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Understanding Your Blood Tests

by Larry Levin, MD

Thyroid lab testing is an area that can be confusing to both patients and physicians. It is a subject of controversy since some people have suggested that standard thyroid lab testing is not reliable and that alternative measures, such as axillary temperatures, or some sort of compilation of symptoms, would be more reliable in diagnosing thyroid dysfunction or monitoring thyroid therapy.

In fact, thyroid testing is an essential component of the evaluation of someone who has an abnormality of the thyroid gland, or anyone who is taking thyroid hormone. If the initial screening thyroid tests do not help provide a definite diagnosis, then there are other lab tests which are available to further evaluate the situation.

These lab tests are very specific and although there certainly are factors that can cause deviations in those lab tests, they are more reliable than some of these projective parameters that have been mentioned. Other parameters, such as axillary temperature or symptoms, can obviously be influenced by many other factors in addition to thyroid function.

Thyroid Physiology

To understand thyroid lab tests, it helps to understand a little bit of thyroid physiology.

Basically, the hypothalamus, which is a higher area of the brain, above the pituitary gland releases TRH, which is thyrotropin-releasing hormone, which stimulates the pituitary gland to release TSH, thyroidstimulating hormone. TSH then combines with a receptor on the thyroid gland and stimulates the thyroid gland to take up iodine and synthesize thyroid hormone and release thyroid hormone.

The thyroid gland releases primarily T4 and very little T3 (T4 is thyroxine, which is the primary hormone produced by the thyroid gland and T3 is Triiodothyronine, which is a hormone produced in small amounts by the thyroid gland but actually is derived by metabolism of T4). T4 is converted in peripheral tissues, primarily the liver, to T3. So, T3 comes primarily from metabolism of T4 out of the tissues and T3 does not come primarily from the thyroid gland.

Thyroid hormones, again primarily T4, circulate in the blond, bound to various proteins, such as thyroid-binding globulin (TBG) and thyroidbinding pre-albumen (TBPA). The only hormone which can really have its effect is the free hormone, or the small amount of hormone that is not bound to protein. The way that this occurs is that the thyroid hormone, T4, is converted to T3 and the T3 goes into the cells of whatever organ is being affected, say the heart muscle or the brain, and the T3 combines with the receptor inside the cell, to have its effect.

The effects of thyroid hormone are not caused just by the level of thyroid hormone but they also are affected by all these details which have to do with the thyroid hormone binding with the receptor. When the hormone binds with the receptor, the receptor does other things which tells the cell what to do. Alterations in the receptor, or what happens after the thyroid hormone binds to the receptor, may determine the ultimate effect.

This is mentioned because some Graves' disease patients may or may not have come into contact with the idea that thyroid hormone levels may be very high and not cause a lot of symptoms in some people; while other people, with just slightly elevated thyroid hormone levels, may have a lot of symptoms. Part of that relates to the fact that it is not just the thyroid hormone level, but the whole process of the thyroid hormone combining with the receptor and the receptor making the cell make proteins and do other things which has its ultimate effect.

In summary, the brain, through stress, temperature, other neurochemicals, and other nerve pathways, may have influence on the hypothalamus. The hypothalamus releases the TRH which then acts on the pituitary gland to release TSH which stimulates the thyroid gland to take up iodine and synthesize and release thyroid hormone. The thyroid hormone, primarily T4, circulates throughout the body and then is converted into T3 in the organs and muscles, primarily the liver. T3 has its effect by going inside cells in the brain, heart, etc., and causing the ultimate end result. In addition, thyroid hormone has a negative feedback loop in that it acts on the pituitary gland and the hypothalamus to reduce the production of TRH and TSH. So, the more T4, then the less TSH. The opposite is also true. This is very important in evaluating the tests.

Blood Tests

Historical. One of the oldest tests was the basal metabolic rate, a way to measure metabolism by measuring oxygen consumption while wearing a mask. However, it was not very accurate in assessing thyroid function since many different things can affect the basal metabolic rate.

The protein-bound iodide (PBI) was a test that measured the amount of iodide bound to protein, which is primarily thyroid hormone, but there are other proteins containing iodine which do not have thyroid hormone activity. This test, though it was roughly correlated with thyroid hormone function, was not very accurate.

