Thyroglobulin (Tg) measurements in tissue and serum play an important role in the management of differentiated thyroid cancers. In the post thyroid ablation scenario, Tg estimation plays a very important role for detection of recurrence and metastasis. Although there could be Tg autoantibody interference in some patients, Tg is a highly specific and sensitive tumor marker for differentiated thyroid cancer in those where the autoantibody is absent. The clinical sensitivity of Tg is greatest when endogenous TSH is elevated before radioiodine imaging. Tg assays and radioiodine scans are independent indicators of residual or metastatic thyroid cancer, indicating that both these tests are complimentary. Immunohistochemical detection of Tg in surgical specimens is useful in the differential diagnosis of tumors of unknown origin. In future, measurement of Tg mRNA to detect circulating tumor cells may help to overcome some of the current limitations.

Key words: Thyroglobulin – Serum estimation -Antibodies – mRNA testing - Differentiated thyroid cancer – Radioiodine – Thyroxine – TSH – Diagnostic use

Thyroglobulin (Tg) is a unique marker which is specific for the thyroid follicle from which it is synthesized. The serum levels estimated reflect the thyroid mass and the extent to which the gland is stimulated. Higher levels during follow up indicate recurrence of the disease. Advances in laboratory technology have helped in overcoming many of the earlier problems in estimation. The specificity of the marker is utilized to identify the tissue of origin when an immunohistochemical study is performed.

Tg is synthesized by the thyroid follicular cell and its synthesis and secretion is the characteristic of a differentiated follicular cell (FRANCESCHI et al. 1996). Tg specifically represents thyroid tissue as there is no evidence of transcription of Tg gene by non-thyroidal tissues. Hence Tg expression a reliable specific indicator of the presence of thyroidal epithelial cells, either benign or malignant (WHITLEY and AN 2004). For clinical usage, although Tg is specific for thyrocyte origin, estimation by either qualitative or quantitative methods would be unable to distinguish between normal gland, tumors and malignancy. Tg has a half life of about 65 hours and it takes about a month for the body to remove Tg completely after total thyroidectomy (HOCEVAR et al. 1997). However under clinical circumstances, it could take a year or more for Tg levels to reach a nadir limits of assay detectability following primary therapy in DTC (OZATA et al. 1994).

Serum measurement of thyroglobulin

Tg estimation is a specific marker for the thyroid gland but not for malignancy, since it can be synthesized either by the remnant normal gland or the tumorous part. The serum Tg is a reflection of the mass of differentiated thyroid tissue present, any inflammation or injury to the thyroid gland which results in the release of Tg and the magnitude of stimulation of the thyroid stimulating hormone (TSH) receptor (BALOCH et al. 2003). Normally, release of Tg is under the control of TSH (VAN HERLE et al. 1979) and can be suppressed by exogenous thyroid hormone. Metastatic DTC commonly produce Tg even when TSH secretion is fully suppressed since TSH is only one of the determinants of serum Tg. Hence measurement is important in follow-up of thyroid cancers (SHEPPARD, 1986). The histo-
logical type of carcinoma influences both basal Tg and responsiveness to recombinant human TSH (rhTSH) (ROBBINS et al. 2004). Partly contradicting this is the opinion that grade of the tumor does not indicate the Tg synthesizing capability. Poorly or moderately differentiated thyroid cancer can produce high levels of serum Tg, whereas highly differentiated carcinomas may produce low serum levels (DRALLE et al. 1985; SCHLUMBERGER et al. 1981). Basal Tg level directly correlates with the number of lesions, and is highest in patients with follicular carcinoma, bone metastasis and lowest in those with papillary thyroid carcinoma and cervical metastases. The increase in the serum Tg after rhTSH treatment is highest in papillary thyroid carcinoma and is not influenced by tumor volume or by the site of metastatic lesions.

Tg is usually measured in serum although measurements can also been made in thyroid cyst fluids and material obtained by fine needle biopsy of thyroid nodules. Two commonly used methods of measurement are radioimmunoassay (RIA) and immunometric assay (IMA). The first clinically useful assay for routine measurement of Tg in human sera by competitive RIA was a rabbit polyclonal antibody (VAN HERLE et al. 1973). The subsequent development of monoclonal antibodies led to IMA. The IMA methods include isotopic immunoradiometric and non-isotopic primarily chemiluminescence (BALOCH et al. 2003). The advantage of IMA method is a shorter incubation time, an extended dynamic range for the assay and a more stable labeled antibody reagent that is less prone to labeling damage compared to that of RIA. In addition, these assays of Tg are more sensitive, a wider concentration range and a potential for automation (WHITLEY and AIN 2004). Recent meta-analysis data suggest that IMA method after thyroid remnant ablation and thyroid hormone withdrawal has the highest pooled sensitivity (EUSTATIA-RUTTEN et al. 2004). The same sensitivity decreased if the patient was tested while on thyroxine therapy. Despite advances in instrumentation and improvements in assay sensitivity and specificity, IMA assays have technical limitations, such as suboptimal sensitivity and precision, high-dose “hook” effects, differences in standardization, and autoantibody interference. IMA methods are more prone to interference by Tg antibodies (TgAb), which cause an underestimation of serum Tg levels (CLARK and BECKETT 2002; SPENCER 1996; SPENCER and WANG 1995; TORRENS and BURCH 2001). Most Tg methods now claim analytical/functional detection limits below 1.0 ng/mL, some as low as 0.2 ng/mL and are able to detect Tg below the lower limit of the euthyroid reference range (about 1–3 ng/mL) (GIOVANELLA and CERIANI 2002; MIKOSCH et al. 1999; MORGENTHALER et al. 2002). To enhance diagnostic sensitivity, the serum Tg response to either endogenous TSH (thyroid hormone withdrawal) or rhTSH can be used (PACINI et al. 2001). Comparing previous and current values may be particularly helpful to detect small changes in serum Tg in high-risk patients.

