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Evaluating Thyroid Disorders

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SYNOPSIS AND RELEVANCE

Thyroid stimulating hormone (TSH) forms the mainstay for assessing thyroid function in most patients. The judicious use of TSH combined with free thyroxine (FT4) levels can help patients by:

1. Accurately classifying thyroid disorders in patients with an intact hypothalamic-pituitary axis.
2. Avoiding over-diagnosis of older patients with mild increases in TSH as hypothyroid.
3. Improving detection of maternal hypothyroidism.
4. Avoiding over-diagnosis of thyroid disease in inpatients who may have physiologically suppressed TSH.
5. Stratifying the risk of progression from subclinical hypothyroidism to hypothyroidism.

OBJECTIVES

1. Encourage use of a TSH-based algorithm for initial workup of thyroid disease in non-hospitalized patients with an intact pituitary-hypothalamic axis.
2. Become familiar with the causes and clinical presentations of hypo- and hyperthyroidism and the most useful ancillary tests to confirm thyroid dysfunction.
3. Discourage the use of thyroid function tests with uncertain clinical utility.
4. Discourage duplicate ordering of ancillary thyroid function tests.
5. Recognize variability of TSH levels in older patients and pregnant patients.

BACKGROUND

Thyroid function is controlled by a classical negative endocrine feedback loop of the hypothalamic-pituitary-thyroid axis. Thyrotropin releasing hormone (TRH) is released by the hypothalamus and stimulates the pituitary gland to secrete thyroid stimulating hormone (TSH), which in turn acts on the thyroid gland to secrete thyroxine (T4) and, to a lesser extent, triiodothyronine (T3). Most T3 is produced peripherally by conversion of T4 to T3. TSH, T4 and T3 levels form the mainstay of modern laboratory testing for thyroid disease.

Hypothyroidism, insufficient production of thyroid hormone by the thyroid gland, is most commonly caused worldwide by a lack of dietary iodine and in the United States by autoimmune thyroiditis (Hashimoto thyroiditis).[1-2] Other causes of primary hypothyroidism include previous thyroidectomy, radioiodine treatment for thyroid tumors, radiation damage from treatment of head and neck cancers, and rarely, medications such as tyrosine kinase inhibitors and checkpoint inhibitors, including programed cell death 1 and anti-cytotoxic T-lymphocyte associated antigen immunotherapy.[3-4] All of these etiologies lead to decreased levels of T4 and consequent increases in TSH.

Central hypothyroidism occurs when there is insufficient production of TSH, usually as a result of injury to the pituitary-hypothalamic axis from, for example, pituitary or hypothalamic tumors, previous surgery or radiation for pituitary or hypothalamic disease, and lymphocytic or granulomatous hypophysitis.[5] In central hypothyroidism, serum FT4 levels are low, but TSH levels can vary. When TSH and FT4 levels are low, the diagnosis of central hypothyroidism is straightforward.

Symptoms of hypothyroidism include cold intolerance, fatigue, depression, and weight gain. Hypothyroidism increases serum LDL levels and increases the risk of coronary artery disease. If untreated in pregnancy, it can lead to growth delays and intellectual impairment in infants.

Subclinical hypothyroidism (SCH) is defined as a mildly elevated TSH level with a normal value of FT4, and is usually asymptomatic. Its prevalence is between 3% to 8% in the general population without known thyroid disease, and increases with age.[6] Patients with SCH are at risk for overt hypothyroidism. In one study, the rate of progression to hypothyroidism was 2.6% annually if anti-thyroid peroxidase antibodies (anti-TPO) are absent and 4.3% annually if anti-TPO are present.[7] SCH is more common in women, but by the 6th decade, the prevalence is equal in men and women. SCH is associated with anti-thyroid antibodies in up to 80% of cases. Other than in pregnancy, there is no consensus whether SCH requires treatment with levothyroxine, especially when TSH levels are less than 10 mIU/L.[8-9] Children of women with SCH during pregnancy have been found to more frequently have a reduced intelligence quotient, and these women more frequently have adverse outcomes during pregnancy.[10] There is no conclusive evidence that treatment of SCH with TSH levels between 5.1 and 10 mIU/L leads to reductions in lipid and cholesterol levels or decreases the risk for coronary artery disease.[11]