Total T4. A more recent development has been the measurement of total T4. It is the measurement of all of the T4, i.e., the T4 which is bound to protein and the T4 which is free. There were some older measurements than this one, called a Murphy technique, but now the technique of radioimmuno assay enables us to get more accurate measurement of total T4.

The limitation with total T4 is that it includes the bound thyroid hormone, which can be affected by anything that can affect the binding protein Some of the things that commonly affect binding proteins are estrogen. As such, birth control pills and pregnancy raise the level of thyroid-binding globulin which, therefore, elevate the total T4 measurement, even when the actual circulating thyroid is normal.

T3 Resin Uptake (T3RU). In trying to deal with the variations in binding of thyroid hormone and the possible effect on the measurement of total T4, a test called the T3 resin uptake

(T3RU) was developed. While this test is not a direct measurement of binding protein, T3RU is helpful in those situations where the level of binding protein may have been influenced by other factors.

T3RIA. It is important not to confuse the T3RU test, which is a measurement of binding protein and not a measurement of thyroid hormone, with the T3RIA test, which is a measurement of the T3 hormone. This has been a source of constant confusion over the years when people say to order a T3 and some people take that to mean a T3 resin uptake and other people take that to mean a T3RIA test.

The T3RIA assay is a measurement of the T3 (triiodothyronine), which is actually the active thyroid hormone (although there is still some controversy about what effects the free T4 has itself). This assay is a measurement of the total hormone, both the hormone bound to the protein and the free unbound hormone.

The T3RIA test is actually not useful in that many cases and this will be emphasized further subsequently. It is affected by protein binding, although not to the same extent that T4 measurements are. In addition, the body converts some T4 into reverse T3, which is not active. In other words, T3 can vary due to various factors, including serious illness and malnutrition, and therefore, the T3RIA level may not really reflect thyroid hormone activity and whether or not the thyroid gland is overactive or underactive.

Limitations of T3RIA. When evaluating thyroid gland function, it's more helpful to look at the free T4 levels than the T3 levels. It is true that in the early stages of hyperthyroidism, T3RIA may become elevated before free T4 does, such as in cases of T3 toxicosis, in which a person is hyperthyroid but has normal T4 levels. Also, T3RIA may help indicate hyperthyroidism in subclinical cases.

However, the T3RIA test certainly isn't useful for evaluating low thyroid function because the T3RIA levels can be normal when, in fact, somebody is hypothyroid. Again, there are factors that affect T3 levels, not just active thyroid hormone binding, but other factors that affect the levels of T3 in the blood. So, the usefulness of the T3RIA test is rather limited.

Free T4 Index. Using the T3 resin uptake test in conjunction with the total T4 measurement, one can make a calculation of a free T4 index. Some labs very ingeniously call this a T7, because they're taking a T3 and a T4 and it becomes a T7. Actually, this index is a multiplication of the T3RU and the total T4. The free T4 index is a good test, even though it is not completely accurate, due to abnormalities in binding or other situations. However, free T4 is definitely a great advantage over the older total T4 or protein-bound iodide tests.

Free T4 Assay. The free T4 assay is a measurement of the actual free, unbound thyroid hormone, thyroxine, that is in circulation. This test gets around any problems with abnormalities in binding because it just measures the free circulating hormone which is the hormone which has the effect. These tests are fairly reliable although the commercial assay, which has to be done on large scale, still has some limitations depending on how it is done and still may be subject to distortions in certain situations where there are abnormal thyroid-binding proteins.

Equilibrium Dialysis. A more accurate technique is something called equilibrium

dialysis. The limitation for a commercial lab is that it usually has to be done overnight and that it is a longer, more tedious procedure. Consequently, it's not well-suited for a large laboratory which has to run hundreds of tests per day or even 20 tests per day.

TSH. The importance of the TSH test gets back to the physiology, for example, when T4 goes up, TSH goes down. And conversely, if T4 is low, TSH goes up. So, this isn't just measuring hormone levels in blood, and saying what is thought to be the normal range; this test is actually an assessment of how the body itself is responding to the level of thyroid hormone. The TSH is very reliable in evaluating overactive and underactive thyroid condition and it gets around abnormalities in thyroid hormone binding.