A hook effect occurs when an excessive amount of antigen overwhelms the binding capacity of the capture antibody. Very high concentrations of Tg may be encountered when patients have advanced metastatic disease (SCHLUMBERGER and BAUDIN 1998). In some IMA assays, excessive amounts of Tg (>1000 ng/ml) can exceed the binding capacity of the capture and signal antibodies, which makes the antibodies unavailable to form antibody-antigen complexes. Known as the high-dose hook effect, this phenomenon results in a severe underestimation of the Tg concentration (SPENCER 1996). For the reasons mentioned earlier, although IMA methods are more popular, some laboratories estimate Tg with RIA methods especially in those with TgAb although no method could be of fool proof. It is common practice to analyze samples in such assays at several dilutions although newer Tg assays do not demonstrate high-dose hook effects except with very high Tg concentrations (>30,000 ng/mL) (WHITLEY and AIN 2004). In addition, heterophilic antibodies can cause significant interference problems in any Tg immunoassay and are difficult to detect (PREISSNER et al. 2003). To improve method-to-method variability, through collaborative effort sponsored by the Community Bureau of Reference of the Commission of the European Communities an international Tg reference preparation (CRM-457, BCR Brussels) was developed in 1994 (FELDT-RASMUSSEN et al. 1996; FELDT-RASMUSSEN and SCHLUMBERGER 1988). Most Tg immunoassays are now calibrated directly or indirectly against this standard. Even then, serum Tg levels that are determined by methods using this standard vary by as much as four-fold (BALOCH et al. 2003). Inter-method differences are likely to remain and particular care should be taken when comparing serum Tg values that are obtained by using different immunoassay systems. For long-term follow-up of patients who have cancer, it is strongly recommended that serial Tg measurements in a patient be made in the same laboratory using the same assay method because Tg values are not interchangeable among laboratories, even when using the international Tg standard. The assay should have a functional sen-
sitivity of at least 1 ng/ml, with a normal lower Tg limit of 3 ng/ml, and the results should be standardized to the CRM-457 standard, which reduces but does not eliminate biases between Tg methods (MAZZAFFERRI et al. 2003; SPENCER et al. 1996).

**Thyroglobulin antibodies**

The most serious technical problem that limits the clinical value of Tg determinations is interference that is caused by endogenous TgAb. These are seen in autoimmune thyroid diseases including Grave’s and Hashimoto’s thyroiditis. TgAb is more common in patients with sporadic goiter, multinodular goiter and cancer than in the general population. TgAb is 330 kd molecule which is often undetectable using older techniques. The TgAb is polyclonal, belongs to IgG class not restricted to a particular subclass, although IgG2 is the predominant class in DTC (CATUREGLI et al. 1994). TgAb are detected in a higher percentage of DTC patients than the general population (25 % versus 10 %, respectively) (SPENCER et al. 1998). The extent and type of interference that is caused by these autoantibodies depends on the specific Tg method that is used by the clinical laboratory. In IMA, reported Tg concentrations can be falsely lowered by autoantibodies that bind Tg and prevent antigen interaction with assay’s antibodies (MARIOTTI et al. 1995). Underestimation of the total Tg concentration is characteristic of noncompetitive IMA assays, presumably because these assays measure free Tg and are unable to quantify Tg that is complexed with TgAb. Overestimation of Tg is typical of most competitive immunoassays that are capable of measuring free and TgAb-bound Tg, although underestimation may also be observed (SPENCER et al. 1998). Hence, in TgAb positive sera while IMA methods generally underestimate the Tg value, RIA methods could yield either a false high or false low values. The clinician must be aware of such interference by TgAb resulting in false values. A false negative result may cause a delay in detecting and treating recurrent or metastatic disease. A false positive result can lead to further clinical studies or therapy and unnecessary patient anxiety. Thus, presence of TgAb invalidates the negative predictive value of Tg.

Exogenous Tg recovery studies have been used in an attempt to identify TgAb interference. Many laboratories choose to prescreen sera for TgAb and restrict the use of faster IMA methods to specimens without TgAb and reflexing TgAb-positive sera for measurement by a slower Tg RIA method. Although no Tg estimation is totally free of autoantibody interference, an assay that uses a high-affinity rabbit polyclonal antibody was reported to give clinically useful results in TgAb-positive patients (BLACK and HOFFENBERG 1983). Recovery studies do not detect TgAb reliably because of heterogeneity of TgAb and recent recommendations have discouraged their use for this purpose (BALOCH et al. 2003; MASSART and MAUGENDRE 2002; SPENCER 1996).