Hyperthyroidism is due to the inappropriately high synthesis and secretion of thyroid hormones by the thyroid gland. Graves disease is an autoimmune disorder, and is the most common cause of hyperthyroidism. It can lead to enlargement of the thyroid gland and produce symptoms of irritability, heat intolerance, rapid heart rate, diarrhea, weight loss, thickening of the skin over the pretibial area, and Graves ophthalmopathy. Graves disease and all other variants of autoimmune related hyperthyroidism are the result of thyroid stimulating hormone receptor (TSHR) autoantibodies that bind and transactivate the TSH receptor on thyroid cells resulting in uncontrolled production of thyroid hormones without the normal feedback inhibition. These antibodies are known as long-acting-thyroid-stimulating hormone or thyroid-stimulating immunoglobulins (TSI). Some patients with Graves disease have TSHR-blocking antibodies, which do not transactivate the TSH receptor. The severity of Graves disease is determined by the balance between titers of TSI and TSHR-blocking antibodies. TSHR-blocking antibodies, and to lesser extent TSI, can also found in hypothyroidism.

Other causes of hyperthyroidism are rare and include toxic multinodular goiter and autonomously functioning thyroid adenoma. Toxic multinodular goiter occurs in regions of iodine deficiency and its prevalence increases with age. Autonomously functioning thyroid adenomas arise from somatic mutations in genes controlling the production of thyroid hormone.[12-13]

Testing Targets

TSH secretion is exquisitely sensitive to minor fluctuations in serum FT4, responding to FT4 in an inverse log-linear relationship so that small changes in FT4 will lead to large changes in TSH. Thus, TSH is powerful in the evaluation of thyroid disease.[14] Third generation TSH assays are immunometric with a typical sensitivity of less than or equal to 0.01 mIU/L.[15] In a disease free “reference population” established from a population study in the United States, 4.12 mIU/L has been suggested as the upper limit of TSH for hypothyroidism. TSH levels increase with age: for every 1-year increase in age after 30-39 years; the 97.5th percentile for age increases by 0.3 mIU/L.[16]

Total T4 is bound, up to 99.7%, to serum binding proteins, mostly to T4-binding globulin. Since levels of total T4 will be affected by many factors that alter binding protein levels, total T4 is not typically useful in the assessment of thyroid function, and has been replaced by free T4 (FT4). FT4 is the biologically active portion of T4; direct measurement of FT4 has replaced surrogate measurements of FT4 such as the serum free T4 index (Please see testing targets of uncertain clinical significance below).[17] A decreased level of FT4 confirms the diagnosis of hypothyroidism whether primary, in which TSH is increased. or central, in which TSH levels are variable.

T3 is generated largely from peripheral conversion of FT4, and it is the biologically active form of thyroid hormone. Similar to T4, T3 is also highly bound by serum binding proteins. However, unlike FT4, measurement of free T3 (FT3) is not widely clinically validated. Assays for FT3 suffer from imprecision, as the concentrations of FT3 are very low, in the picomolar range, making measurement difficult. The use of total T3 versus FT3 will depend upon the specific performance characteristics of the assay. The measurement of T3 is typically only useful to confirm rare cases of early hyperthyroidism when TSH is decreased, FT4 is normal and T3 is elevated (T3 thyrotoxicosis). T3 measurement has no place in the evaluation of hypothyroidism. Other tests to determine T3 levels such as the resin T3-uptake test and the reverse T3 test are not useful (see testing targets of uncertain clinical significance below).

Antibodies
Until recently, the measurement of TSI antibodies was a relatively complex procedure involving tissue culture followed by immunoassay.[18] An automated semi-quantitative TSI specific assay has become available.[19] This assay has shown good sensitivity and specificity in the detection of Graves disease and may be useful and economical to confirm autoimmune hyperthyroidism when T3 and T4 are discordant with TSH levels or when the clinical presentation is confusing. Testing for TSI antibodies is also useful to confirm the presence of Graves ophthalmopathy, monitor response to therapy, and to predict hyperthyroidism in neonates.[20] Neonatal thyrotoxicosis is due to maternal TSI antibodies crossing the maternal-placental barrier and binding to the fetal thyroid gland.[21]

The presence of anti-TPO and anti-thyroglobulin antibodies (anti-TG) in subclinical hypothyroidism is associated with a greater risk for the development of overt hypothyroidism. While the presence of either antibody signals increased risk of progression to overt hypothyroidism, anti-TPO is more sensitive and specific than anti-TG in risk stratification.

