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Thyroid antibodies: which, why, when and who?

'This editorial will try to address the questions by concentrating on which antibodies we measure, why they might be useful, when it is useful to measure them and who is best suited to do so.'

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Clinical laboratories are asked to measure a number of different thyroid antibody types. This editorial will try to address the title questions by concentrating on which antibodies we measure, why they might be useful, when it is useful to measure them and who is best suited to do so.

Which?

There are three major types of thyroid antibodies: thyroid peroxidase (TPO) antibodies, thyroglobulin antibodies and antibodies to the thyroid-stimulating hormone receptor (TSHR). The antigen for the first of these, described originally as thyroid microsomal antigen, was identified as TPO in 1985 [1]. TPO is a 107-kD, 933-amino-acid glycoprotein present as a dimer on the apical surface of the thyroid follicular cells [2], as well as in the cytoplasm. It is probably the cell-surface antigen involved in cell-mediated cytotoxicity [3]. There are multiple B cell-reactive epitopes present on human TPO, since different antisera recognize different forms of the antigen in its naive and/or denatured states. These epitopes may be genetically determined and are believed to be stable within each patient [4].

The antigen for the second antibody is thyroglobulin, which is a 670-kD protein with two polypeptide chains of approximately 2768 amino acids each, from which the thyroid hormones tri-iodothyronine (T3) and thyroxine (T4) are produced. Only four to six epitopes are believed to be recognized by B cells and involved in the antibody response to thyroglobulin [5].

The antigen for the third major antibody, the TSHR, is a 74-amino-acid glycoprotein related to the receptors for other hormones, such as luteinizing and follicle-stimulating hormones [6]. The TSHR exists as two subunits, an extracellular A and a trans-membrane B subunit. Some studies have suggested that thyroid-stimulating antibodies preferentially recognize the A subunit, whereas those antibodies recognizing binding sites nearer the cell surface appear to function as blocking antibodies [7]. Antibodies to the TSHR are primary antibodies (i.e., antibodies that are the cause of the disease; others include glomerular basement membrane antibodies in Goodpasture's syndrome and antibodies to the acetylcholine receptor in myasthenia gravis) so they cause disease by binding to the TSHR and either stimulating or blocking the thyroid cells. Patients may have both stimulating and blocking antibodies present and the relative potency of each antibody determines the clinical picture.

Anti-TPO and thyroglobulin antibodies can be detected using complement fixation or by immunofluorescence on thyroid tissue sections, but concentrations are estimated most commonly using an enzyme-linked immunoassay. TSHR antibody assays are more involved, taking the form of bioassays in mice, measurement of thymidine uptake by thyroid cells or inhibition of TSH action on thyroid cells and inhibition of TSH binding to cells or membranes.

The role of heredity in autoimmune thyroid disease is clear, since there is an increased prevalence among family members, first-degree

relatives and twins of patients with the illnesses [8,9]. The most important factor recognized so far is the inheritance of certain major histocompatibility complex (MHC) class II genes. Inheritance of human leukocyte antigen (*HLA*)-*DR3* carries a two- to sixfold increased risk of occurrence of Graves' disease or autoimmune thyroiditis in Caucasians and, in some studies, inheritance of *HLA-DR4* and *DRDR5* has been found to be associated with an increased prevalence of goitrous hypothyroidism [10,11]. In addition, it has been speculated that environmental aspects, such as virus infection, are etiological factors in thyroid autoimmune disease by causing cell destruction and liberating antigens or by forming altered antigens, causing molecular mimicry, inducing DR expression or by inducing CD8⁺ T-cell responses to viral antigens expressed on the cell surface.

Why & when?