The TgAb concentration should be measured in all patient sera prior to Tg analysis because low levels of TgAb can interfere with serum Tg measurements causing either falsely low, undetectable or high values depending on the Tg method used (BALOCH et al. 2003). Quantitative measurement of TgAb in serum is used widely to identify antibody interference when patient specimens are submitted for Tg analysis (HITIANNAKIS et al. 1999). However it should be appreciated that TgAb levels need not correlate with degree of interference. This variability is in part due to heterogeneity of TgAb itself. Approximately 40 different immunoreactive areas (epitopes) have been identified within the Tg molecule, although only a few sites react with the majority of naturally occurring Tg autoantibodies (TORBEN and BURCH 2001). On the flip side, serial serum TgAb measurements may by itself be an independent prognostic indicator of the efficacy of treatment for, or recurrence of, DTC in TgAb-positive patients (RUBELLO et al. 1992; SPENCER et al. 1998). It is important for the laboratory to measure TgAb in every specimen sent for Tg measurement. Patients who have TgAb are not able to rely upon the absence of measurable serum Tg levels as an index of disease status. In addition, a patient’s TgAb status may change from positive to negative or vice versa, and the trend in TgAb values over time (i.e. 6 to 12 months) gives additional information on how well the tumor is responding to treatment. While one study showed that thyroid cancer patients expressing TgAb may have a better prognosis than those without (BAKER and FOSSO 1993), the persistence of TgAb following thyroideectomy and radioiodine ablation may in itself indicate the presence of residual thyroid tissue and an increased risk for recurrence (SPENCER et al. 1998). However a downtrend in TgAb levels over time (years) is a good sign that treatment is effective (CHUNG et al. 2002). Antigenic stimulation should cease after the thyroid is completely ablated and autoantibody production is expected to decrease with time. A patient who had Tg antibodies detected at diagnosis becomes anti-
body negative within 1 to 4 years of being rendered athyreotic. The continued presence of TgAb after treatment suggests that the patient has persistent disease (Hryiannakis et al. 1999). An increasing level of TgAb is often the earliest sign of residual or recurrent disease (Whitley and Ain 2004).

Thyroglobulin mRNA testing

To overcome the interference of TgAb, methods that detect Tg messenger ribonucleic acid (Tg mRNA) circulating in the peripheral blood of patients with thyroid cancer are being developed, although their utility has been questioned (Takano et al. 2001). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of tissue specific mRNA has been used to detect circulating cancer cells in the peripheral blood of patients with melanoma, prostate and breast malignancies (Baloch et al. 2003). The availability of Tg-specific primers now allows the application of this technique to the detection of Tg mRNA transcripts in blood (Biscola et al. 2000; Ringel et al. 1999). Both quantitative and qualitative methods have been developed (Wingo et al., 1999). Quantitative methods may be required to interpret positive results because detectable Tg mRNA usually is found in all subjects, including those who do not have a thyroid (Bugalho et al. 2001). Some reports suggest that Tg mRNA complements FNA cytology in the preoperative differentiation of benign from malignant thyroid disease and their combined use may save unnecessary surgeries (Chinnappa et al. 2004; Wagner et al. 2005). Contradictory reports of both correlation and non-correlation with presence and absence of metastasis have been reported (Biscola et al. 2000; Eszlinger et al. 2002; Grammatopoulos et al. 2003; Ringel et al. 1999; Span et al. 2003). In addition, poor correlation between serum Tg and Tg mRNA have been reported suggesting non-thyroidal origin (Eszlinger et al. 2002; Takano et al. 2001). Lymphocytes and granulocytes could also express Tg (Bugalho et al. 2001). Another study suggested that this method could be useful only in papillary thyroid carcinomas (Bellantone et al. 2001). Many problems need to be addressed before Tg mRNA is widely used in clinical practice. Some such issues include sensitivity, tissue specificity, discordances between serum Tg and Tg mRNA and prognostic significance of Tg mRNA in those with undetectable Tg. Tg mRNA test is more expensive than a serum Tg measurement. It is likely that if Tg mRNA measurements are shown to be clinically useful, these tests will be reserved for high-risk or TgAb-positive patients in whom serum Tg measurements are diagnostically unreliable. Currently, Tg mRNA is not useful in the follow-up of DTC (Elisei et al. 2004; Verburg et al. 2004).

Thyroglobulin in Diagnosis

Patients with DTC may produce excessive amounts of Tg or release stored Tg in increased amounts into the blood stream. It is suggested that a value twice the normal is indicative of malignancy in the absence of thyrotoxicosis. Unfortunately there is considerable degree of overlap in measured values with a number of benign conditions. In a prospective study of 100 patients, Tg levels were significantly higher in patients with thyroid carcinoma than in those with benign disorders, but the predictive value was low (Christensen et al. 1984). Since Tg is raised in a variety of benign thyroid disorders as well as DTC over a wide range, pre thyroidectomy estimation of Tg is of little usefulness for the purposes of diagnosis (Refetoff and Lerner 1983). A major value of measurements of serum Tg level is in the follow up management of DTC.

