proportional to [TgAb]

Tg by LC-MS/MS



Cost Savings and Lab Utilization

AJCP / ORIGINAL ARTICLE

Thyroglobulin Antibody Screen Prior to Mass Spectrometry Provides Measurable Cost Savings and Optimal Laboratory Utilization

Jennifer L. Powers, PhD, Frederick G. Strathmann, PhD, and Joely A. Straseski, PhD

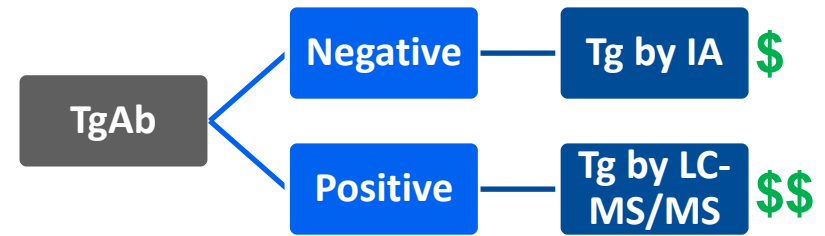
From the Department of Pathology, University of Utah, Salt Lake City.

Key Words: Thyroglobulin; Autoantibody; Test utilization; Cost savings; Reflex testing; Mass spectrometry

Am J Clin Pathol March 2017;147:309-314

DOI: 10.1093/AJCP/AQW228

Reflexive Workflow: Thyroglobulin (Tg)



- Analysis of Tg testing patterns (> 100,000 orders)
 - 89% utilized reflex testing
 - 89% were TgAb negative
 - Reflex to IA vs. MS directly saved > \$3 million*
 - If ordered separately (TgAb + Tg by LC-MS/MS)
 - Reflex would save almost \$9 million*
 - 11% ordered TgAb and Tg by LC-MS/MS separately
 - 90% were TgAb negative
 - Reflex (to IA) would have saved almost \$250,000*

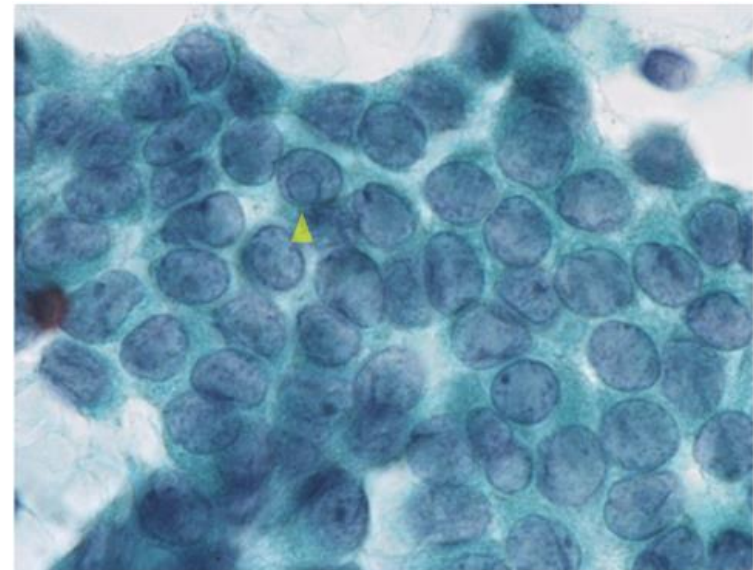
* Cost analysis based on average list price from 3 laboratories