TPO antibodies

Low-level immune reactivity to autologous thyroid antigen is probably a normal event and cross-reacting epitopes, aberrant T- or B-cell regulatory mechanisms, inheritance of specific immune response-related genes, malignancy, viral infection and aberrant HLA-DR expression on thyroid epithelial cells may all be involved in the development and progression of thyroid autoimmunity. These are processes that may wax and wane for years as thyroiditis may be apparent clinically, only for it to disappear periodically. As well as being useful in confirming an autoimmune cause for hyperthyroid results, the presence of TPO antibodies makes autoimmune hyperthyroidism more likely in subacute thyroiditis (which may manifest as transient thyrotoxicosis). They will also determine an autoimmune cause (Hashimoto's thyroiditis) in patients with primary thyroid failure and for postpartum thyroiditis in at-risk women. The Whickham study has demonstrated that the presence of antithyroid microsomal (TPO is the antigen involved in the 'microsomal' response) antibodies or raised serum TSH alone was associated with a highly significant increased risk of developing hypothyroidism at 20 years of age [12]. It has also demonstrated that, independent of age, the higher the serum TSH above 2 mU/l, the greater the prognostic significance for the development of overt hypothyroidism in subjects with or without antithyroid microsomal antibodies. Cooper suggests that, given the high rate of conversion of subclinical hypothyroidism to overt hypothyroidism in the presence of circulating anti-TPO antibodies, it makes sense to treat asymptomatic patients with positive antibody tests, even if they have normal serum cholesterol levels [13]. However, because an elevated serum TSH is associated with an increased risk of overt hypothyroidism, even in the absence of anti TPO antibodies, positive antibodies should not be the sole criterion for therapy. He also considers it reasonable to treat subclinical hypothyroidism in pregnant women and in women who have ovulatory dysfunction with infertility.

'It is not generally useful to measure levels of thyroid peroxidase antibodies repeatedly, as treatment is aimed at correcting the consequence rather than the cause.'

Interestingly, Surks and colleagues, in their review and guidelines for diagnosis and management of subclinical thyroid disease, could not either recommend or contest the estimation of TPO antibodies in this context, stating that presence or absence of antibodies does not change the diagnosis or the expected efficacy of treatment [14]. This is in contrast to the recommendations of the Royal College of Physicians [15], the American Association of Clinical Endocrinologists [16] and the American Thyroid Association [17], who are all in favor of treating antibody-positive patients. Subclinical hypothyroidism is, by definition, a laboratory-based phenomenon and not associated with convincing clinical signs. The instigation of treatment, therefore, remains a controversial subject, probably because of the possible associations of subclinical hypothyroidism with comorbidities, such as atherosclerosis, cardiovascular disease and hyperlipidemia. However, the majority, if not the consensus, view seems to favor using the results of TPO antibody assays to give extra confidence to the decision to treat. This is because it is probable that the TSH level is less likely to return to normal without the aid of external thyroxine support in TPO antibody-positive patients. In addition, antibody presence appears to matter more than the antibody titer.

My own unpublished observations suggest that the TPO antibody titer has little bearing on the TSH concentration with modestly increased antibody titers being associated with grossly elevated TSH concentrations and vice versa [SINCLAIR D, UNPUBLISHED DATA]. This suggests that the

disease is T cell-mediated and that the antibodies are merely markers of disease rather than being causative. As far as serial monitoring of TPO antibodies is concerned, they may remain positive for years and do not always indicate normal or abnormal thyroid function. Furthermore, some patients with well defined Hashimoto's disease may have undetected antibodies and, conversely, patients with positive TPO antibodies may never develop thyroid disease. It is not generally useful to measure levels of TPO antibodies repeatedly, as treatment is aimed at correcting the consequence rather than the cause [101].

Thyroglobulin antibodies

Antibodies to thyroglobulin are very common in autoimmune thyroid disease but the major drawback with their clinical application is the lack of specificity, with 11% of the general population having detectable antibodies [18]. As a result, it has been suggested that they have no role to play in routine diagnosis of autoimmune thyroid diseases [19]. However, it has long been recognized that endogenous antithyroglobulin antibodies have the potential to interfere with the immunometric estimation of thyroglobulin. This is the most serious technical problem affecting the use of thyroglobulin as a tumor marker in disseminated thyroid carcinoma, as 25% of such patients have these antibodies [20]. Although radioimmunoassay is less prone to the interfering effect of these antibodies, for safety and