Thyroglobulin in differentiated thyroid cancer

Amongst factors influencing serum Tg, TSH is the single most important regulator. Hence it is difficult at times to interpret Tg values without the knowledge of corresponding TSH values. Similarly, there is a relationship between thyroid mass and serum Tg. As a guide, 1 g of normal thyroid releases 1 ng/ml of Tg when TSH is normal, while 0.5 ng/ml of Tg is released when TSH is suppressed and below 0.1 mU/l (Baloch et al. 2003). It is useful to obtain a preoperative Tg level in DTC in order to recognize those tumors capable of secreting Tg. The blood sample must be drawn prior to fine needle aspiration or a fortnight following the procedure for the values to be representative (Baloch et al. 2003). About two-thirds of patients with DTC have raised Tg values. The post surgical monitoring of Tg levels is useful only in these patients. Normal values in the remaining third is less assuring during follow up as these tumors may lack the ability to secrete Tg and a detectable post-operative serum Tg in these patients could represent a large tumor volume. Low preoperative Tg values may correlate with less differentiated tumors having a poorer prognosis (Torrens and Burch 2001).
The sensitivity of post-operative serum Tg monitoring for detecting recurrence will be highest when the tumor is relatively small (≤ 2 cm diameter) and the pre-operative serum Tg value is high. Accepting that pre-operative values are somewhat useful, how practical is it to estimate Tg in all goiters before diagnosis, or how feasible is it to wait for a fortnight following diagnosis only for estimation of Tg?

In the first few weeks following surgery, the Tg level is dependent on the completeness of surgery and whether the patient receives thyroxine therapy. In a typical case following total thyroidectomy, and thyroxine therapy, the serum Tg levels fall with a half life of 2-4 days (Hocevar et al. 1997). The surgical trauma related Tg is likely to settle within a month or two. Hence estimation can be carried out at the end of two or three months with suppressive thyroxine therapy being initiated post surgically. The pre-surgical value and the 6-8 week post surgical value may provide the information that guides the overall treatment. One study has suggested that a serum Tg level of 70 ng/ml or higher at months after initial surgery (Schlumberger and Baurdin 1998). The residual gland after thyroidectomy would ideally be less than two grams. The serum Tg value is expected to be less than 2 ng/ml (expected TSH being <0.1mU/l) (Baloch et al. 2003). Higher values suggest larger thyroid remnant or metastatic disease. Hence the appearance of Tg in patients without residual normal thyroid tissue like in post surgery post radioiodine ablative status, indicates the development of metastasis and should lead to further evaluation for additional therapy (Spencer and Wang 1995). Two Tg levels, one just before ablative therapy and another about 6-12 months from surgery measured under TSH stimulation have a predictive value of disease progression (Tourbeau et al. 2004). In the multivariate analysis, the levels at 1 year from surgery were found to be most important. A non-suppressed serum Tg level greater than 3 ng/ml measured 1 year after operation was an independent risk factor for tumor recurrence irrespective of other factors in DTC (Bohm et al. 1999).

During thyroid hormone suppression therapy, Tg levels are undetectable in 98% of patients and less than 5 ng/ml in 100% of patients without clinical evidence of disease after total thyroidectomy and radioiodine therapy (Torrens and Burch 2001). The pattern of serial serum Tg measurements, made when the patient has a stable TSH, is more clinically useful than an isolated Tg value. When the TSH level is stable during thyroxine therapy, any change in the serum Tg level will reflect a change in tumor mass. Recurrence of tumors is usually associated with a progressive rise in serum Tg in those who have elevated Tg levels pre-operatively (Spencer and Wang 1995). Tg assessment while on suppression of TSH by thyroid hormone is insensitive but is specific for delineating presence of thyroid carcinoma. On the other hand, absence of Tg while on thyroid hormone suppression, is no guarantee of absence of disease. Some studies have evaluated the utility of Tg estimation in those patients with TSH suppression and thyroid remnant (De Vathaire et al. 1988; Harvey et al. 1990; Schlumberger et al. 1981; Van Wyngaarden and McDougall 1997). The risk of relapse increased significantly when the suppressed serum Tg level was greater than 10 ng/ml. Compared to only 4% of patients with Tg less than 10 ng/ml had recurrent disease, 21% of patients with Tg more than 10 ng/ml had recurrence which increased to more than 43% when Tg was higher than 30 ng/ml (Torrens and Burch 2001).

The most sensitive use of serum Tg determination is when the patient is no longer receiving thyroid hormone and TSH level is elevated (Ozata et al. 1994). Under these circumstances, the absence of serum Tg indicates that little functioning tissue is present although poorly differentiated thyroid carcinoma may not synthesize this protein. The sensitivity of detecting disease by measurement of Tg during thyroid hormone withdrawal is 85% to 95% but may be as low as 50% during TSH suppression or with dedifferentiated tumors (Haugen et al. 1999). Instead of thyroid hormone withdrawal and resultant symptomatic hypothyroidism, rhTSH could be used for both Tg estimation and diagnostic radioiodine scan (Pacini and Lippi 1999). This permits testing without withdrawal of thyroxine suppressive therapy, thus avoiding symptoms of sustained hypothyroidism (Dow et al. 1997). In addition, the theoretical disadvantage of thyroid hormone withdrawal resulting in raised TSH may not be desirable as it could unfavorable in respect to enhancement of tumor proliferation (Lukinac et al. 1996). Comparable accuracy between rhTSH stimulated Tg and Tg estimation after withdrawal of suppressive therapy have been reported (Mazzaferri et al. 2003; Robbins et al. 2001). Activation of TSH receptors on the transformed thyroid folli-
cicular cells stimulate production of Tg resulting in an increase in serum Tg. Levels of Tg could increase 5 to 10 fold when TSH is high as after withdrawal of suppressive doses of thyroxine compared with an estimation done on TSH suppression (SCHLUMBERGER et al. 1980). The threshold TSH value that is used to assess Tg levels conventionally has been 30 mU/l (SCHNEIDER et al. 1981). Serum Tg levels rise with TSH stimulation, but the duration of stimulation is generally longer in the hypothyroid state, resulting in higher serum Tg levels than occur 72 h after the last of two 0.9-mg doses of rhTSH in the euthyroid state (MAZZAFERRI et al. 2003). Tg measurements 4 days after the first rhTSH injection is a very sensitive indicator of presence of malignant thyroid tissue with 100 % sensitivity if the cut off value is 2 ng/ml and 97 % sensitivity if the value is 5 ng/ml (HAUGEN et al. 1999; MAZZAFERRI and KLOOS 2001). A lower cut off value of 1 ng/ml has also been suggested to increase the sensitivity of prediction of disease status (PACINI et al. 2001). Other means suggested to improve accuracy include rhTSH dose depending on the body mass and repeated Tg measurements on days 2 through 7 (PELLEGRITI et al. 2003). The recent meta-analysis showed that when rhTSH was used instead of thyroxine withdrawal, sensitivity remained high while specificity decreased (EUSTATIA-RUTTEN et al. 2004). However when results are doubtful it is advisable to have Tg levels measured after withdrawal of thyroxine for improving diagnostic accuracy (PELLEG-RITI ET AL. 2003).