In terms of the details of the assay, it is true that the earlier TSH assays were not as accurate at the lower range and that you couldn't really distinguish between below normal and abnormally low. As such, TSH assays were not as helpful when T4 levels were high (Remember, when T4 levels are high, TSH is low). However, we now have TSH assays that are more sensitive at the lower range and as such, can distinguish between somebody whose thyroid levels are normal and somebody who has too much thyroid hormone circulating in their system. The nomenclature varies, but people call them super-sensitive, sensitive, 2nd generation or 3rd generation TSH assays. In many respects, the sensitive TSH assay may be the best screening test to decide if somebody has a normal, overactive or underactive thyroid, although it would be better if the patient also gets a free T4 level.

One little point is that the sensitive TSH test cannot be completely relied on when monitoring someone being treated for an overactive thyroid. Somebody who has an overactive thyroid has a suppressed (i.e., very low) TSH level and there is quite a bit of lag time before that TSH starts to come up to normal. So, even though the thyroid hormone levels may be in the normal range, this TSH level may still be suppressed just because of this lag time. So, it's important not to rely on the TSH level when assessing treatment of hyperthyroidism.

Limitations of TSH. The TSH test is generally the most useful test for screening and usually helps give us the diagnosis. However, in terms of monitoring hypothyroidism, we can't rely on TSH levels because of the lag time.

The other thing, which hasn't been mentioned in detail, is monitoring thyroid hormone therapy. The TSH level is the most useful test for monitoring thyroid hormone therapy to decide if someone is getting enough or not enough thyroid hormone.

TRH Test. The TRH test involves drawing blood samples to measure TSH and then giving an intravenous injection of TRH to stimulate the pituitary gland to release TSH. The TSH is measured after the injection at various intervals. I usually use just 15 and 30 minutes, although the standard test says 15, 30, 45 and 60 minutes. By measuring the response of TSH to TRH, one can usually tell whether someone has an overactive or underactive thyroid.

This is a very, very sensitive test. In addition, it is not useful as a screening test because it involves at least three blood samples, which is an expense, and giving an injection, and the material itself is somewhat expensive – it costs about $30 — $35. So, to do one of these TRH tests, even in a limited version, is probably $120 — $150, depending on how much it

costs to run the TSH levels. When there's a question, though, the TRH test is very helpful.

Thyroglobulin Test.

Thyroglobulin is the protein matrix in which the thyroid hormone is stored within the thyroid cells. The main value of measuring this is as a tumor marker in someone who has had thyroid cancer and has had their thyroid gland removed. Since there is no normal thyroid tissue remaining once the gland is removed, if these levels start to go up in the blood, it must mean that there is thyroid cancer somewhere in the body that is producing this thyroglobulin. (Note: It's important not to confuse thyroglobulin, which is the protein matrix material, with anti-thyroglobulin level, which is the antibody against the thyroglobulin.)

Thyroid Antibodies

Thyroid antibodies are proteins made by the immune system which have an affinity for thyroid tissue. Some of these antibodies actually stimulate the thyroid gland to become more active. These antibodies combine with the TSH receptor on the thyroid gland and actually stimulate the thyroid gland to work harder. Some of these antibodies initiate an inflammatory type of reaction which may destroy the thyroid tissue and/or cause swelling and nodularity. Some of these antibodies may react against both thyroid tissue and eye tissue. There is some thought that there are common antibodies that react against both tissues and people have been trying to identify these antibodies.

There's a class of antibodies called thyroid-stimulating antibodies, or thyroidstimulating immunoglobin (TSI). These are the antibodies that bind to the TSH receptor and stimulate the thyroid gland to become more active. As such, they are a marker for Graves' disease because that's what Graves' disease is – a production of these antibodies which stimulate the thyroid gland to become overactive. These antibodies may even be useful in monitoring one's response to treatment and prognosis of whether or not someone will stay in remission after treatment for an overactive thyroid gland.