environmental reasons we are keen to move away from assays that require radiolabeled constituents. Interference always causes underestimation of thyroglobulin concentration, as shown by studies on antibody-positive patients with disseminated thyroid carcinoma who have no thyroglobulin detected by immunoassay [21]. The interference can occur even at low antibody concentrations but the continued presence of antibodies in patients who have undergone a total thyroidectomy hints that the immune system is still sensing the presence of thyroglobulin, as complete ablation of thyroid tissue results in the disappearance of antibodies to all the major thyroid antigens [22]; that is, as thyroid tissue is the only source of thyroglobulin, any persisting antibodies must indicate the continued presence of thyroglobulin, for example, possible metastasis. All sera sent for thyroglobulin estimation should, therefore, be tested for the presence of antithyroglobulin antibodies and laboratories should not report undetectable serum thyroglobulin values if antibodies are present and the method produces inappropriately low or undetectable serum thyroglobulin values in patients with disseminated thyroid carcinoma and antithyroglobulin antibodies [23]. A number of studies note that serial estimation of antibody titers can act as surrogate tumor markers in patients with disseminated thyroid carcinoma who have antithyroglobulin antibodies. This is because the antibody titer responds to changes in thyroglobulin concentration but the apparently paradoxical increase after increased thyroglobulin antigen release following radioactive iodine treatment must be taken into account.

Therefore, the investigation of thyroid carcinoma is the only indication for retaining antithyroglobulin antibody testing. Its poor specificity for autoimmune thyroid disease precludes its use in more routine areas.

TSHR antibodies

Antibodies that compete with TSH for TSHR binding sites can either mimic or block the effect of TSH. In addition, because these antibodies can react with multiple epitopes on the TSHR molecule, antibodies that block or mimic the effect of TSH can be present at the same time in the same patient. As a consequence, measuring TSHR antibodies has potential clinical implications in the differential diagnosis of Graves' disease, predicting the outcome of Graves' disease after antithyroid drug treatment and predicting fetal/neonatal hyperthyroidism or neonatal hypothyroidism [24]. However, the fact that most laboratories do not offer this service routinely is testimony to the difficulties inherent in measuring these antibodies. Patients with positive TSHR antibodies, especially stimulating antibodies, and negative TPO antibodies are particularly at risk of Graves' ophthalmology [25]. TSHR antibodies can be used to distinguish postpartum thyroiditis from Graves' disease in postpartum thyrotoxicosis and to predict the probability of either fetal or neonatal thyrotoxicosis in women undergoing treatment or those who have had previous thyroid ablation [26].

'Measuring thyroid-stimulating hormone receptor antibodies has potential clinical implications in the differential diagnosis of Graves' disease.'

Most authorities report that there is a failure to detect TSHR antibodies in approximately 10% of Graves' patients, with the most likely explanation being a lack of sensitivity in the assay, as there are reports of sensitive assays being positive in 98% of untreated patients [27]. By contrast, a study from Birmingham (UK) demonstrated a relatively low prevalence (45.6%) of these antibodies in a stringently defined group of Graves' patients using a radioactive inhibition assay and suggested that they have a limited role in the diagnosis of Graves' disease [28].

Neonatal thyrotoxicosis is suggested by either maternal signs (e.g., tachycardia, exophthalmia and weight loss) or fetal problems (e.g., tachycardia, intra-uterine growth retardation or pre-term birth) or both. The condition disappears with clearance of the transferred maternal antibodies, with clinical signs usually disappearing during the first 4 months of life. The most frequent neonatal clinical signs of thyrotoxicosis are tachycardia, goiter, hyperexcitability, poor weight gain, hepatosplenomegaly, stare and eyelid retraction. To confirm the nature of hyperthyroidism, thyroid-stimulating immunoglobulins can be assayed and the disappearance of the antibodies is a sign of recovery. Indeed, there are reports that maternal Graves' disease has been diagnosed by this route [8]. Guidelines for the use of

tests for antithyroid antibodies in pregnancy have been suggested [29]: euthyroid women on no medication and treated previously with antithyroid drugs alone should not be tested for TSHR antibodies as the risk of fetal hyperthyroidism is low; women treated previously with radioiodine or surgery for Graves' disease should have TSHR antibodies measured early in preg-

nancy and, if they are high, the fetus should be monitored closely for signs of hyperthyroidism and thionamide treatment considered for the mother. Antibody levels can be repeated in the last trimester to predict the risk of neonatal disease. Women treated currently with antithyroid drugs should have their treatment adjusted to achieve a normal free T4 concentration. This should avoid any risk of significant hyperthyroidism in the mother or hypothyroidism in the fetus, which can result from over-treatment of maternal hyperthyroidism. The guidelines suggest that antibodies can be checked in the last trimester and, if they are raised, a formal evaluation of the developing baby could be carried out [30].

Associations with other diseases

Autoimmune thyroid diseases are associated with a number of other organ-specific and systemic autoimmune disorders. For example, the presence of these antibodies is a risk factor for the development of frank autoimmune thyroid disease in patients receiving lithium [31] and amiodarone treatment [32].

