Thyroglobulin as Specific Tumor Marker in Differentiated Thyroid Cancer

13

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13.1 Introduction

Thyroglobulin (Tg) is an iodoglycoprotein with a molecular mass of 660 kDa which is exclusively produced in thyrocytes or tumor cells of thyrocyte origin and which is necessary for the synthesis and storage of thyroid hormones. For a long time it was assumed that no secretion or leakage from the healthy thyroid occurs. In the 1960s, more-sensitive detection tools were developed: the specific hemagglutination-inhibition tests [34] and especially radioimmunoassays [65, 81]. Using these new tests, the detection sensitivity was sufficient to prove the presence of Tg also in the blood of healthy subjects. The reference range (95-percentile of the healthy population) extends to about 50 ng/ml, with quite a high interindividual variance. Some thyroid diseases release considerable amounts of Tg into the blood, particularly differentiated, follicular cell-derived thyroid cancer (DTC). However, also benign thyroid diseases may be associated with (highly) increased serum Tg levels (e.g., thyroid enlargement, thyroid nodules, hyperthyroidism, thyroiditis), and there is a very wide overlap between the serum Tg levels in benign or malignant disease. In Hashimoto's thyroiditis, for example, Tg values up to 22,000 ng/ml have been reported [52].

Like the glandular secretion, tumoral Tg secretion mostly displays a TSH dependency, because follicular-cell derived tumor tissue mostly preserves TSH receptors [72]. Consequently, Tg values measured under maximum TSH stimulation, obtained 3–4 weeks after levothyroxine or 2 weeks after triiodothyronine withdrawal ("off-Tg"), exceed Tg values under TSH suppression ("on-Tg") by one order of magnitude (in poorly differentiated tumors, less than factor 3, and in highly differentiated tumors, factor 10 and more [29, 30, 54, 57, 70, 75]). In an unpublished study of 356 patients in our department, a median stimulation factor of 8 was determined. In less-differentiated carcinomas, when TSH receptors are reduced, the stimulation factor can be much lower or absent.

Even in healthy subjects without thyroid disease, circulating Tg displays a molecular heterogeneity, e.g., in respect of iodine content, which obviously depends on iodine alimentary support. Furthermore, structural distinctions between the predominant Tg forms in benign thyroid diseases and DTC have been detected, which are in part due to the process of release from the thyroid cell [17]. However, assays relying on those structural differences, which measure

exclusively or at least preferably "malignant" Tg, are not available yet or in the foreseeable future.

Thus, Tg can be used as a tumor marker only after total thyroidectomy – apart from a few exceptions. In the case of a carcinoma of unknown origin and proven distant metastases which might be thyroid cancer, the level of serum Tg may be a useful indicator. A high Tg value suggests thyroid cancer; a value in normal range nearly excludes at least a well-differentiated carcinoma derived from follicular cells [18]. Furthermore, Spencer et al. [75] proposed the measurement of serum Tg levels in all patients with DTC prior to surgery (in the presence of the primary tumor) in order to achieve information about the Tg secretion activity of the tumor.

The specificity of Tg measurement in the follow-up of thyroid cancer is highest after thyroidectomy and adjuvant radioiodine ablation of the thyroid remnants. Corresponding to the serum half-life, on average 65 h [35], during the first days and weeks after thyroidectomy, still measurable but decreasing Tg values are expected. Thus, depending on the initial level of serum Tg, the full specificity and accuracy is established only several weeks after radioiodine ablation of the thyroid remnants [21]. Assuming the pretherapeutic serum Tg level to be 100 ng/ml arising from the benign thyroid or thyroid carcinoma, Tg might be measurable for approximately 4 weeks even after complete cure.

In thyroid cancer patients with subtotal or "total" thyroidectomy but no radioiodine ablation, Van Wyngaarden and McDougall [82] detected persistent, measurable Tg values in 38% of the patients being judged tumor free, even under TSH-suppressive medication. The proportion of Tg-positive patients necessarily increases with the sensitivity of the Tg test used. In a group of 33 patients who were only thyroidectomized but had no radioiodine ablation, we found measurable serum Tg values under TSH suppression in 50% of the subjects using a test with a lower detection limit of 0.5 ng/ml and even in 100% using a test with a functional sensitivity of 0.03 ng/ml [31].