Testing (Initial Workup)
For the initial evaluation of thyroid disease in non-hospitalized patients without pituitary disease, TSH is the most useful test as it is abnormal in both hypothyroidism and hyperthyroidism. If TSH is normal and there are no risk factors for central hypothyroidism, then no additional workup is required. If central hypothyroidism is possible, then FT4 levels should also be ordered.

If TSH is below the reference range, then hyperthyroidism is suspected and confirmatory FT4 should be ordered. If FT4 is normal or minimally abnormal, then total T3 should be ordered to exclude T3 thyrotoxicosis.

If TSH is above the reference range, then hypothyroidism is suspected. Generally, TSH values over 10 mIU/L are indicative of overt hypothyroidism, almost always associated with decreased FT4 levels. Elevations less than 10 mIU/L with normal values of FT4 are usually associated with subclinical hypothyroidism.

Testing Caveats
Screening for hypothyroidism in hospitalized patients is generally not recommended as many of the testing targets are spuriously affected. TSH levels may be suppressed in acutely ill patients; levels below 0.1 mIU/L in conjunction with depressed levels of FT4 may be seen in critically ill patients. Levels of T3 are also low in acute illness because of reduced peripheral conversion of T4 to T3.[1]

In pregnancy, TSH levels vary by trimester and trimester-specific reference ranges are encouraged.[22] TSH should be measured in conjunction with measurement of serum total T4 or FT4. Total T4 may be more useful in pregnancy than direct immunoassay of FT4 because method-specific reference ranges for FT4 may not have been established for each trimester.

The American College of Obstetrics and Gynecology emphasizes that method-specific total and free T4 reference intervals should be provided by the laboratory to ensure proper interpretation of these tests.[23]

Testing Targets of Uncertain Clinical Significance
Except for extremely rare cases of deiodinase defects, reverse triiodothyronine (rT3) is not helpful in assessing thyroid dysfunction. It is formed by peripheral deiodination of T4, is metabolically inert, and tends to follow T4 levels. An increased level of rT3, typically seen in critically ill, hospitalized patients, who often have low levels of FT4, was thought to exclude a diagnosis of hypothyroidism, but this has not found to be clinically useful.[24-25]

Resin T3 uptake was historically used as a surrogate to estimate thyroid binding globulin. Free thyroxine index (FTI) is determined by the product of total T4 and resin T3 uptake. The two tests comprising FTI have been replaced by FT4, which is a more accurate measurement.

INSIGHTS
1. The initial assessment of thyroid function in most patients should be performed using an algorithm for reflexive testing based on sensitive (sTSH), or third generation, TSH levels (see **Appendix A**)
    - If the TSH is < 0.10mIU/L, suspect hyperthyroidism.
    - If the TSH is borderline low (0.10 - 0.20 mIU/L), measure FT4.
    - If the TSH is > 10 mIU/L, the presumptive diagnosis is hypothyroidism.
    - If the TSH is inconclusive, with mildly elevated TSH levels (4.2 - 10 mIU/L), measure FT4.

    - If the FT4 level is decreased, suspect hypothyroidism.
    - If FT4 level is normal, consider subclinical hypothyroidism.
2. T3 has no role in the evaluation of hypothyroidism.
3. Discourage routine testing for hypothyroidism in hospitalized patients, unless there is a high index of suspicion.
4. Use trimester-specific reference ranges for TSH in pregnancy. Choose a secondary target (free T4 vs. total T4) to test in pregnancy based on the availability of trimester-specific reference ranges.
5. Discourage simultaneous ordering of free and total T4 and free and total T3.
6. Eliminate reverse T3 and T3 uptake test options if present.
7. Eliminate the FT4 index test option if present.
8. In order to stratify risk of progression from SCH to overt hypothyroidism, recommend measurement of anti-TPO rather than anti-TG.
9. Discourage repeat anti-TPO or anti-TG testing, as these antibodies seldom resolve.

INTERVENTIONS

Note: These are suggestions that may not be applicable to your practice setting. Other interventions, if needed, should be considered that offer the best opportunity to improve testing practices.