**Thyroglobulin in tissue specimens**

Immunohistochemical (IHC) methods of detecting Tg in tissues indicate thyroid as the tissue of origin although it would be unable to differentiate between benign and malignant conditions. IHC methods of detecting Tg include peroxidase-antiperoxidase and avidin-biotin-peroxidase methods. Both polyclonal (raised in rabbits) and monoclonal (raised in mice) Tg antibodies are used. Although monoclonal antibodies are costly, they are highly specific and they have reduced background staining. In addition, there is absence of contaminating antibodies and consistency between production lots. The antibodies stain the lumen of thyroid follicles and apical surface of thyrocytes. In carcinomas, anti-Tg antibodies also may label the thyrocyte cytoplasm. Formalin fixed, paraffin-embedded tissue sections and fine needle aspirates give satisfactory results, but frozen tissue may not because Tg is a soluble protein and is washed away easily (TUNG et al. 1995). Most DTC (> 95 %) synthesize Tg and demonstrate positive immunostaining for Tg. In contrast, Tg is rarely detected in medullary or anaplastic (undifferentiated) thyroid carcinomas but some monoclonal antibodies react specifically with Tg in poorly-differentiated carcinomas (BEIJARANO et al. 2000; DE MECCO et al. 1987). Tg which expressed in metastatic lesions and stains for this marker particularly valuable in establishing the origin of metastatic tumors (PACINI and PINCHERA 1999). Positive Tg staining indicates thyroid origin, whereas the absence of staining suggests non thyroidal origin. Thus, metastases of thyroid carcinomas can be distinguished from metastases of adenocarcinomas of non-thyroidal origin with similar histologic appearances, such as ovarian, renal cell, breast, and salivary gland malignancies (LOGMANS and JOBSIS 1984).

Another technique proposed is to estimate Tg in the washout of the needle used for fine needle aspiration of nodes in thyroid cancer and compare with serum Tg. Higher level of Tg in the former would indicate metastasis in the nodes with a sensitivity of 81.4 % (BASKIN 2004; URIU NO et al. 2005). The non-immunoassay approach has been applied to fine needle aspiration biopsy specimens that were obtained from small cervical lymph node metastases. This PCR based technique by amplification of thyroid specific transcripts TSH receptor and Tg was more sensitive for the detection of thyroid cancer than measurement of Tg in the aspirate (ARTURI et al. 1997).

**Thyroglobulin versus radionuclide scan**

Secretion of Tg is TSH dependent resulting in raising Tg levels when suppressive therapy is withdrawn (SCHNEIDER ET AL., 1981). Hence serum Tg is a sensitive marker of persistent or recurrent thyroid carcinoma after total gland ablation by surgery and radioactive iodine. One study compared Tg levels ‘off’ thyroxine treatment and post-ablative radioiodine scan in patients who had undergone a total thyroidectomy. Post-ablative radioiodine scan showed ectopic uptake in 11% of patients with Tg levels below 5 ng/ml, in 24% of those with Tg levels ranging from 6 to 15 ng/ml, and in 46% of those with Tg levels above 15 ng/ml indicating that Tg is a sensitive investigation to detect occult disease foci (TENENBAUM ET AL., 1996). In the post thyroidectomy setting, radioiodine ablation increases the sensitivity of Tg compared to thyroidectomy alone on follow up (SCHLUMBERGER AND BAUDIN, 1998). This is proba-
bly due to ablation of non tumoral thyroid remnants. Following total thyroid ablation, Tg estimation is a better prognosticator compared to radioiodine scan (MENENDEZ TORRE ET AL., 2004). Tg measurement after TSH stimulation often identifies the presence of cancer well before whole body radioiodine scan can localize the tumor (CAILLEUX ET AL., 2000; HAUGEN ET AL., 2002; MAZZAFERRI AND KLOOS, 2002; PACINI ET AL., 2002; PACINI ET AL., 2001). Isolated elevated values of Tg despite radioiodine scans being negative suggests presence of disease in patients who have undergone total thyroidectomy and radioiodine ablation (SCHNEIDER ET AL., 1981). When neck recurrences are studied, neither Tg nor radioiodine scans alone can be considered as reliable indicators for absence of disease. However when both are negative, there is very little chance of persistent disease (BACHELOT ET AL., 2005).