Nowadays, immunometric assays (IMAs; with isotope or nonisotope technique) are most frequently used for Tg measurement in Europe. Many of the commercialized kits offer a functional sensitivity of about 0.5 ng/ml. However, this lower limit is only a technical threshold and would lead to a high rate of false-positive findings if a single Tg value were to be assessed underlying such low value as cutoff. Therefore, arbitrary cutoff values are introduced intending to find an optimal compromise between diagnostic sensitivity and specificity. Often an arbitrary threshold of about 2 ng/ml is proposed [21, 29, 42, 84]. According to our experience, a significantly lower value can be used for the assessment of *serial* Tg measurement in the individual patient, if the performance of the Tg test is under permanent control. If the test is working perfectly, a newly detected measurable Tg value in the follow-up suggests recurrence.

In their recent meta-analysis concerning the diagnostic value of serum Tg measurements in the follow-up of DTC, Eustatia-Rutten et al. [21] reported for IMAs an average diagnostic sensitivity of 0.778 ± 0.023 (mean ± SE), a specificity of 0.977 ± 0.005, and an accuracy of 0.933 ± 0.007 for on-Tg in patients with thyroid ablation (9 underlying series, with a total of 1,613 patients, median cutoff 2 ng/ml). The respective values for off-Tg were 0.961 ± 0.013, 0.947 ± 0.007,

and 0.952 ± 0.006 (12 underlying series, with a total of 1,602 patients, median cutoff 3 ng/ml)

13.2 Thyroglobulin Measurement: Methodology and Problems

Tg measurement – aiming for high sensitivity and keeping up high specificity – is challenging for several reasons that are explained in this section, and encumbered with more serious consequences as compared to many other analytes. High laboratory standards are essential, including the definition of the functional sensitivity, based on a 20% interassay coefficient of variation during an application period of the assay over at least 6 months [73], duplicate measurement of the serum samples, restoring frozen sera for future validation, quality controls, and methods for recognizing interfering/disturbing factors and a high-dose hook effect.

The actual serum Tg level is influenced by various factors, including the amount of active tumor tissue, the histological tumor type (in papillary carcinoma mostly lower than in follicular carcinoma [16, 55, 77]), the grade of tumor differentiation (which may change during the course), the stimulation of tumoral TSH receptors (e.g., through TSH or TSH receptor antibodies), the acute attack of Tg-releasing processes (e.g., irradiation), and the serum clearance rate of Tg. Thus – except for analytical interferences and problems – a false-negative Tg finding could be caused by a deficient release of Tg from the tumor, by a very small amount of tumor tissue, or by a lack of sensitivity of the Tg assay. Bachelot et al. [2] report a ratio of 0.5–1 ng/ml serum Tg concentration per gram of neoplastic tissue. However, the serum Tg concentration largely depends on the abovementioned biological parameters.

For these reasons, not only high laboratory standards are necessary, but the reporting physician must also be aware of interfering factors and needs detailed knowledge of the patient, such as mass of thyroid remnant, possible stimulation factors for Tg secretion, previous and actual therapeutic measures (kind of surgery, radioiodine therapy, external radiation, and other interventions), time interval to therapeutic measures, tumor biology, and the course of follow-up.

The most frequent analytical problems in Tg measurement are due to interfering factors in patient's sera (especially anti-Tg autoantibodies; far less frequently, heterophilic antibodies), also to the high-dose hook effect, and in rare cases to the presence of an abnormal Tg not recognized by the assay antibodies [68, 73]. In the majority of one-step immunoradiometric assays (IRMAs), the high-dose hook effect results in false-low values in cases of excessive high serum Tg levels (depending on the Tg test used, typically for serum Tg exceeding the upper detection limit of the test more than 100-fold), as all binding-sites of the first (e.g., solid-phase) assay antibody are saturated. In consequence, a meaningful proportion of the second, signal antibody binds to unbound Tg in the supernatant and is decanted prior to measurement, resulting in a decreased measurement signal. The high-dose hook effect is uncovered by the recovery test: the recovery is remarkably diminished in such cases. In some modern two-

step assays, the high-dose hook effect has been overcome by additional wash procedures [51, 87].