1. Provide educational information about the recommended testing algorithm for the diagnosis of hypothyroidism and hyperthyroidism in non-pregnant patients and about testing in pregnant patients.
2. Discourage the use of resin T3 uptake, reverse T3, and FT4 index testing. Attempt to obtain consensus to eliminate these tests from the laboratory test menu.
3. Review standing orders, panels, etc. that contain thyroid function tests to confirm they are appropriately designed and used. Modify or eliminate orders as needed to improve utilization.
4. Consider using the hospital or laboratory information system to develop reflexive algorithms, once approved by institutional leadership, to assess thyroid function.
5. Create an alert (ie, a “pop-up”) that fires whenever thyroid testing is requested for inpatients, indicating possible spurious results in acutely ill patients. The clinician may override the alert at the point of computerized order entry (soft stop).
6. Create an alert (ie, a “pop-up”) that fires whenever a T3 is ordered to indicate that it is only useful in rare cases of suspected T3 thyrotoxicosis. The clinician may override the alert at the point of computerized order entry (soft stop).
7. Create an alert (ie, a “pop-up”) that fires whenever total and free T3 or total and freeT4 levels are ordered together. The clinician may override the alert at the point of computerized order entry (soft stop).
8 Discourage the use of repeat anti-TPO and anti-TG testing, as these antibodies seldom resolve. Consider implementation of a best practice alert (i.e. a “pop-up”) whenever anti-TPO or anti-TG testing has been previously ordered. The clinician may override the alert at the point of computerized order entry (soft stop).

INTERVENTION ANALYSIS

One approach is to focus on the impact of discouraging thyroid tests with little clinical utility and on the benefit that a reflexive testing algorithm may have in reducing unnecessary thyroid tests. Depending on your goals for this project, it is relatively straightforward to assess current thyroid function testing practices. Thyroid function testing can then be assessed post-intervention along with annual volume change. **Appendix B** illustrates how to calculate the change.

1. Perform a pre-intervention assessment of current thyroid function testing practices.
    - Unnecessary T3 tests ordered: Calculate the number of T3 tests (total and free) ordered with FT4 when TSH is high.
    - Unnecessary FT4 tests: Calculate the number of free T4 and TSH tests ordered simultaneously when TSH is within normal limits.

2. Implement interventions as suggested from the interventions list provided or those developed in your institution as agreed by your clinicians.