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Case Study

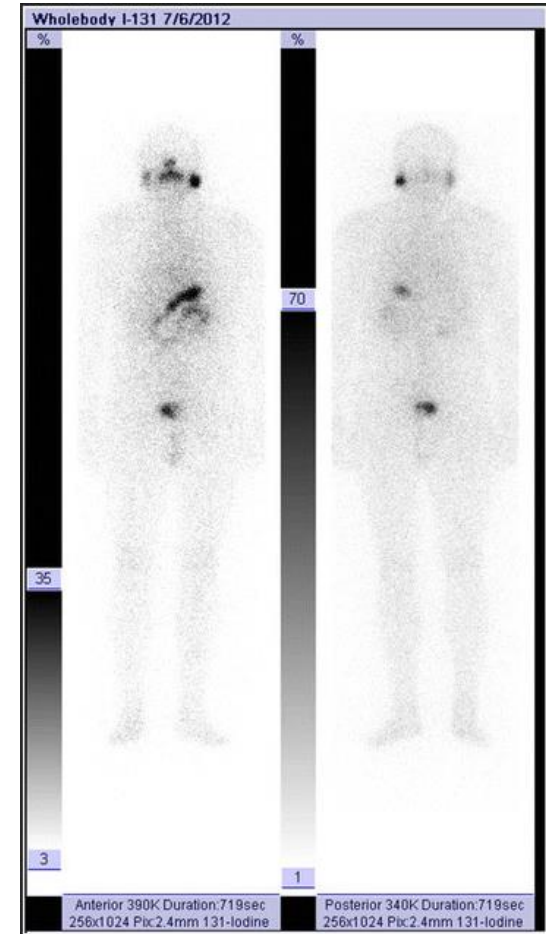
- 46-year-old female with painless, asymmetric neck mass detected on routine physical exam
- Ultrasound detection of 3.2 cm mass in right thyroid and 2 suspicious lymph nodes
- Laboratory Data:
 - TSH: 1.5 mU/L (0.27-4.20 mU/L)
 - Thyroxine: 6.7 μ g/dL (4.50-11.70 μ g/dL)
 - FT4: 1.1 ng/dL (0.7-1.3 ng/dL)
- Fine needle aspiration
 - Malignant



<https://www.pathologyoutlines.com/topic/thyroiddiagnostic.html>

Case Study Continued

- Papillary thyroid carcinoma
- Total thyroidectomy with lymph node dissection in 2021
- Radioactive iodine ablation
- Thyroid cancer surveillance
 - History and physical negative
 - Imaging US and whole-body scan following ^{131}I (negative)
 - Laboratory tumor markers Tg and TgAb



[Negative whole body scan \(WBS\) 24 h after radioiodine I-131 showing no... | Download Scientific Diagram \(researchgate.net\)](#)

Patient Results

- Surveillance labs:

- TgAb

- Negative

- Tg

- Within reference interval

- No reflex

- Clinical history

Date/Test	Marker	Result	Reference Interval
8/30/2021	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
Tg w/reflex	Tg (CIA)	15.5 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL

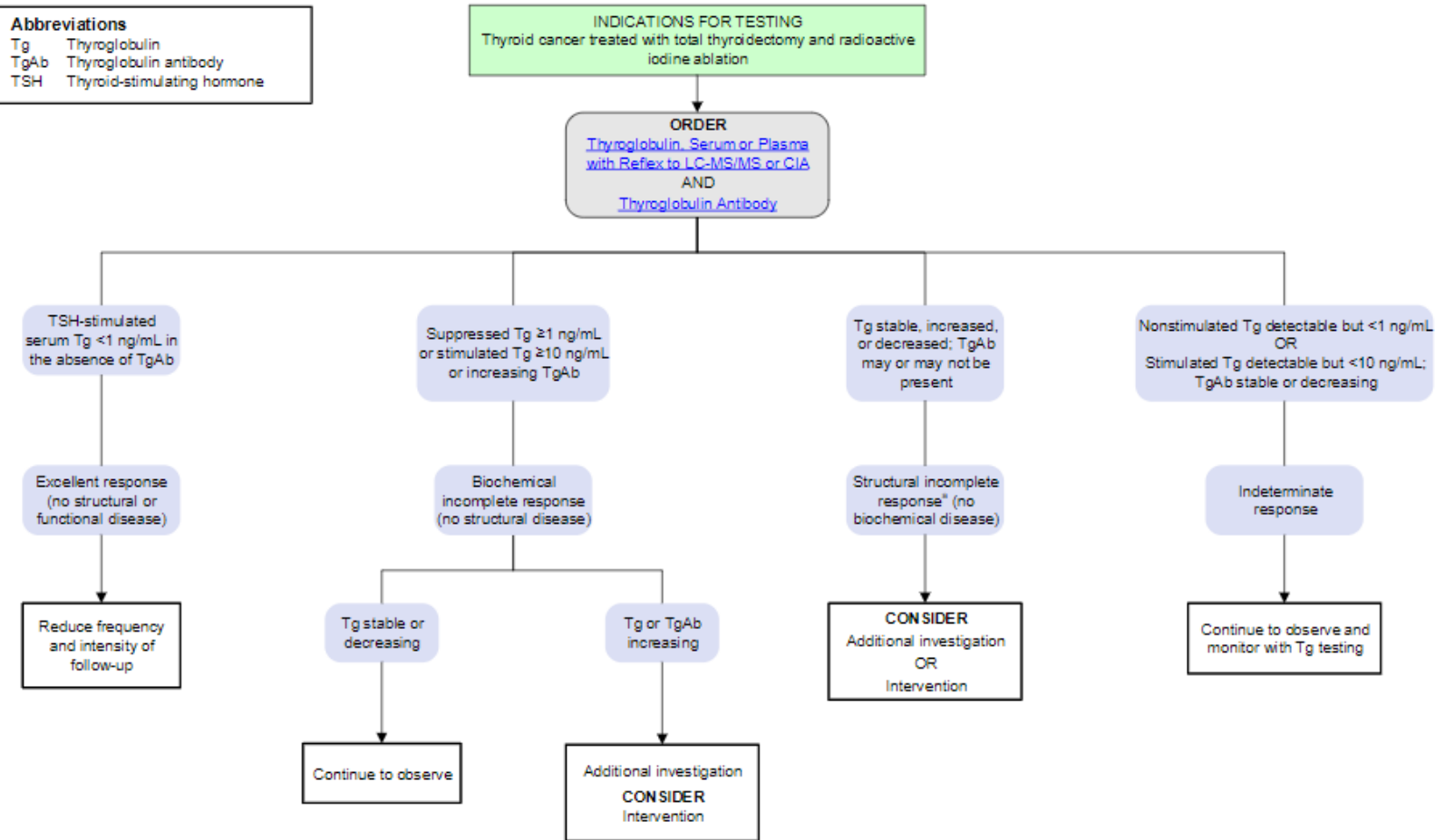
Patient Results Continued

- Interval labs:
 - Roughly 6 months later
 - TgAb
 - Negative
 - Tg
 - Increased/high
- No reflex
- Concerning for...

Date/Test	Marker	Result	Reference Interval
8/30/2021 Tg w/reflex	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
	Tg (CIA)	15.5 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL
3/10/2022 Tg w/reflex	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
	Tg (CIA)	49.7 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL

Abbreviations

Tg Thyroglobulin
 TgAb Thyroglobulin antibody
 TSH Thyroid-stimulating hormone



*Evidence of disease observed on imaging.

Reference

Filet S, Durante C, Hartl D, et al. ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019;30(12):1856–1883. Pub Med

Patient Results Continued

- Interval labs:
 - Roughly 6 months later
 - TgAb
 - Negative
 - Tg
 - Within reference interval
- No reflex
- Client contacts client services
 - Path on call
 - Clinical history obtained
 - They tested outside and Tg was negative

Date/Test	Marker	Result	Reference Interval
8/30/2021 Tg w/reflex	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
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	Tg (CIA)	27.9 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL

Patient Results Continued

- Investigation
 - New CIA
 - Request to run LC-MS
- Discordant results
 - CIA
 - LC-MS
- What could increase Tg?

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	Tg (LC-MS)	N/A	1.3-31.8 ng/mL
10/12/2022	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
Tg w/reflex	Tg (CIA)	25.5 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL
10/12/2022	TgAb	N/A	0.0-4.0 IU/mL
Tg w/reflex	Tg (CIA)	N/A	1.3-31.8 ng/mL
	Tg (LC-MS)	<0.5 ng/mL	1.3-31.8 ng/mL

