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Clinical Challenges of Transitioning to High Sensitivity Thyroglobulin Assay

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Abstract

Background: Patients treated for Differentiated Thyroid Cancer (DTC) are followed by thyroglobulin (Tg) testing with standard Tg assays after a stimulation test (Tg-ST), which is expensive and time consuming. High-Sensitivity Thyroglobulin (HS-Tg) assays are purported to replace Tg-ST; acceptable cut-offs however may vary according to assays and patient population. We aimed to evaluate the HS-Tg assay (Roche Elecsys® Tg II) and apply it to clinical use.

Method: Analytical evaluations were performed following CLSI standard protocols. Clinical evaluation was done prospectively on 35 DTC patients subjected to Tg measurements both pre- and post- Tg-ST Clinical accuracy performance of HS-Tg was compared to that of conventional Tg-ST protocol utilizing the Siemens Immulite 2000 XPI platform.

Results: HS-Tg assay showed an excellent precision (CV=2-3%). The assay reached CV of 11.0% in pooled samples at a mean of 0.048µg/L. HS-Tg results are slightly higher than those from the conventional Siemens Tg assay. HS-Tg ≥ 0.2µg/L showed clinical sensitivity of 1.0 and specificity of 0.81 for predicting recurrence of DTC, which is superior to the Tg-ST protocol using 2µg/L as the cutoff. Two of six patients with HS-Tg results between 0.06-0.2 had a Tg-ST result of >2µg/L, but no recurrence.

Conclusions: Although the HS-Tg cut-off of 0.2ug/L is a reliable alternative to Tg-ST in most cases, these tests show divergent results in a proportion of patients making transition from one test to another challenging. Further studies are required to determine the clinical significance of HS-Tg between 0.06-0.2 µg/L.

Keywords: High-sensitivity thyroglobulin; Clinical value; Thyroid cancer

Introduction

Serum Thyroglobulin (Tg) is a sensitive marker of Differentiated Thyroid Carcinoma (DTC) activity after the initial management [1]. Despite an excellent overall prognosis, persistent or recurrent disease occurs in up to 20% of DTC patients thus requiring long-term follow-up using a test with preferably high negative predictive value [1]. Traditionally, due to poor analytical sensitivity of conventional Tg assays, Tg levels are measured after 'stimulation' (Tg-ST). This is done through either prolonged withdrawal of L-thyroxine (THW) or administering recombinant human Thyroid Stimulating Hormone (rhTSH) [1]. Indeed, the current guidelines suggest that all patients should undergo Tg-ST 6-12 months after remnant ablation1. However, both Tg-ST protocols are inconvenient for patients, time consuming, expensive and require patients to follow strict protocols.

Recently novel High-Sensitivity Tg (HS-Tg) assays have been developed reporting sensitivities of up to ten-fold of conventional assays, which may obviate the need for Tg-ST [2-6]. Consequently, American Thyroid Association (ATA) guidelines suggest that negative imaging and HS-Tg levels of <0.1-0.3µg/L obtained 6-12 months after primary treatment indicate excellent response to treatment. However, there are considerable challenges in interpreting the appropriate cutoff of HS-Tg in comparison with Tg-ST, making it difficult to assess patients transitioning from one test to another. For instance, the suggested cutoff of 0.1µg/L requires an analytical sensitivity of 0.05µg/L for the HS-Tg assay [1,5,7-9], which may vary in equivalence to the Tg-ST among medical centers and laboratories [1]. A recent meta-analysis reported that while HS-Tg with a functional sensitivity of <0.1µg/L had a high negative predictive value, it lacked the accuracy and positive predictive value to provide a reliable alternative to Tg-ST [10]. The aim of our study was two-fold: a) We sought to establish an appropriate clinical cut-off for HS-Tg assay using the Elecsys® Tg II HS-Tg assay, and b) Illustrate the clinical challenges of transitioning to the new assay.