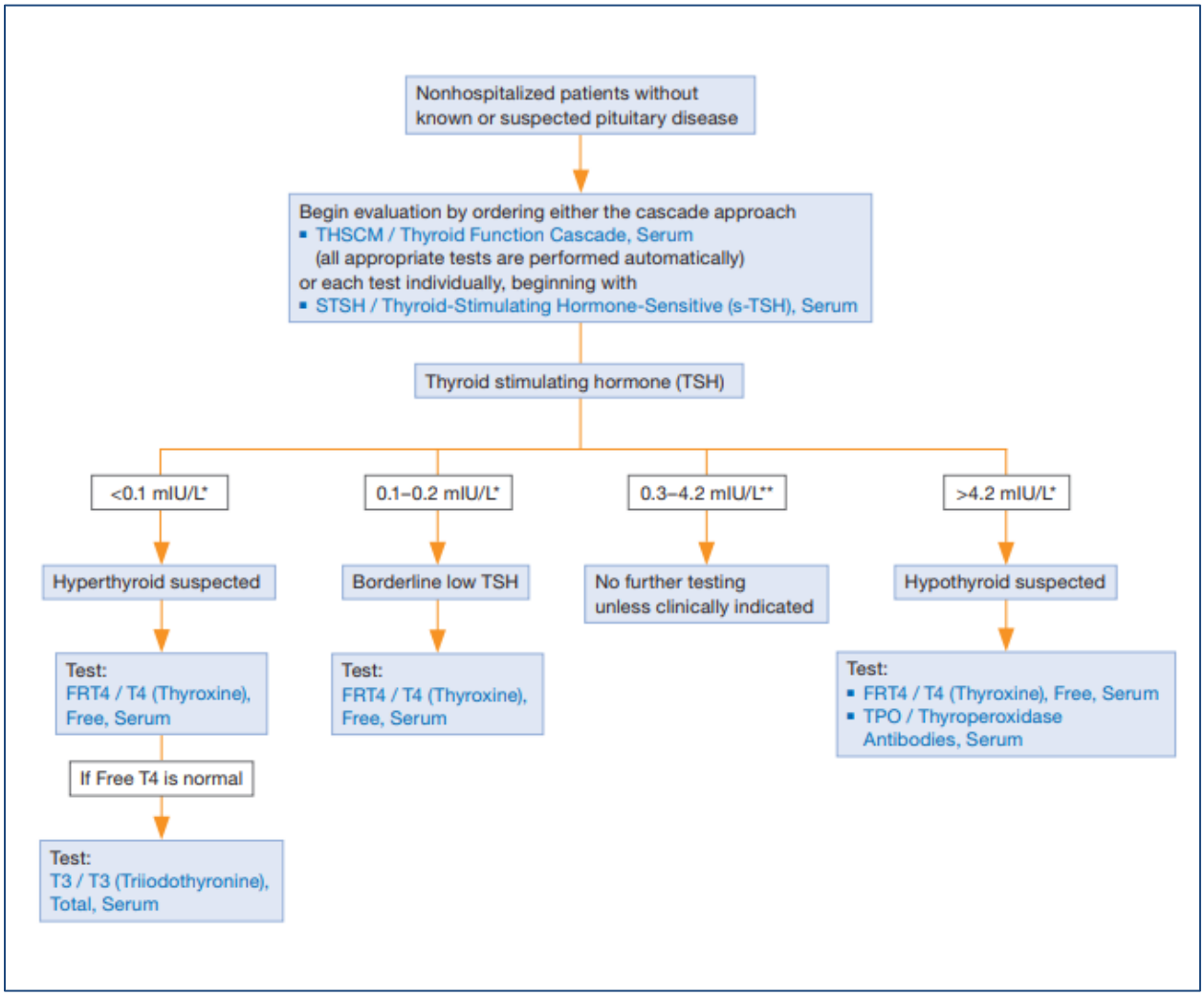
3. Perform a post-intervention assessment of thyroid function testing based upon your institution’s reflex testing algorithm.
    - Unnecessary T3 tests ordered: Calculate the number of T3 tests (total and free) ordered with FT4 when TSH is high.

- Unnecessary FT4 tests: Calculate the number of free T4 and TSH tests ordered simultaneously when TSH is within normal limits.

4. The annual volume change can be calculated by comparing your pre-intervention and post-intervention performance.

5. This impact study can be repeated for each major intervention or reflex testing algorithm update.

APPENDIX A: THYROID FUNCTON ORDERING ALGORITHM



* For 12 hours before specimen collection for any thyroid test listed in this algorithm, the patient should not take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.
** Adult s-TSH reference ranges. For pediatric intervals, see STSH / Thyroid-Stimulating Hormone-Sensitive (s-TSH), Serum

APPENDIX B: INTERVENTION IMPACT

	Pre Intervention	Post Intervention	Annual Volume Change
# T3 (Free and Total) tests performed with FT4 when TSH is elevated	**B1**	**C1**	**(B1*(12/B3)) – (C1*(12/C3)) = D3**
# of FT4 and TSH tests ordered simultaneously when TSH is normal	**B2**	**C2**	**(B2*(12/B3)) – (C2*(12/C3)) = D4**
Time period (months)	**B3**	**C3**	

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE
Recognize which ancillary tests are not useful to confirm thyroid dysfunction.

QUESTION 1
Which of the following tests is not useful for routine screening and/or workup of thyroid disorders?
A. TSH
B. Free T4
C. Reverse T3
D. Free T3
E. Total T3

The correct answer is C. Increased levels of reverse T3 are seen in critically ill, hospitalized patients, who often have low levels of FT4. An increased reverse T3 was thought to exclude a diagnosis of hypothyroidism, but this has not found to be clinically useful.
A is incorrect. TSH forms the mainstay of thyroid function screening.
B is incorrect. Free T4 is used to confirm abnormal TSH levels.
D is incorrect. If the assay in use is clinically validated, then free T3 is useful in confirming T3 thyrotoxicosis, but this test has no utility in evaluating hypothyroidism.
E is incorrect. If clinically validated, then FT3 is preferred. However, specific FT3 assays may not be clinically validated. In this case, total T3 is used to confirm T3 thyrotoxicosis.