Heterophilic antibodies (e.g., human anti-mouse antibodies, HAMA) in the patient's serum may disturb the measurement (e.g., as the assay employs murine antibodies). In times of in vivo diagnostics and therapy with monoclonal murine antibodies, Tg measurements compromised in that way may actually occur more frequently. In this case, using the IMA methodology, the measured Tg values as a rule overestimate the real serum concentration [61]. Meanwhile, the assays of most manufacturers contain blocking agents to prevent this phenomenon, but the efficacy of these measures is not guaranteed in each case.

Modern Tg assays rely on monoclonal antibodies that are highly epitope-specific. Therefore, there is at least a theoretical risk that "atypical" Tg might not be recognized, as it might not express the tested epitope. That is why there is discussion about whether the use of polyclonal or polyvalent antibodies, or the combination of different antibodies might be advantageous. However, up till now, the proof of clinical relevance of this aspect is missing.

Tg-TgAb interference is a definitely more relevant challenge, and numerous efforts have been undertaken to overcome this problem:

A. The development of assays which employ monoclonal capture antibodies with specificities for Tg epitopes not involved in the autoimmune response [60]
B. Adding a Tg fragment which neutralizes the presence of autoantibodies [39]
C. The use of various polyclonal and monoclonal antibodies to increase the number of epitopes on the Tg molecule that are recognized and possibly not affected by Tg-TgAb interference [19]
D. The use of assay antibodies which bind with markedly higher affinity to the Tg epitopes than the autoantibodies. Thus Tg is stripped from interfering human TgAb and bound to the assay antibodies [4].

Nevertheless, the problem of Tg-TgAb interference could not be eliminated through these modifications, but merely somewhat diminished [44, 67, 86].

TgAb interference can produce either under- or overestimation of serum Tg depending on the assay architecture and therefore can cause discordant results between IMAs and RIAs. Tg IMAs typically underestimate serum Tg when sera contain Tg antibodies (TgAbs), presumably because the endogenous Tg complexed with TgAbs cannot interact with the assay antibodies, whereas RIAs can under- or overestimate Tg depending on the characteristics of the respective assay antibodies [74, 86]. In contrast, Mariotti et al. [44] postulated that low Tg values observed in some patients who also have TgAbs could also be due to an accelerated metabolic clearance rate of the Tg-TgAb complexes from the blood via the reticuloendothelial system. Consequently, very low or negative Tg values in the presence of circulating TgAbs should be interpreted carefully. Some authors recommend serial TgAb measurement in such cases as surrogate tumor marker test, but there is controversy over the clinical relevance [11, 62].

On the other hand, circulating TgAbs – even in high serum concentrations – must not necessarily interfere with the Tg measurement [74], and moreover not only TgAb interference, but other factors, too, may also cause unreliable Tg values. Finally, not only Tg assays, but also TgAb assays can produce unreliable results, e.g., because of interfering high Tg concentrations [24] or epitope incompatibility between TgAbs and the radioligand (Tg) on the one hand and radioligand and assay antibodies on the other hand [6]. Thus, estimation of TgAbs is no ideal tool to authenticate Tg measurements.

In the light of this background, Tg recovery tests have been introduced, allowing control over whether a defined amount of Tg added to the patient's serum sample could be measured adequately. However, there exists no consent about the usefulness of those recovery tests and their validity for recognizing Tg-TgAb interferences. Some authors [74, 86] stress that recovery tests – at least in the usually performed manner concerning the amount and origin of added Tg and the incubation time – cannot be used to validate a Tg measurement in serum containing TgAbs. Particularly, they postulate that the epitopes on the exogenous Tg molecules (glandular origin) may differ from the epitopes of endogenous tumor Tg in the patient's serum. In contrast, other authors emphasize the importance of recovery tests instead of TgAb measurements [10, 41].