Materials and Methods

Study population

The prospective study was conducted at the Interdisciplinary Thyroid Oncology Clinic (ITOC) in Halifax, Nova Scotia, Canada between 2017 and 2020, which follows all patients with DTC in this province (approximate population = 1 million) using a standardized protocol. All patients had undergone total thyroidectomy followed by radioactive iodine (I-131) therapy for remnant ablation between 6-12 months prior to the index Tg test. All tests were conducted using the standardized rhTSH Tg-ST protocol. Fifty patients who were scheduled for rhTSH test were contacted for consent to enroll

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in the study. None of the patients declined. The Nova Scotia Health Authority research ethics board approved the study.

Study design

Analytical validation of the HS-Tg assay on the Cobas e-411 (Roche Diagnostics, Laval, QC), followed the relevant Clinical and Laboratory Standards Institute (CLSI; PA, USA) standard method evaluation protocols. Two levels of quality control materials (BioRad, QC, CA) were tested to assess precision. Functional sensitivity was validated by testing pooled patient serum samples at just above the analytical sensitivity defined by the vendor. Linearity was tested by serial dilution of a patient sample that is greater than the upper end of measuring range (500µg/L) as defined by the vendor. Forty anonymized serum samples spanning the linear range were selected for comparison of results of the HS-Tg assay against our conventional Tg assay (Immulite 2000 XPI; Siemens, Oakville, ON).

For clinical validation, only patient-samples with both pre and post stimulation Tg results were included (n=35). Baseline blood samples were collected from the study patients within two days prior to rhTSH injections. Two intramuscular injections of 0.9mg rhTSH were then given 24 hours apart. Post stimulation blood samples were collected on day 5 after the first injection [2]. All samples were centrifuged within 1 hour of collection and the serum samples were aliquoted and stored at -20°C. Each sample was analyzed using both the conventional Tg assay (Siemens) and the HS-Tg assay. Clinical performance of HS-Tg was evaluated based on the Tg-ST results as well as patient clinical and radiographic data. Serum Tg-ST cut-off values of <1µg/L and <2µg/L were regarded as excellent and satisfactory post-treatment responses, respectively, based on the commonly used cut-off values [10]. All patient samples tested negative for thyroglobulin antibody on the Immulite 2000 XPI.

Statistical analysis

Analyze-it® software was used for statistical analysis of the data for the analytical and clinical performance of the HS-Tg assay including Passing-Bablok regression and ROC curves for sensitivity and specificity.

Results

Analytic validation

Total CV for the Elecsys® Tg II assay was 2.0 % and 3.0 % at mean of 4.2µg/L and 41.2µg/L (N=24) respectively. The assay demonstrated a CV of 11.0% for pooled samples at a mean value of 0.048µg/L (N=10), hence functional sensitivity was established to be around 0.05µg/L. The linearity for the analytical range was 0.04-500 µg/L (R^2 = 0.99). Comparison of patient results from the HS-Tg with our conventional Tg assay showed a Passing-Bablok fit of Y= 1.02x-0.44 (N=80). However, at values lower than 1.5µg/L, there was significant scatter between the two methods with poor correlation.

Clinical validation

Demographic, clinical and pathological characteristics as well as the TNM staging of the enrolled DTC patients are summarized in Table 1. Of these patients, 26 had classical variant Papillary Thyroid Cancer (PTC), 8 had follicular variant of PTC, 1 had predominant tall cell variant of PTC and 1 had metastatic disease (pulmonary) at the time of diagnosis. Median follow-up was 28 months.

Table 1: Clinical validation cohort study demographics.