REFERENCE
Lee S, Farwell AP. Euthyroid sick syndrome. *Compr Physiol*. 2016(2):1071-1080.

QUESTION 2 OBJECTIVE
Recognize causes and clinical presentation of hypothyroidism.

QUESTION 2
Which of the following statements regarding hypothyroidism is false?
A. The most common cause in the United States is due to nutritional deficiency of iodine.
B. It is associated with increased levels of LDL.
C. Untreated in pregnancy, it can lead to growth delays and intellectual impairment in infants.
D. It is associated with cold intolerance, fatigue, weight gain and clinical depression.
E. Central hypothyroidism occurs when there is insufficient production of bioactive TSH.

The correct answer is A. In the United States, autoimmune destruction of thyroid gland by lymphocytes (Hashimoto thyroiditis) is the most common cause of hypothyroidism. Worldwide, iodine deficiency is the most common cause.
B is incorrect. LDL levels are increased in hypothyroidism. Patients with newly identified elevated LDL levels should be screened for hypothyroidism.
C is incorrect. Untreated, hypothyroidism in pregnancy can have devastating effects on the fetus leading to decreased IQ levels and delay in growth.
D is incorrect. Cold intolerance, fatigue, weight gain and clinical depression are common in hypothyroidism.
E is incorrect. Central hypothyroidism usually results from injury to the pituitary-hypothalamic axis resulting in decreased levels of bioactive TSH. Typical causes are from pituitary or hypothalamic tumors, previous surgery or radiation for pituitary or hypothalamic disease, and lymphocytic or granulomatous hypophysitis.

REFERENCES

1. Haddow JE, Palomaki GE, Allan WC, Wi JR. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Eng J Med.*1999;341:549-555.
2. Vanderpump MP, Tunbridge WM, French JM, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol (Oxf)*.1995;43:55-68.
3. Willard DL, Leung AM, Pearce EN. Thyroid function testing in patients with newly diagnosed hyperlipidemia. *JAMA Intern Med.* 2014;174:287.

QUESTION 3 OBJECTIVE

Recognize causes of variability in TSH levels.

QUESTION 3

Which of the following statements regarding TSH is false?

A. TSH levels are extremely sensitive to changes in serum FT4 levels.
B. TSH levels increase with age.
C. TSH levels vary by trimester in pregnancy.
D. An elevated TSH level is diagnostic of clinical hypothyroidism.
E. TSH levels may be suppressed in acutely ill patients.

The correct answer is D. Elevated TSH levels alone do not reflect necessarily indicate hypothyroidism. While TSH levels over 10 mIU/L are generally associated with overt hypothyroidism, mild increases in TSH are not uncommonly seen with increasing age and seen in subclinical hypothyroidism. The latter is characterized by lack of symptoms, elevated TSH levels (but less than 10 mIU/L) and normal FT4 levels.

A is incorrect. TSH secretion is exquisitely sensitive to minor fluctuations in serum FT4, responding to FT4 in an inverse log-linear relationship.

B is incorrect. TSH levels increase with age.

C is incorrect. TSH levels vary by trimester in pregnancy so that trimester specific reference ranges for TSH should be established.

E is incorrect. TSH levels may be suppressed in acutely ill patients.

REFERENCES

1. de los Santos ET, Starich GH, Mazzaferri EL. Sensitivity, specificity, and cost-effectiveness of the sensitive thyrotropin assay in the diagnosis of thyroid disease in ambulatory patients. *Arch Intern Med*. 1989;149:526-532.
2. Witherspoon LR. Clinical utility of sensitive TSH measurements. *Lab Med.* 2005; 36:711-715.
3. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. *Endocrinol Metab Clin North Am.* 2001;30:265-289.