If TgAbs were measured in order to authenticate Tg values, one should use a highly sensitive assay and even report very low TgAb values, as there is no established correlation between the TgAb titers and the influence of Tg-TgAb interference on the measured serum Tg level [74]. In their cross-sectional study, Spencer et al. [74] found TgAbs in approximately 25% of the DTC patients as compared to 10% in the general population by using this sensitive assay. Tg-TgAb interference could not be excluded for measurable TgAb within the normal range for subjects without thyroid disease at all, and even TgAb concentrations below the lower detection limits of common assays may result in significant interferences. Spencer et al. [74] also proposed a RIA/IMA discordance test to uncover interfering TgAbs. Other authors recommend performing recovery tests and TgAb measurements in parallel [88], since recovery tests additionally allow the detection of otherwise disturbing factors in Tg measurement, e.g., a possible high-dose hook effect.

If recovery tests are performed, they should not be normalized to the expected concentration of added Tg (e.g., 50 ng/ml), but one should take the actually measured Tg results into consideration in order to diminish possible (e.g., pipetting) errors, using the formula:

$$\frac{Tg_{(\text{patient's serum + recovery buffer})} - Tg_{(\text{patient's serum})}}{Tg_{(\text{Tg-free serum + recovery buffer})}} \times 100\% = \text{recovery (as a rule, a recovery 70–130\% is defined as undisturbed).}$$

In our laboratory, in each assay run, at least half of the patients show up with negative serum Tg levels and a long-term, established complete remission. All the recovery values are finally normalized to the average of those values, resulting in a narrower distribution of the recovery tests, which enables us to reduce the normal interval to 80–120% and to compensate for day by day fluctuations. Because also economical aspects are of increasing importance in our health care

system, further data may be helpful on which a pragmatic approach concerning the authentication of Tg values could be based (e.g., low-dose recovery tests; recovery tests using special Tg forms; "cold" preincubation to reach equilibration between TgAbs, assay antibodies, serum Tg and recovery Tg; only initially performed TgAb measurement, and restriction on further controls in cases of initially elevated TgAbs values).

For a long time, the results from different Tg assays and different laboratories could not be compared numerically, as no valid international standard was available [23]. In 1996 the European reference preparation CRM 457 was introduced in order to overcome this drawback [25, 26], and recent assays are calibrated on this preparation. However, the comparability was increased but still is not perfect as, for example, the antibodies of different assays are directed against different epitopes, and the variation between the different assays may still be up to factor 1.5 despite the calibration on the same standard preparation [53].

From a statistical point of view, the abovementioned analytical difficulties cause problems of only minor relevance in the clinical routine of our department, which takes care of far more than 2,000 DTC patients per year, and it has to be emphasized that Tg measurement – performed with assays now available under high laboratory standards – is a highly reliable diagnostic tool in the follow-up of DTC. Nevertheless, one should be aware of the possibility of the above-outlined problems, since they may cause fatal health consequences in some patients, mainly due to unnecessary or delayed diagnostic and therapeutic measures.

13.3 Diagnostic Value of Thyroglobulin in the Spectrum of Follow-Up Methods

Since recurrences in DTC may occur even after decades and the chance for a curative therapy commonly increases with the earliness of the detection of a relapse, most expert organizations recommend a lifelong follow-up. Diagnostic radioiodine whole-body scintigraphy (dWBS), Tg measurement, and neck ultrasonography are the most important tools of the thyroid cancer aftercare found in numerous procedure guidelines (e.g., National Cancer Centre Network [45], German Cancer Society [38], American Associations of Clinical Endocrinology and of Endocrine Surgeons [78]). Subsequent to the ablation of thyroid remnants, both dWBS and Tg measurement have a high specificity regarding the detection of tumor tissue, because the ability to secrete Tg and to concentrate iodine are characteristic features of differentiated carcinomas. However, both features may not always be associated [16] and, in cases of dedifferentiation, the capacity of iodine concentration is more often lost than the Tg secretion.

Data from the literature concerning the sensitivity and specificity for the detection of tumor tissue of dWBS with ^{131}I as compared to Tg measurement under TSH-suppressive thyroid hormone supplementation ("on-Tg") are given in Table 13.1 (studies which only examine the prediction of a positive dWBS as "gold standard" by a positive on-Tg were excluded). The data are highly dependent on the sensitivity of the applied Tg assay and the performance of dWBS;

moreover, the question of the ideal "gold standard" is a point of discussion. In the majority of cases, on-Tg measurement is superior to dWBS, but some authors favor a combination of Tg measurement and routinely performed dWBS in order to maximize the sensitivity, especially concerning patients with interfering TgAbs. Of course, the additional opportunity to measure off-Tg should always be used when thyroid hormones are withdrawn in preparation to dWBS.