Another type of antibody is the antimicrosomal antibody which binds to a specific part of the thyroid cell and initiates this inflammatory reaction. These antibodies are significantly elevated in a condition known as Hashimoto's thyroiditis. That is basically the pathophysiology of Hashimoto's thyroiditis that a high level of these antibodies is produced and causes damage in the thyroid gland which causes it to then become underactive.

Now there are also antithyroglobulin antibodies which act in the same way, although these antimicrosomal antibodies are more sensitive and more specific. Consequently, I rely primarily on measuring these antimicrosomal antibodies.

In summary, thyroid antibodies are useful in diagnosing Hashimoto's thyroiditis and in subclinical cases of hypothyroidism. In cases where someone may be borderline or slightly underactive, measuring thyroid antibodies may be helpful if one has a very sensitive assay. If the antimicrosomal antibodies are significantly elevated, then that might help confirm the diagnosis of borderline hypothyroidism. In fact, even if the patient is not hypothyroid at the time, if they have a very high titer of those antibodies, they are likely to develop hypothyroidism later on. So, the thyroid antibody tests are very useful.

As far as the thyroid-stimulating immunoglobins, they tend to be used, in a sense, as a marker for Graves' disease and in borderline cases, if the thyroid-stimulating immunoglobin is elevated, that may help to confirm a diagnosis of overactive thyroid. It may also serve to provide a prognosis as to whether or not someone is going to stay in remission after stopping therapy for an overactive thyroid.

Some Case Histories

1. In this case, screening tests were borderline but the patient's symptoms suggested hypothyroidism (underactive). The free T4 was 1.1, which was normal but in the lower third, and the TSH level was 3.8, which was also normal but in the upper third. Since the individual was symptomatic, a TRH test was done and measured 3.6. It was decided to inject TRH, and the maximum TSH afterwards rose to 45.1, which was consistent with the diagnosis of hypothyroidism. So based on this test, the patient was started on Synthroid ® and subsequently, TSH went to 1.3 and the free T4 went to 1.8 and the patient felt better. This is a situation where a sensitive antimicrosomal thyroid antibody test might have been helpful because if that was significantly elevated, there would have been further justification to start thyroid hormone therapy.

2. Another patient had obvious signs and symptoms of hyperthyroidism (overactive). Free T4 was 3.7, which was elevated (upper limits of normal were 2.0) and TSH was 0.1, which was low (the lower limits of normal were 0.4), which was consistent with hyperthyroidism. The patient opted to have radioactive iodine therapy and received 7.4 millicuries and, soon afterward, the free T4 went to 1.7, which was interesting because it was already in the normal range. Then, three months after treatment, free T4 was low (although TSH was still in the normal range) and the patient was beginning to experience symptoms suggestive of hypothyroidism. After TSH started to rise and the free T4 got lower, the patient was started on thyroid hormone therapy.

3. This person was hyperthyroid (overactive). Free T4 was greater than 4.5 (meaning that it was sort of off the scale), and clearly high, and the TSH was essentially unmeasurable. The T3RIA was 600 (normal was up to 230 for this lab). So the patient's laboratory findings were consistent with hyperthyroidism. This patient opted to have treatment with propylthiouracil (PTU) and, subsequently, free T4 became measurable, although it was still elevated, and T3RIA was still elevated. With a further increase in the antithyroid medication, the free T4 went into the normal range, but TSH remained suppressed. So, we didn't want to just look at the TSH and conclude that the thyroid levels were still too high, but we wanted to look at the free T4. Then the patient's free T4 started to get low and so medication was cut back. Then the patient actually started to become hyperthyroid symptomatically and interestingly, the T3RIA was elevated (at 400) but the free T4 was still normal. Then, based on the laboratory findings and the clinical evidence, the patient was judged to be hyperthyroid again and this time treatment with radioactive iodine was given. Subsequent to that, the free T4 levels came down, into the low range actually, and the TSH became elevated, thus indicating that the thyroid gland had become underactive as a result of radioactive therapy and that it was appropriate to begin thyroid hormone therapy.

