Serum Thyroglobulin and Thyroid Cancer

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Thyroglobulin is a large glycosylated protein (MW 660,000 daltons) which is synthesised and secreted by the thyroid cell into the follicular lumen. In the thyroglobulin molecule the iodotyrosines undergo oxidative coupling to form the iodothyronines thyroxine (T4) and triiodothyronine (T3). Production of thyroid hormones results from micropinocytosis of iodinated thyroglobulin from the colloid into the thyroid cell and its hydrolysis by lysosomal enzymes. The resulting T4 and T3 diffuse across the basal membrane into the bloodstream whilst iodotyrosines are de-iodinated and the iodine recycled. Thyroglobulin thus acts as the template for thyroid hormone synthesis and a storage form of inactive thyroid hormones and iodine. Thyroid function and activity are under the positive control of thyrotrophin (TSH) which stimulates many steps of thyroid cell metabolism including protein synthesis, iodothyronine synthesis, thyroglobulin secretion into the follicular lumen, and replacement of micropinocytosis of thyroglobulin by massive colloid macropinocytosis [1]. The action of TSH on the thyroid cell is mediated via adenylate cyclase activation and recent work has shown that transcription of the thyroglobulin gene is regulated by cyclic AMP [2].

For many years thyroglobulin was thought to be confined to the thyroid gland but the sensitive radioimmunoassays now available have demonstrated that the protein is unequivocally present in the peripheral circulation of man. It is assumed that some of the protein escapes the potent lysosomal mechanism of the thyroid gland and crosses the basal membrane with the thyroid hormones to appear intact in the circulation. The first radioimmunoassays for thyroglobulin in human, monkey and rat serum were reported in 1967 [3]. A wide variation in the normal range of human serum thyroglobulin levels has been reported but a recent international co-operative study evaluating serum thyroglobulin standards has shown this to be due to remarkable interlaboratory variations [4]. No age-related variation of thyroglobulin levels has been reported (except in children) although several studies have reported higher values in women than men, higher values in pregnant women at delivery than non-pregnant controls, and elevated thyroglobulin levels in cord blood from newborn infants. Serum concentrations in man appear to be closely regulated within a narrow range suggesting a mechanism for controlling thyroglobulin release. Thyroid hormone administration suppresses thyroglobulin levels and after injection of thyrotrophin releasing hormone, TSH or thyroid stimulating immunoglobulins, the levels of serum thyroglobulin rise. The data support the concept that thyroglobulin is a normal secretory product of the thyroid gland under the control of TSH, but the mechanism of this 'secretion', rather than simple diffusion, remains unknown [3].

Once radioimmunoassays for thyroglobulin had been established and validated, it was of immediate interest whether serum concentrations of thyroglobulin invariably reflected the
functional status of the thyroid gland and would therefore be a useful adjunct to tests of thyroid function. It quickly became apparent, however, that serum thyroglobulin concentrations are elevated in a variety of thyroid disorders thus severely limiting its clinical application. High levels were found in patients with endemic goitre and multinodular goitre, during the acute phase of thyroiditis, in subjects with congenital thyroxine-binding globulin deficiency, and in patients with benign thyroid adenomas as well as differentiated follicular or papillary carcinomas. In contrast thyroglobulin levels in patients with undifferentiated tumours or medullary carcinoma of the thyroid are normal. In addition serum concentrations are high in patients with Graves' disease and although it has been shown that the mean serum thyroglobulin concentration at the end of anti-thyroid treatment is lower in patients who sustain a remission, measurement of serum thyroglobulin concentration in individual patients is of little use in predicting eventual outcome [5]. Its clinical usefulness may be further limited in this situation (and in autoimmune hypothyroidism) by the presence of endogenous anti-thyroglobulin autoantibodies which interfere in some assay procedures.