MODULE REFERENCES

1. Garber JR, Cobin RH, Gharib H et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocrn Pract. 2012;*18(6): 998-1028.
2. Vanderpump MP, Tunbridge WM**.** Epidemiology and prevention of clinical and subclinical hypothyroidism. *Thyroid*. 2002;12:839-847.
3. Desai J, Yassa L, Marqusee E, et al. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. *Ann Intern Med.* 2006;145:660-664.
4. Corsello SM, Barnabei A, Marchetti P, et al. Endocrine side effects induced by immune checkpoint inhibitors. *J Clin Endocrinol Metab.* 2013;98:1361-1375.
5. Beck-Peccoz P, Amr S, Menezes-Ferreira MM, et al. Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. Effect of treatment with thyrotropin-releasing hormone. *N Engl J Med.*1985;312:1085-1090.
6. Karmisholt J, Andersen S, Laurberg P. Variation in thyroid function tests in patients with stable untreated subclinical hypothyroidism.*Thyroid*. 2008;18(3):303-308.
7. Kong WM, Sheikh MH, Lumb PL, et al. A 6-month randomized trial of thyroxine treatment of women with mild subclinical hypothyroidism. *Am J Med.* 2002;112(5):348-354.Erratum: *Am J Med*. 2002;113(3):264; Naoumova P. *Am J Med.* 2002;113(5):442.
8. Surks MI, Oritz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA.* 2004;291(2):228-238.
9. Chu JW, Crapo LM. The treatment of subclinical hypothyroidism is seldom necessary. *J Clin Endocrinol Metab.* 2001;86(10):4591-4599.

10. Haddow JE, Palomaki GE, Allan WC, Wi JR. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Eng J Med.*1999; 341:549-555.
11. Vahab F. Subclinical hypothyroidism: an update for primary care physicians. *Mayo Clin Proc.* 2009;84(1):65-71.
12. Bahn RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid association and American Association of Clinical Endocrinologists. *Endocr Pract.* 2011;17(3):457-520.
13. Davis PJ, Davis FB. Hyperthyroidism in patients over the age of 60 years. Clinical features in 85 patients. *Medicine* (Baltimore*).* 1974;53:161-181.
14. de los Santos ET, Starich GH, Mazzaferri EL. Sensitivity, specificity, and cost-effectiveness of the sensitive thyrotropin assay in the diagnosis of thyroid disease in ambulatory patients. *Arch Intern Med*. 1989;149:526-532.
15. Witherspoon LR. Clinical utility of sensitive TSH measurements. *Lab Med*. 2005; 36:711-715.
16. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.*2002;87:489-499.
17. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. *Endocrinol Metab Clin North Am.* 2001;30:265-289.
18. Morshed SA, Latif R, Davies TF**.** Characterization of thyrotropin receptor antibody-induced signaling cascades. *Endocrinology.*2009;150:519-529.
19. IMMULITE 2000 TSI Assay Package Insert. Accessed September 5, 2025. https://www.healthcare.siemens.com/clinical-specialities/thyroid/tsi-assay
20. Tozzoli R, D'Aurizio F, Villalta D, Giovanella L. Evaluation of the first fully automated immunoassay method for the measurement of stimulating TSH receptor autoantibodies in Graves disease. *Clin Chem Lab Med*. 2017; 55(1):58-64. doi:10.1515/cclm-2016-0197
21. Fu J, Jiang Y, Liang L, Zhu H. Risk factors of primary thyroid dysfunction in early infants born to mothers with autoimmune thyroid disease. *Acta Paediatr.* 2005; 94:1043-1048.
22. Lee RH, Spencer CA, Mestman JH, et al. Free T4 immunoassays are flawed during pregnancy. *Am J Obstet Gynecol*. 2009;200:260 e261-266.
23. Practice bulletin no.148: thyroid disease in pregnancy. *Obstet Gynecol*. 2015; 125(4):996-1005. doi: 10.1097/01.AOG.0000462945.27539.93
24. Gomes-Lima C, Burman KD. Reverse T3 or perverse T3? Still puzzling after 40 years. *Cleve Clin J Med*. 2018; 85(6):450-455. doi: 10.3949/ccjm.85a.17079
25. Burmeister LA. Reverse T3 does not reliably differentiate hypothyroid sick syndrome from euthyroid sick syndrome. *Thyroid*. 1995; 5(6):435–441. doi:10.1089/thy.1995.5.435
26. Mayo Foundation for Medical Education and Research. Thyroid function ordering algorithm. Published October 2017. Reviewed 11/2023. Accessed September 5, 2025. https://www.mayocliniclabs.com/it-mmfiles/Thyroid_Function_Ordering_Algorithm.pdf