Autoimmune thyroid disease is strongly associated with pernicious anemia. It has been known for decades that a large proportion (45%) of patients with autoimmune thyroiditis have circulating antigastric parietal cell antibodies [33] and the reverse association is almost as strong [34]. Up to 14% of patients with

pernicious anemia have primary hypothyroidism and the prevalence of pernicious anemia is increased in patients with autoimmune hypothyroidism [35]. Thyroid antibodies are associated with other autoimmune conditions, such as Sjogren's syndrome, systemic lupus erythematosus, rheumatoid arthritis and coeliac disease. Up to 20% of patients with autoimmune hepatitis or primary biliary cirrhosis have thyroid disease (Hashimoto's or Graves' disease) and a further 10–20% present with thyroid autoantibodies without evidence of thyroiditis. Indeed, liver specialists often receive referrals of such patients with previously 'asymptomatic' autoimmune hepatitis or primary biliary cirrhosis from endocrinologists who have noted abnormal biochemical liver function tests in thyroid disease patients [MCFARLANE I, PERS. COMM.]. As in patients with autoimmune thyroid disease, rheumatoid arthritis and Sjogren's syndrome are also very common in autoimmune hepatitis and primary biliary cirrhosis.

Thyroid antibodies are also found in disparate and seemingly unrelated areas, such as breast cancer, urticaria, mood and anxiety disorders, polycystic ovarian syndrome, recurrent abortion, seasonal allergic rhinitis and systemic vasculitis, among many others. Loose associative relationships do not prove cause and effect and further investigation into these relationships is required before it can be recommended that thyroid antibodies are assayed in each case. These relationships are often with other organ-specific conditions but antithyroid immunity can be seen clearly in association with the non-organ-specific collagen diseases. This indicates that it is a defect in the immune system rather than defects in each organ, probably indicating a shared immunoregulatory defect, which may have a genetic origin, as these diseases often share similar HLA associations [36].

Based on the current state of knowledge on this subject, I would propose the following as a general algorithm for requesting antithyroid antibodies.

TPO antibodies:

- All patients from primary care who have a TSH level between the upper limit of the laboratory reference range and 10 MU/l with a normal free T4 and which persists on repeating at 6 months
- Patients with goiter, regardless of thyroid function tests
- New cases of primary thyroid failure and thyrotoxicosis
- Predicting postpartum thyroiditis samples in at-risk women

I do not believe the estimation of TPO antibodies is indicated:

- If the thyroid function tests are normal and the patient does not have goiter
- In the absence of clinical data on the request form and when the thyroid function tests are normal
- Without knowledge of the thyroid function tests
- If the antibodies have been investigated previously

Indications for requesting TSHR antibodies:

- Predicting the risk of neonatal hyperthyroidism in a woman known to have had Graves' disease
- Distinguishing postpartum thyroiditis from Graves' disease in postpartum thyrotoxicosis

Indication for requesting antithyroglobulin antibodies:

- To assess the risk of interference with thyroglobulin estimation in patients with disseminated thyroid carcinoma

Who?

This is actually two questions: who should instigate these tests and who should carry them out? An audit of subclinical hypothyroidism in Scottish laboratories proposed a scheme for laboratories commenting on such results and advocated that TPO antibodies should be measured if the TSH remains high for some months. If the antibodies are found to be raised, a comment should be added by the laboratory staff to indicate that the patient is at greater risk of developing overt thyroid disease [37]. Our own work [38] and that of Lock and colleagues [39] have shown that it is more effective if laboratory staff themselves take the lead in ensuring that these antibodies are estimated to investigate subclinical hypothyroidism. This can be carried out by reflex testing, following review of the thyroid function tests and the clinical aspects of the patient. Subsequent estimation of the TPO antibody titer can be performed using the same thyroid function test sample. Hence, these antibodies may be offered most efficiently by departments who have ready access to thyroid function test results and the relevant samples. Thyroglobulin antibodies must be measured in conjunction with thyroglobulin itself, so the department offering the tumor marker estimation must also be able to offer the antibody assay to accompany it. TSHR assays are much more complicated and, because of these complexities, their estimation is best left to specialist laboratories and should not be offered in a routine setting.

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Website

- 101 Spencer CA. Assay of thyroid hormones and related substances
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