The interest in thyroglobulin measurements in thyroid cancer originated from initial observations that serum concentrations were often elevated in patients with differentiated thyroid carcinoma and thus it was apparent that follicular or papillary neoplasms, like normal thyroid, produce thyroglobulin that may be measured in peripheral blood by sensitive radioimmunoassay techniques [3, 6, 7]. The mechanism responsible for this finding is not known although several have been proposed: a circulating thyroid stimulator which induces release of thyroglobulin by the tumour, a deficiency of protease activity needed for the hydrolysis of thyroglobulin, increased tumour vascularity or capillary permeability, or alteration of tumour cell polarity with consequent reduction of thyroglobulin storage in colloid spaces. The magnitude of the serum thyroglobulin rise has been related to tumour mass, degree of differentiation, proportion of thyroglobulin-synthesising tumour cells, and location of metastases, those in bone being associated with the highest values. It is clear, however, that measurement of serum thyroglobulin is of no use in the differential diagnosis of thyroid carcinoma at presentation. Patients with histologically-confirmed differentiated thyroid cancer may have serum Tg values within the normal range whereas high serum thyroglobulin levels occur as frequently in the presence of benign as in malignant nodules. Because thyroid tissue, normal or neoplastic, is presumed to be the only source of circulating thyroglobulin it was suggested that after complete surgical and radioiodine ablation, serum thyroglobulin should not be detectable unless tumour tissue remained. After incomplete thyroidectomy, or after thyroidectomy and incomplete radioiodine ablation, serum thyroglobulin levels obtained while patients are not receiving thyroid hormone replacement do not discriminate between the presence of residual normal thyroid tissue or tumour. Conversely, when blood samples are taken during thyroid hormone suppression of normal thyroid tissue, there are several reports of an excellent discrimination [8–12]. The observations that in patients with known papillary or follicular thyroid carcinomas who have undergone apparently definitive treatment, serum thyroglobulin concentrations fall to normal or undetectable values in the absence of residual tumour, but remained abnormally high in the presence of known metastases or recurrence, have led several authors to propose that serum thyroglobulin measurement is a valuable tumour marker in the continued observation of patients with treated differentiated thyroid carcinoma. It is in this area therefore that the measurement of serum thyroglobulin has found an important and valuable place in the management of patients with thyroid cancer.

Data from Birmingham compiled in collaboration with Royal Liverpool, Royal Marsden and Guy's Hospitals [9] have shown an excellent correlation between serum thyroglobulin measurements and disease activity in over 400 patients with differentiated thyroid cancer treated by surgery with or without radioiodine who have now been observed for up to seven years. Serum
thyroglobulin concentrations (less than or greater than 5 μg/l) reflected the presence or absence of cancer assessed on clinical, radiological and radiiodine scanning criteria in 83 per cent of patients not receiving thyroxine replacement; this concordance improved to over 95 per cent when serum thyroglobulin measurements were obtained whilst patients were taking thyroxine. This improved specificity of a high serum thyroglobulin measurement in patients taking thyroxine is thought to be due to suppression of TSH secretion and thus presumably suppression of any residual normal thyroid tissue, assuming that the tumour is autonomous. It is probable that for maximum discrimination the dose of thyroxine must be sufficient to suppress TSH secretion and not simply to return elevated values to the normal range; this aspect may become clearer with the advent of radioimmunoassays for TSH which have markedly improved specificity. Although a low thyroglobulin value after presumed definitive treatment usually predicted continuing freedom from cancer this was not always the case as evidenced by five patients in whom cancer reappeared; however in all instances this was associated with a rise in serum thyroglobulin. A recent large study from Sweden has confirmed these findings [11]. Over 250 patients were evaluated and serum thyroglobulin levels measured during thyroxine treatment accurately predicted the results of ¹³¹I total body scans in all but two cases; the authors concluded that determination of serum thyroglobulin concentrations can replace total body iodine scans in most patients who have undergone ablative treatment for differentiated thyroid cancer. There are, however, some discrepancies in these and other studies between the concentration of serum thyroglobulin and disease status in both directions. Serum thyroglobulin can occasionally be detected in the serum of patients without clinical, radiological or scan evidence of metastases and in the absence of residual thyroid tissue demonstrable by total body scan [5, 9, 13]. Accepting that non-specific interference in the thyroglobulin assay has been excluded it is possible that the scanning methods were not sensitive enough to detect small amounts of residual or metastatic thyroid tissue because of technical limitations or dose of ¹³¹I used, that the tumour tissue did not trap iodine, or that patients do harbour occult tumour which subsequently declares itself. We believe that the finding of raised levels of thyroglobulin in the serum of patients thought to have no tumour may indicate the presence of cancer before it has become detectable by other means. We have seen eight patients in whom a persistently high serum thyroglobulin concentration heralded the appearance of a previously occult cancer up to three years later.