The utility of stimulated Tg measurements in the absence of scanning may be sufficient to allow testing without scans in low-risk patients and hence one extreme view is that Tg can replace radioiodine scan (ERICSSON ET AL., 1984; ROBBINS ET AL., 2002). However such a view is not generally accepted. As many as 13% of Tg positive and negative whole-body scan patients are found on further evaluation to have recurrent or metastatic disease (PACINI ET AL., 1987; SCHLUMBERGER ET AL., 1988). In one study of 233 cases, 43 patients were found to be Tg positive without radioiodine uptake and another three with radioiodine uptake without raise in Tg (RONGA ET AL., 1986). In another study, 76% of patients had high Tg levels but metastasis were detected by means other than radioiodine scan (GIRELLI ET AL., 1985).

It is estimated that about 1.3% to 9.2% of patients with metastasis have low values of Tg when ‘off’ thyroxine (TORRENS AND BURCH, 2001). Such metastatic disease is usually found in the nodes of neck or mediastinum but could be in the bone or lungs. In addition, one study found 21% of patients had no elevation of Tg while radioiodine had picked metastasis (Moser ET AL., 1988). Although the incidence of positive scans with negative Tg is low, it could be important.

Most DTC produce Tg and take up radioiodine. However in some, these functions appear to be separated (SHEPPARD, 1986). Just as thyroid carcinomas may express Tg in the absence of radioiodine uptake, they may concentrate radioiodine in the absence of Tg production. The causes are listed in Table 1. Alternatively, DTC may produce Tg but not secrete the protein into the serum (WESTBURY ET AL., 2000). It is not surprising that neoplastic tissue may lose one function independent of the other. Approximately 90% of all differentiated thyroid cancers but only 60% of their metastases concentrate radioiodine (AIELLO AND MANNI, 1990). These reports indicate that Tg levels alone cannot replace radioiodine scan. In essence, the serum Tg level does not predict radioiodine uptake nor does uptake predicts the Tg level.

The understanding of transcription factors of Tg expression would perhaps throw more light on radioiodine scan. Malignant transformation of thyroid follicular cells may alter expression of thyroid-specific transcription factors and genes that are responsible for differentiated functions, such as responsiveness to TSH, iodide transport, and Tg secretion (WHITLEY AND AIN, 2004). This results in reduced expression of Tg mRNAs in malignant thyroid tissue and decreases progressively as the tumor becomes less differentiated (RINGEL ET AL., 2001; WHITLEY AND AIN, 2004). A key feature of the alterations in gene expression of various thyroid specific transcription factors is that they are not necessarily linked. It is not unusual for some thyroid cancers to lose sodium iodide symporter expression, yet still produce Tg. This results in the clinical situation of negative radioiodine scans, yet elevated serum Tg levels (PINEDA ET AL., 1995). Conversely, although less common, serum Tg levels may be undetectable in the presence of iodide concentrating tumors with positive nuclear scans (MERTENS ET AL., 1999; MULLER-GARTNER AND SCHNEIDER, 1988; ROELANTS ET AL., 1997). Loss of Tg expression by thyroid carcinomas may be correlated with mutation of the N-ras oncogene (BASOLO ET AL., 2000). Thus, Tg assays and radioiodine scans are independent indicators of residual or metastatic thyroid cancer, contrary to recommendations that serum Tg assays can replace scanning for detection of recurrent disease (PACINI, 2002). Hence it is generally viewed that radioiodine scan and Tg are complementary to each other and one should not be used to replace the other (RONGA ET AL., 1986).

The presence of measurable serum Tg with negative radioiodine whole body scans in a patient who has had appropriate thyroid ablation constitutes evidence of presence of thyroid carcinoma. This constitutes patients who have hidden macroscopic or micrometastatic disease and warrants additional diagnostic and therapeutic procedures. It is suggested that thallium may be useful in localizing metastasis in those patients with negative radioiodine scan and raised Tg (HOEFNAGEL ET AL., 1986). Alternative diagnostic approaches use other radiographic and nuclear techniques, including positron-
Table 1
Causes of discordant thyroglobulin and $^{131}$I scans*

<table>
<thead>
<tr>
<th>Elevated Tg &amp; negative whole body scan</th>
<th>Positive whole body scan &amp; negative Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse small metastases too small to be detected despite radioiodine uptake</td>
<td>Tg antibodies</td>
</tr>
<tr>
<td>Intact Tg synthesis with decreased or impaired iodine trapping</td>
<td>Tumor incapable of Tg synthesis or secretion</td>
</tr>
<tr>
<td>Iodine contamination</td>
<td>Immunologically inactive Tg</td>
</tr>
<tr>
<td>Serum TSH adequate to induce Tg synthesis but not sufficient to stimulate radioiodine uptake</td>
<td>False-positive scans due to other causes</td>
</tr>
<tr>
<td>• Tg antibodies</td>
<td>• Head and neck conditions</td>
</tr>
<tr>
<td>• Thyroid tissue remnant increasing the Tg synthesis and decreasing the radioiodine uptake in the metastatic deposits</td>
<td>• Thoracic conditions</td>
</tr>
<tr>
<td>• False-positive elevation of Tg</td>
<td>• Gastrointestinal disorders</td>
</tr>
<tr>
<td></td>
<td>• Renal disorders</td>
</tr>
<tr>
<td></td>
<td>• Ovarian and scrotal disorders</td>
</tr>
<tr>
<td></td>
<td>• Skin contamination or inflammation</td>
</tr>
</tbody>
</table>