Heterophile Antibody Testing

- Specimen pretreated with heterophile blocking reagent

- Result for the CIA

- 90% lower
 - Interpretation?
 - Abnormally normal

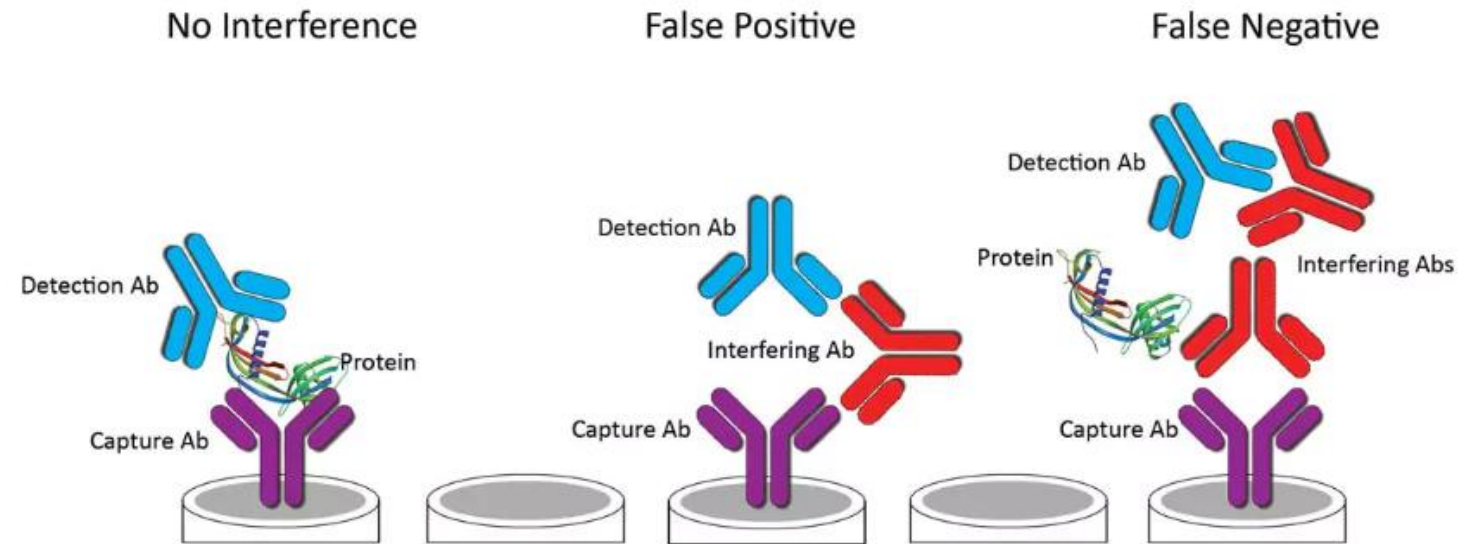
- No reflex to LC-MS

- No TgAb detected

Date/Test	Marker	Result	Reference Interval
10/12/2022	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
Tg w/reflex	Tg (CIA)	25.5 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL
10/12/2022	TgAb	<0.9 IU/mL	0.0-4.0 IU/mL
Heterophile	Tg (CIA)	2.4 ng/mL	1.3-31.8 ng/mL
	Tg (LC-MS)	N/A	1.3-31.8 ng/mL
10/12/2022	TgAb	N/A	0.0-4.0 IU/mL
Tg (LC-MS)	Tg (CIA)	N/A	1.3-31.8 ng/mL
	Tg (LC-MS)	<0.5 ng/mL	1.3-31.8 ng/mL

Heterophile Antibody

- Erroneously elevated or decreased patient results from immunoassays
 - Serum or plasma contains heterophile antibodies
 - Bind animal antibody in immunochemistry assay
 - False positive > false negative
 - Diagram
 - Analyte with positive result
 - Heterophile false positive
 - Heterophile false negative
 - Heterophile blocking tubes (HBT)
 - Significant change = false result



<https://www.dianova.com/en/faq/heterophilic-blocking-reagents-hama/>

Summary

- Thyroid carcinoma workup and surveillance is multifaceted the lab is integral in both and helping thyroid cancer patients in with treatment and quality of life in survivorship.
- Thyroglobulin levels are used to monitor thyroid cancer treatment, recurrence, antithyroid autoantibodies and heterophile antibodies may interfere with CIA in different ways.
- LC-MS can overcome TgAb, however, health care costs should prompt initial testing of TgAb with a reflex to CIA or LC-MS dependent on TgAb result.

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- American Thyroid Association
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