Of more concern are those patients with residual, recurrent or metastatic thyroid carcinoma in whom serum thyroglobulin values are undetectable, i.e. false negative determinations. Some thyroid cancers may not produce thyroglobulin at all or they may produce abnormal molecular forms that are not recognised by the antibody used. Several individual case reports have indicated that undetectable serum thyroglobulin measurements during thyroid hormone replacement treatment do not exclude the presence of metastases and some authors have suggested that administration of hormone should be stopped before measurement of serum thyroglobulin to minimise the number of false negative test results [5, 13]; this procedure would of course increase the number of false positive results and may be related to the regimen of thyroid hormone suppressive treatment. A review of published reports up to 1982 [14] noted that in only eight were patients reported as taking thyroid hormone replacement at the time of blood sampling: in six of these very good correlation was reported between serum thyroglobulin value and thyroid status. In two papers non-concordance was reported and in both of these series, samples were taken whilst patients were on triiodothyronine, not thyroxine; in all cases the predominant discrepancy was in false negative results, i.e. serum thyroglobulin concentration was very low or undetectable despite positive iodine scans. The reason for this discrepancy is not clear. Triiodothyronine in 'physiological' doses in fact does not suppress TSH as effectively as does thyroxine. An earlier study in 1977 showed that pre-treatment of normal subjects
with triiodothyronine impairs the acute thyroglobulin response to TSH showing that there is a direct suppressive effect of triiodothyronine on the thyroid gland [15]; this observation may have some relevance to triiodothyronine suppression of neoplastic tissue and needs further study. Our view is that serum thyroglobulin monitoring in patients with thyroid cancer may not be as helpful in those patients taking triiodothyronine.

The results that we have reported were obtained from a series of patients regardless of the presence of circulating anti-thyroglobulin autoantibodies. Most workers who have evaluated the usefulness of serum thyroglobulin in monitoring patients with thyroid cancer have excluded from their studies those with detectable endogenous anti-thyroglobulin antibodies, resulting in the exclusion of up to 25 per cent of patients. In our studies the correlation between clinical status and thyroglobulin measurement appears excellent whether anti-thyroglobulin antibody is present or not. The assay of serum thyroglobulin is not influenced in any consistent way by the addition of different sera containing varying concentrations of anti-thyroglobulin [16]. It is not clear why this is so but may relate to the affinity of the antiserum or its antigenic recognition sites. A recent study has reported the production of a monoclonal anti-thyroglobulin antibody which recognises a determinant different from those recognised by anti-thyroglobulin autoantibodies and thus did not cross-react with anti-thyroglobulin antibodies [17]. This method was excellent for measuring serum thyroglobulin concentration regardless of the presence of anti-thyroglobulin autoantibodies. It is important for all laboratories to evaluate their own assays to determine whether sera with endogenous anti-thyroglobulin antibodies need to be excluded from clinical trials of the usefulness of serum thyroglobulin assay to aid in the detection of thyroid carcinoma. There are a number of other factors which make comparative analysis of published series difficult. These include variations in the sensitivity and precision of the thyroglobulin assay used, the reliability of the methods employed to determine the presence (or confirm the absence) of residual tumour or metastases, heterogeneity of the treated groups, patient selection, population size, and lack of information concerning compliance and adequacy of thyroid hormone suppressive treatment at the time that thyroglobulin measurement was obtained.

On the basis of several carefully conducted studies with thoroughly characterised radioimmunoassays it seems reasonable to propose that the practice of performing routine neck or whole body scans in all patients after withdrawal of suppressive thyroid hormone treatment is no longer necessary. It is now possible to measure thyroglobulin in blood samples taken at regular intervals while suppressive thyroxine treatment is continued and to perform radioiodine scans only when the patient’s serum thyroglobulin remains above a level defined for each assay, or where there is clinical evidence suggesting recurrence. Monitoring of serum thyroglobulin would allow more frequent testing and in most patients would obviate the need for complex and costly investigations. These simple steps are proving to alter significantly the management of patients with differentiated thyroid carcinoma.

The next stage of our understanding of potential links between thyroglobulin and thyroid cancer lies in detailed studies of the thyroglobulin gene [18]. The thyroglobulin chromosomal gene is extremely large and has been assigned to the long arm of chromosome 8 close to the oncogene c-myc. The relative proximity between the thyroglobulin gene and the c-myc oncogene on human chromosome 8 is probably no more than coincidence but it will be important to examine the possibility that certain thyroid cancers could involve gene rearrangement placing the c-myc oncogene under the control of the thyroglobulin promoter. A series of polymorphic sites have been demonstrated at the 5' end region of the gene and characterisation of restriction fragment length polymorphism at the thyroglobulin gene locus will allow testing of the hypothesis that a given thyroid disorder (including thyroid cancer) is linked with the thyroglobulin gene.
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