Table 2
Normal values

<table>
<thead>
<tr>
<th>Gland</th>
<th>TSH</th>
<th>Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.4-4.0 mIU/L</td>
<td>3-40ng/ml</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;0.1 mIU/L</td>
<td>1.5-20ng/ml</td>
</tr>
<tr>
<td>Lobectomy</td>
<td>&lt;0.1 mIU/L</td>
<td>&lt;10 ng/ml*</td>
</tr>
<tr>
<td>Total thyroidectomy</td>
<td>Either &lt;0.1 Miu/L or high values</td>
<td>&lt; 2ng/ml</td>
</tr>
</tbody>
</table>

* value of < 10 ng/ml decreases (but does not eliminate) the chances of recurrence.

Table 3
Thyroglobulin estimations in different settings

<table>
<thead>
<tr>
<th>Tg measured during TSH stimulation</th>
<th>more sensitive for detecting residual or metastatic DTC than a basal Tg measurement made during thyroxin treatment. Higher sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnitude of the serum Tg increase in response to TSH</td>
<td>provides a gauge of the TSH sensitivity of the tumor</td>
</tr>
<tr>
<td>Tg concentration during thyroxine treatment</td>
<td>more stable indicator of tumor mass than a serum Tg measured when the TSH is high.* Higher specificity</td>
</tr>
<tr>
<td>Pattern of change in serum Tg values (on thyroxine)</td>
<td>better indicator of a change in tumor burden than any single serum Tg value</td>
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</table>

* exact amount of thyroid tissue needed to increase the serum Tg is not known

emission tomography (PET) (Helal et al., 2001). Recently PET could detect recurrent or metastatic disease in 60% of patients enabling surgical resections and showed promising results when Tg levels are higher than 10ng/ml (Muros et al., 2000; Ruiz Franco-Baux et al., 2005).

One approach to this situation wherein Tg is elevated but no anatomical disease is identified is to administer a therapeutic $^{131}$I dose and evaluate the post therapy scan 2–10 days later. Subsequent $^{131}$I measurements can be used to identify the sites of tumor. Efficacy of treating the disease could also be documented by diminished Tg levels (de Geus-Oei et al., 2002; de Keizer et al., 2001; Koh et al., 2003; Pineda et al., 1995; Torrens and Burch, 2001). In one study, 16 of the 17 patients showed a positive scan after therapeutic dose of radioiodine (Pacini et al., 1987). Therapeutic doses of radioiodine in such patients with raised Tg and negative diagnostic radioiodine scan could be beneficial in those with micrometastasis (Kabasakal et al., 2004).
Interestingly, 65% to 75% of Tg positive and scan-negative patients with positive post-therapy scans have improvement in Tg levels after iodine-131 ablation (PA-CINI ET AL., 1987; PINEDA ET AL., 1995; SCHLUMBERGER ET AL., 1988). But, contradictory reports of neither survival benefit or tumor reduction has also been reported under similar situations (VAN TOL ET AL., 2003). However, the effect on long term survival is not obvious and unknown(KAMEL ET AL., 2004). The treatment of Tg positive and scan negative patients with radiiodine continues to be controversial for several reasons. The risk for disease-related morbidity or mortality is not well defined. Radiiodine ablation in patients without obvious residual disease may not necessarily increase survival and there is a risk for the development of secondary malignancies in young patients after a large cumulative dose of radiiodine (McDOUGALL, 1997; ROBBINS, 1999; SCHLUMBERGER ET AL., 1996; WARTOFSKY ET AL., 1998). Therapies of these patients need to be individualized based on risks versus benefits until evidence is available for or against a modality.

Lobectomy and thyroglobulin

The presence of a thyroid remnant, defined as surgically treated patients without subsequent radiiodine ablation, decreases the specificity and the clinical utility of the Tg level (OZATA ET AL., 1994; TORRENS AND BURCH, 2001). After lobectomy, the Tg level is undetectable during thyroxine treatment in only half of the patients. A guide to levels of Tg following thyroid surgery is given in Table 2. In the majority of patients with detectable Tg levels, ultrasound examination of the remnant lobe could show clinically unsuspected micronodules. Due to their small size, fine needle biopsy may be impossible and in case of progression, surgery may be warranted. Citing these data, some suggest total thyroidectomy for all patients with DTC. Following thyroid hormone withdrawal, the Tg level is poorly informative in these patients, because it can be produced both by normal and by neoplastic thyroid tissue (SCHLUMBERGER AND BAUDIN, 1998). One study reported 84 patients on follow up after hemithyroidectomy and found 3 of them with recurrence to have Tg levels above 10 ng/ml; however, the number of patients having this level without recurrence was not indicated (HARVEY ET AL., 1990). Levels above 10 ng/ml carries a 5.5 fold increased risk of recurrence (de VATHAIRE ET AL., 1988). In conclusion, Tg estimation in patients undergoing hemithyroidectomy or lobectomy is not very useful and has a low predictive value.