4. In terms of thyroid disease, thyroid cancer is much less common than hyper – or

hypothyroidism. This person had their entire gland removed and then thyroid levels were adjusted. This person, for some reason, required very high levels of Synthroid®, up to 300 mg, and TSH was brought into the normal range (at 2.1). This is a case where measuring the thyroglobulin level is helpful because if this thyroglobulin level starts to go up, then we start looking for possible recurrences of thyroid cancer. In terms of monitoring thyroid hormone levels, the patient had to be taken off of thyroid hormone so that a scan could be done to look for any recurrence of tumor and, off the thyroid hormone level, the TSH went up to 25.6, which was consistent with not having sufficient amounts of thyroid hormone. After the scan, the patient was put back on 200 mg of Synthroid®, which is a very large dose (average dose was more in the range of 75 - 125) and the TSH became 0.8, which is suppressed, but which is probably pretty good for someone who has had their thyroid gland removed. However, if the patient hadn't had their thyroid gland removed, we wouldn't want to suppress the TSH too much, because that could mean the person was getting too much thyroid hormone.

Note: There are studies that suggest that excessive levels of thyroid hormone will increase loss of calcium from bone and that over many years, this could conceivably produce osteoporosis. There is a new awareness to avoid overtreating. For many years, a lot of people have been overtreated with very high doses. One advantage of the sensitive TSH assay is that we can make sure that people aren't being overtreated. Before we really couldn't get an accurate TSH measurement at the lower levels so we couldn't say if people were being overtreated – now we can. So, this supersensitive TSH assay is very useful for monitoring thyroid hormone treatment.

Conclusion

Clearly, there is no thyroid lab test or medical test which is perfect. There are always limitations and exceptions. However, this does not mean that tests should be thrown out or that decisions regarding diagnosis and therapy should be made based on strictly subjective parameters that are influenced by many different factors. If there is a discrepancy between the laboratory tests and the symptoms and physical findings, or if a diagnosis isn't clear, then more detailed lab testing can be done. Also, more investigation of other possible causes of the symptoms can be done. Or, sometimes simply using the test of time is best, i.e., waiting and reevaluating after one, two, or three months and then repeating tests and evaluating in terms of symptoms and exams. But these lab tests are helpful, especially the newer tests.

Questions and Answers

Q If a person is exhibiting signs of hyperthyroidism, but the lab tests indicate that a person should be feeling fine, what should you do?
A If someone is having symptoms of getting too much hormone when the TSH test indicates that person is barely getting enough, it is important to repeat the test, maybe use a different laboratory in case there was an error, and also look for other potential causes of those symptoms.

Q Should women on estrogen replacement or birth control pills take thyroid supplements at separate times?
A Those substances interfere with thyroid blood tests but they don't really cause an increase or decrease in the effective circulating thyroid hormone, but they affect the measurement of the total T4 because they increase the thyroid binding proteins which then cause an increase in the

measurement of the total T4. Pregnancy, oral contraceptives estrogen therapy, hepatitis, and perhaps some other medications may raise the binding protein and therefore will increase the total T4. In most cases those things will not affect the free T4 assays. There are situations where there is abnormal binding of free T4 and free T4 may still be thrown off by that, depending upon the details of how the free T4 test is run, but I'm not saying that people on estrogen have abnormally elevated levels of thyroid hormone. Now pregnancy is a little bit different but there are things that suggest that pregnant women may require more thyroid hormone, but that is a completely different situation.

Q If your blood tests are normal, but you still have symptoms of fatigue, etc., how do you feel about patients fiddling with their Synthroid® dosage under a doctor's care, as sometimes happens with diabetics?
A There is a real pitfall in adjusting hormone dosage based solely on symptoms. The symptoms of hypothyroidism, tiredness, fatigue, food retention, can be caused by other things, which should always be considered. Adjusting dosage when lab tests were normal may be how a lot of people ended up getting excessive levels of thyroid hormone. It's important to use the laboratory test, specifically the TSH test. There may also be some lag time of several months before reaching equilibrium after dosages have been adjusted.