It has to be underlined that values for diagnostic sensitivity of dWBS and on-Tg from the literature mostly derive from patients with active tumor disease in general. Diagnostic sensitivity of these two tools concerning early detection of recurrences in patients thought to be in complete remission is a distinctly different situation. Focusing on these patients, significantly lower values for diagnostic sensitivity have been reported, e.g., virtually 0% [79], 14% [36], 27% [46], and 41% [63] for dWBS. Apart from the limited sensitivity for detection of still small amounts of relapsing tumor tissue after diagnostic doses of ^{131}I in general, this could be explained by less radioiodine accumulation in late recurrences, the proportion of which ranges from one-half to two-thirds of all cases [63]. In a comparative study of 44 DTC patients who were in complete remission after primary therapy, we found a diagnostic sensitivity for early detection of recurrences for on-Tg of 27% (RIA with a lower detection limit of 6 ng/ml) and 58% (IRMA with a lower detection limit of 1 ng/ml), respectively, and for dWBS of 2.7% [50].

Schlumberger and Pacini performed a meta-analysis of various studies (with underlying assay sensitivities or cutoff values between 1 and 3 ng/ml) and reported that 20% of the local metastases and 2–5% of the distant metastases were Tg-negative under TSH-suppressive conditions [69]. Under maximum TSH stimulation, the respective rates were 5% and 0%. Thus, particularly local and regional recurrences to a certain extent are missed by on-Tg measurements.

Focusing on detection of neck recurrences, Frasoldati et al. [27] reported a significant superiority of high-resolution ultrasonography (sensitivity 94%) relative to Tg measurement, even when performed under TSH stimulation (sensitivity 57% for an underlying cutoff of 2 ng/ml; 67% for 0.25 ng/ml) and dWBS (sensitivity 45%). The important additional role of ultrasonography is confirmed by the results of other recent studies [14, 80]. In a recent publication [32], we introduced rules for judging the dignity of cervical lesions in thyroid cancer patients using a logistic regression model based on dichotomized B-mode and color flow Doppler criteria. Performing high-end ultrasonography and applying these rules, a diagnostic sensitivity of 90% (95%-CI: 76–97%) and specificity of 82% (95%-CI: 57–96%) could be reached.

13.4 Tg Measurement Under Exogenous TSH Stimulation

Approval for the use of recombinant human TSH (rhTSH) for diagnostic purposes in DTC was granted in 1998 in the USA and in 2001 in Europe. If injected i.m. following the established application scheme (each 0.9 mg rhTSH at days 0 and 1) the median serum TSH-peak (>150 ng/ml) is reached at days 2 and 3, followed by a quite rapid decline. The maximum median serum Tg concentration is

Table 13.1. Data from the literature concerning the diagnostic value of on-Tg compared with diagnostic ^{131}I whole body scintigraphy (WBS) for the detection of follicular cell-derived thyroid cancer (DTC) tumor tissue

Authors	Patients	On-Tg			Diagnostic ^{131}I WBS			On-Tg + WBS
		Lower detection limit or cutoff, respectively	Diagnostic sensitivity	Diagnostic specificity	Dose of ^{131}I	Diagnostic sensitivity	Diagnostic specificity	Diagnostic sensitivity
Ashcraft and van Herle 1981 [1]	Overall 36 (18 with active tumor disease)	1 ng/ml	100%	29%	185 MBq	58%	100%	Not reported
Colacchio et al. 1982 [12]	Overall 67 (37 with recurrences)	15 ng/ml	78%	100%	74 MBq	84%	100%	Not reported
Hüfner et al. 1983 [36]	28 with recurrences	10 ng/ml	71%	Not reported	74 MBq	14%	Not reported	78%
Reiners et al. 1984 [63]	55 with active tumor disease	5 ng/ml	89%	Not reported	74 MBq	58%	Not reported	Not reported
	22 with late recurrences		86%	Not reported		41%	Not reported	Not reported
Sulman et al. 1984 [76]	Overall 115 (23 with active tumor disease)	6.25 ng/ml	83%	95%	37 MBq	35%	100%	83%
Müller-Gärtner et al. 1988 [55]	Overall 374 (30 with active tumor disease)	3 ng/ml	50%	99%	370–740 MBq	57%	Not reported	Not reported
Ronga et al. 1990 [66]	Overall 61 (30 with active tumor disease)	5 ng/ml	83%	58%	74–148 MBq	77%	100%	96%