Long term follow-up

A precondition for use of Tg in the follow up of DTC includes total ablation the thyroid. This includes surgical ablation by total thyroidectomy, followed by a radiiodine ablation of the remnant to a dose of at least 300 Gy, to ensure that any measurable Tg values represent residual malignant disease (MAXON ET AL., 1983). Although some controversy exists about routine radiiodine ablation for thyroid remnants, this procedure has been adopted by various organizations because of beneficial effects on recurrence and mortality in patients with higher tumor stages and in those with residual tumor (EUSTATIA-RUTTEN ET AL., 2004; SAMAAN ET AL., 1992; SCHLUMBERGER, 1998; SINGER ET AL., 1996; TAYLOR ET AL., 1998). A higher specificity for Tg measurements have been found after thyroid remnant ablation than after surgery alone in a recent meta-analysis (EUSTATIA-RUTTEN ET AL., 2004). For patients who were treated with a total thyroidectomy and adequate radiiodine ablation, there is no “normal” range of serum Tg values; any measurable Tg is considered sufficient evidence for persistent thyroid carcinoma. A lower cut off value has high sensitivity but low specificity. However, further action is recommended when serum Tg exceeds 5 – 10 ng/ml (TORRENS AND BURCH, 2001). Conversely, in patients with absence of TgAb, low or undetectable serum Tg levels in the presence of known residual disease documents the insensitivity of this method for assessment in these patients.

There is no value in using risk-stratification to decide when to measure TSH stimulated levels; in one study, 22% of “low-risk” patients who had undetectable Tg levels on thyroid hormone had elevated Tg levels when TSH was increased by thyroxine withdrawal (DUREN ET AL., 1999). A particular threshold value may not be as important as the change in the Tg level over time (BLACK ET AL., 1987; SCHLUMBERGER ET AL., 1997). A clinical judgement on the value of Tg in a given patient assumes importance based on various criteria important amongst them being the prognostic variants. On follow-up, patients with poor prognostic factors like metastasis prior to primary treatment, serum Tg values more than 2ng/ml ‘on’ thyroxine and more than 5ng/ml ‘off’ thyroxine warrants intervention. If the patient were to be prognostically good on evaluation, Tg values of 5ng/ml ‘on’ thyroxine and 10 ng/ml ‘off’ thyroxine could be considered for further evaluation and therapy.
Despite universal application of Tg measurements in DTC, there is no consensus on frequency, threshold values and whether Tg measurements must be done ‘on’ or ‘off’ thyroxine (EUSTATIA-RUTTEN ET AL., 2004). Tg is estimated both ‘on’ thyroxine and ‘off’ thyroxine. The significance of Tg under various settings is listed in Table 3. As a general observation, pattern of change is a better indicator of the disease process than a single value. When on thyroxine, raised Tg levels is a more stable indicator of tumor mass; while pattern of change indicates the tumor burden (SPENCER AND WANG, 1995). When the patient is ‘off’ thyroxine, Tg levels are more sensitive indicators—specially pertaining to neck disease (HAUGEN ET AL., 1999; SPENCER ET AL., 1999). The magnitude of raise caused by TSH determines the sensitivity of the tumor to TSH. Among patients with undetectable Tg levels following thyroxine withdrawal more than 2 years after initial treatment, long-term follow-up showed a relapse in fewer than 1%. An undetectable Tg level in this situation is therefore an excellent criterion of cure. In these patients, thyroxine in replacement doses rather than suppressive doses is adequate and any other test is unnecessary as long as Tg remains undetectable (SCHLUMBERGER AND BAUDIN, 1998). But, if Tg levels are undetectable on thyroxine and detectable on thyroxine withdrawal, the long term relapse is about 10%. In such patients, radioiodine total body scan (TBS) with 100mCi if the Tg level is above 40 ng/ml ‘off’ thyroxine treatment. If TBS is negative, thyroxine treatment is maintained at suppressive doses, and another radioiodine TBS performed with 100mCi only if it becomes detectable during thyroxine (SCHLUMBERGER AND BAUDIN, 1998).

For patients with low risk DTC, with no evidence of disease over a 12 month follow up, diagnostic radioiodine scan may not be required if Tg is undetectable in the absence of antibodies. In such patients, rhTSH can be used to increase productivity monitor Tg for reference, while ultrasound exam of the neck may be more sensitive in detecting nodal disease (FATEMI AND LoPRESTI, 2003; MAZZAFERRI ET AL., 2003; SCHLUMBERGER ET AL., 2004).

Conclusions

Tg estimation plays a very important part in the management of DTC. Tg estimations are done with IMA and there could be TgAb interference with estimation. Newer developments have focused on alternative measures like TgRNA status. However, many issues need to be addressed before this marker can be used routinely. Tg is a reliable specific tumor marker for thyroid. Estimations are of value as prognosticator and indicative of recurrence when elevated. Estimations need to be done both with and without TSH stimulation. TSH stimulated values are more sensitive, while TSH suppressed values indicate thyroid mass. Radioiodine scans and Tg estimations are complementary. Tissue Tg detected by immunohistochemistry is useful for diagnosing unknown primary. Tg from needle aspirations are being evaluated.

Given certain limitations, the immunoassay has been standardized. With the availability of rhTSH, estimations can be done without thyroxine withdrawal for TSH stimulated values. The current need is to have recommendations on Tg usage both in terms of frequency of estimation and TSH stimulated and suppressed states.

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