Q Can heart damage result from increased dosages of thyroid hormone?
A Heart damage is not likely to result, especially if someone is not already hyperthyroid and if the increased dosage is moderate and the hormone levels are monitored again in two to three months. However, if someone already has heart disease, you have to be very careful.

Q How do you know whether T4 is actually converting into T3 in a particular person? How can a person be hypothyroid when they are taking T4?
A We don't have a good handle on assessing on how T4 is converted into T3 other than measuring the T3. And just measuring levels of T3 in the blood doesn't really tell us what's happening. In terms of the ultimate effect of thyroid hormone, as discussed earlier, it depends not only on the amount of thyroid hormone and conversion of T4 to T3, but also on the binding of hormone to the receptor and the action of the receptor. So, there are a lot of steps in there. However, some patients insist that the thyroid hormone that they take has some T3 – Synthroid® does not.

Q How important is it to have Synthroid® introduced to the body on a daily basis? What about vacations?
A The half-life of Synthroid ® is seven days which means it takes 21 days to get rid of half of the thyroid hormone. So, if you miss a day, that shouldn't make much difference. If you miss a day every week, then you will essentially be reducing your dosage by 1/7. Some people say if they miss a dose, they feel terrible, although theoretically, that shouldn't happen. You might not even see a measurable change if you missed up to seven days.

Q Are you saying that a free T4 and a TSH can replace the "old" T3, T4, TSH? How can you tell if you're getting a "sensitive" TSH? If someone is already on Synthroid®, and the free T4 and TSH are normal, but the person is exhibiting signs of either hyper – or hypothyroidism, is that when you recommend doing a TRH test?
A Yes, a sensitive TSH assay and a free T4 assay should be sufficient for screening tests

and for monitoring thyroid hormone therapy. Regarding a sensitive TSH assay, I believe most labs are using a sensitive assay, but one way to check would be to look at the normal range indicated on the lab results. If the normal values go down to 0.01, then it is a sensitive assay. If someone is already on Synthroid®, the T3RIA test is not helpful, although the TSH test alone, is probably the most reliable test for monitoring thyroid hormone therapy.

Q I was hyperthyroid and had tried both PTU and Tapazole®. However, each of them made me feel worse, possibly because I also have systemic Lupus. Consequently, I had my thyroid gland radiated, but I still have most of the same Graves' disease symptoms that I had before the ablation was done and I feel terrible. My blood tests are normal. I'm on Synthroid® (for thyroid replacement) and prednisone for the Lupus. I have edema, I have difficulty withstanding heat and I know about the sun and Lupus. A Prednisone has a lot of side effects.

Additional Physician Comment: If the TSH test is normal, that really is a reflection of what's going on in your tissues in terms of your thyroid status. Altering dosage in the face of that invariably turns out to be counterproductive since it's not the answer. The fact is that we are often not certain what the cause of your symptoms are except that it isn't your thyroid status specifically. It may be some basal, autoimmune diathesis which may be a part of Graves' disease that we don't know anything about, because a lot of patients with this condition do have an antibody called antinuclear factor and whether there is some basal state going on in patients that we can't measure in any way, we can't rule that out. But we can say safely that if the values of TSH are really in the middle of the normal range, fiddling with a dose of thyroxine doesn't solve much. I've never seen it really solve anything; the symptoms may go away for a while because of the placebo effect only to return again later.

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Glossary of Thyroid Terms.
TFT Thyroid function test.
T4 Thyroxine, which is the primary hormone produced by the thyroid gland.
T3 Tri-iodothyronine, which is a hormone produced in small amounts by the thyroid gland but actually is derived by metabolism of T4.
T3RIA A radioimmuno assay of T3.
TSH Thyroid-stimulating hormone.
TRH Thyrotropin-releasing hormone.
TBG Thyroid-binding globulin.
TBPA Thyroid-binding pre-albumen.
TAB Thyroid antibody.
Anti-M TAB Antimicrosomal thyroid antibody.
TSI Thyroid-stimulating immunoglobin.
US Ultrasound, which is an imaging technology which is used to evaluate the thyroid, primarily for anatomy.
RAI Radioactive iodine.
RAIU Radioactive iodine uptake test.
T3RU T3 resin uptake test.