Table 13.1. *Continued*

Authors	Patients	On-Tg			Diagnostic ^{131}I WBS			On-Tg + WBS
		Lower detection limit or cutoff, respectively	Diagnostic sensitivity	Diagnostic specificity	Dose of ^{131}I	Diagnostic sensitivity	Diagnostic specificity	Diagnostic sensitivity
Berding et al. 1992 [7]	Overall 70 (9 with recurrence)	5 ng/ml	67%	91%	370 MBq	56%	Not reported	78%
		10 ng/ml	56%	94%	370 MBq	56%	Not reported	78%
Lubin et al. 1994 [43]	Overall 261 (59 with active tumor disease)	10 ng/ml	88%	Not reported	185 MBq	55%	Not reported	88%

observed later, with a peak at day 4 [58, 59]. Tg is stimulated by rTSH on average by a factor of 10–20 compared with the "on-Tg" value [75]. Thus, on the average, a similar sensitivity and specificity to endogenously stimulated Tg ("off-Tg") is reached [21]. The advantage of rTSH stimulation is the absence of hypothyroid symptoms induced by thyroid hormone withdrawal, especially the reduction of health-related quality of life [15, 33, 40].

Some authors [3, 56, 85] propose in recent papers the application of rTSH solely to increase the sensitivity of the Tg determination (without dWBS) using assays with a functional sensitivity of about 0.5 ng/ml or cutoff values of 2.0 ng/ml. Based on the results of previous studies, a group of thyroid experts in the United States [47] and in Europe [71] recently proposed a revised follow-up protocol for low-risk DTC patients without interfering TgAbs, where Tg measurement under rhTSH stimulation in combination with neck ultrasonography is routinely performed 6–12 months after primary therapy in all patients without evidence of disease, whereas the routine use of dWBS in the majority of those patients should be abandoned.

However, this proposed follow-up regime was criticized by other thyroid cancer experts for several reasons, including the functional sensitivity of the applied Tg assays and the cutoff values for Tg, the definition of low-risk patients, and the questionable cost-efficiency and impact on the clinical outcome of this paradigm [22, 49]. Yet, there exists no international consent about the routine use of rhTSH-stimulated Tg measurement in DTC aftercare. The broad application of rTSH stimulation (costs: about 900 US dollars) solely in order to increase sensitivity of Tg detection needs a critical discussion, especially if significantly more sensitive Tg assays become available. Already today an increase in the assay sensitivity by a factor of 10 is feasible. This increase in sensitivity is about equivalent to the stimulation effect. In addition, the increased assay sensitivity is of high value for (less differentiated) tumors which do not or only insufficiently react on TSH stimulation due to missing TSH receptors [75].

Moreover, the extent of the Tg increase after TSH stimulation does not correlate with the dignity of the Tg source. The response does not reliably allow differentiation between thyroid remnant and recurrence in each case. In a representative sample of our DTC patients, we found no significant difference of the extent of the Tg response to maximum TSH stimulation: factor 0.6–25.7 in tumor-free patients and 3.2–33.8 in patients with active tumor. In their authors' response to the controversial comments on the abovementioned revised follow-up protocol, Mazzaferri et al. [48] conceded that in the future the availability of Tg assays even more sensitive than used in their studies may "render TSH stimulation unnecessary to identify patients with persistent tumor."

13.5 Ultrasensitive Thyroglobulin Measurement

Meanwhile, Tg assays are commercially available whose sensitivity is one order of magnitude higher than the present assay generation still with a satisfying intra- and interassay precision [31, 37, 51, 87]. Iervasi et al. [37] examined the

diagnostic performance of a fully automated, chemiluminescent immunoassay and found the analytical sensitivity to be 0.01 ng/ml and the functional sensitivity (at 20% coefficient of variation) to be 0.1 ng/ml. The ILMA evaluated by Morgenthaler et al. [51] had a lower detection limit of 0.02 ng/ml and a functional sensitivity of 0.06 ng/ml. Wunderlich et al. [87] evaluated an immunoenzymometric assay (IEMA) in a cross-sectional study and found the functional sensitivity to reach as low as 0.03 ng/ml (calibrated on the European Tg-reference preparation CRM 457).

The diagnostic value of such sensitive assays is no longer based on a single measurement (the rate of Tg-positive but tumor-free patients becomes quite high – even after radioiodine ablation), but relies on an early suspicion of recurrence, which is proven by the Tg course in the follow-up. The high clinical value of serial Tg measurements under TSH suppression has already been stressed by various authors at times using less sensitive Tg assays [8, 9, 73, 75]. DTC usually grow quite slowly. By analyzing the Tg course of 20 patients with established metastases which for various reasons did not get any kind of treatment (with the exception of suppressive thyroxine medication), we calculated the median time for doubling the serum Tg concentration to be 6 months (range 1–42 months).

Applying the same ultrasensitive IEMA which was already reported by Wunderlich et al. [87] in a longitudinal study, we could demonstrate that the scale of this "metabolic tumor doubling-time" is also applicable to the very low range of Tg (<1 ng/ml), corresponding to very early development of tumor recurrences. We used the deep-frozen sera of seven patients collected over 5 years in our serum bank and calculated the potential time profit by using the highly sensitive Tg assay, which naturally depends on the growth characteristics of the tumor and the follow-up intervals. A gain of 5–15 months for the detection of the recurrence could be calculated compared with the established assay generation [31]. Zöphel et al. [89] found a similar range using the same IEMA: 6–12 months.

The validity and reliability of the results of this ultrasensitive IEMA are also proven by the fact that TSH stimulation increases the Tg values in the lowest range by the same factor established for higher values. In addition, an excellent intraindividual reproducibility of the lowest values in clinically stable patients (including those who are likely to be in full remission) was found, as well as a continuous and smooth increase in Tg values with progressive disease [31].

The availability of assays with increasing sensitivity which are clinically applicable (stable intra- and interassay precision at very low Tg levels) will enhance the importance of the documentation of the change in Tg levels in serial measurements under TSH suppression for the early detection of recurrence. The often-proposed arbitrary cutoff value of 2 ng/ml (which today is not adequate) definitely needs to be abandoned in the face of high functional sensitivity. However, the reasonably lower normal Tg level needs to be defined in larger prospective studies; the same holds for the threshold of the respective Tg value, or dynamic pattern of consecutive Tg values, which requires further diagnostics or therapeutic interventions (e.g., continuous Tg increase at three consecutive examinations or doubling of the previous value), dependent on the tumor type and individual risk profile.

13.6 Thyroglobulin messenger RNA as Alternative Tumor Marker

In 1996 Ditkoff et al. [13] described for the first time the qualitative detection of circulating thyroid cells in peripheral blood, applying reverse transcription polymerase chain reaction (RT-PCR). As molecular genetic methods become more common in medical diagnostics, this finding has increasing meaning also for thyroid cancer patients. An increased independence from the extent of TSH stimulation and no interference from circulating TgAbs are expected by the molecular genetic approach, displaying potential advantages over Tg measurement.

In the past 8 years, numerous papers have been published, concerning both the qualitative [5, 28] and the quantitative [20, 64] detection of circulating thyroglobulin messenger RNA (mRNA). The results of the different laboratories and therefore their attitude towards clinical usefulness are at least controversial. One reason might be the lack of standardization. Due to an unfavorable "signal to noise ratio," the average specificity is significantly inferior to that of state-of-the-art Tg assays (e.g., "thyroid-specific" mRNAs – even when the applied primer does not recognize splice variants for Tg – are less specific as initially assumed). In a recent review, Verburg et al. [83] concluded that at present Tg-mRNA detection is not a useful tool in the follow-up of DTC, but that the concept of using RT-PCR measurements during follow-up still warrants further research.

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