Patients	**35**	
Age	Median: 48	Range: 25-72
Iodine therapy	100mCi	0-200 mCi
Parameters	Number	%
Gender		
Female	24	77.4
Male	11	22.6
Histological type		
PTC	26	83.9
PTC variant	9	16.1
FTC	0	0
Stage (T)		
Ia	6	17.1
Ib	6	17.1
II	10	28.6
IIIa	3	8.6
IIIb	1	2.9
III	8	22.9
IV	1	2.9
Stage (N)		
Nx	6	17.1
N0	12	34.3
N1a	11	31.4
N1b	6	17.1
Stage (M)		
M0	34	96.8
M1	1	3.2
Focality		
Unifocal	15	45.2
Multifocal	20	54.8

PTC: Papillary Thyroid Carcinoma; FTC: Follicular Thyroid Carcinoma; TNM staging from AJCC 8th edition [11].

Four patients had evidence of residual disease/clinical recurrence and demonstrated a baseline HS-Tg >0.2µg/L and Tg-ST >2.0µg/L; ROC analysis showed both protocols to have 100% clinical sensitivity, and clinical specificities of 81% and 71%, respectively. The total clinical performance indicated by Area under the Curve (AUC) was 0.96 for HS-Tg and 0.98 for Tg-ST (Table 1). The performance of the HS-Tg assay against pre-specified Tg-ST cut-off values of <1µg/L (excellent response) and <2µg/L (satisfactory response) is shown in Table 2. When using Tg-ST ≥2µg/L as the reference, HS-Tg of ≥0.2µg/L showed the same clinical sensitivity of 76.9%, but better specificity (100% *vs.* 86.4%), positive predict value (PPV, 100% *vs.* 76.9%) and Negative Predict Value (NPV 88% *vs.* 86.4%) than HS-Tg of ≥0.1µg/L When using Tg-ST ≥1µg/L as the reference, HS-Tg of ≥0.2µg/L showed slightly lower sensitivity than that of ≥0.1µg/L, but again better specificity (100% *vs.* 87.5%), PPV (100% *vs.* 84.6%) and similar NPV of 64%.

Twenty-five patients had a HS-Tg value of <0.2µg/L, 19 (76%)

Table 2: Performance of two cut-off values for the HS-Tg assay against two different cut offs for the Tg-ST as reference.

	Against Tg-ST ≥ 2 µg/L as reference		Against Tg-ST ≥ 1 µg/L as reference	
HS-Tg Performance	HS-Tg ≥ 0.2µg/L (N=10)	HS-Tg ≥ 0.1µg/L (N=13)	HS-Tg ≥ 0.2µg/L (N=10)	HS-Tg ≥ 0.1µg/L (N=13)
TP (Sensitivity)	0.769	0.769	0.526	0.579
TN (Specificity)	1	0.864	1	0.875
PPV	1	0.769	1	0.846
NPV	0.88	0.864	0.64	0.636

of these were undetectable at a value below the functional sensitivity we established (<0.06µg/L). The six other patients had a HS-Tg value between 0.06-0.2 µg/L, two of whom demonstrated a Tg-ST of ≥2µg/L. However, none of the 25 patients demonstrated any clinical or radiological evidence of recurrence during the follow-up period.

Discussion

Despite routine clinical use of Tg in monitoring of DTC, discordance between Tg measurements and clinical or radiographic recurrence is known to occur [9]. HS-Tg assays have up to 10 times improved analytical sensitivity over traditional assays [2-4,12,13]. There are potential advantages to this improved sensitivity including: basal unstimulated HS-Tg may be used instead of the expensive and cumbersome Tg-ST, and an upward trend in HS-Tg may indicate early detection of recurrence. In fact, other studies have shown that recurrence can be detected 6-12 months earlier using HS-Tg assays [8]. Additionally, evaluation of the slope of rise over a basal Tg level measured by HS-Tg assays over 3-12 month intervals can accurately discriminate patients with and without recurrence of DTC [2,8]. However, this additional sensitivity comes at a price, in that, minute levels of circulating Tg may be detected, but its true clinical implication remains unclear. Furthermore, in many cases serum Tg would spontaneously normalize over time [14] and may never require therapy.

The Roche HS-Tg assay has been evaluated previously and found to have comparable or superior analytical performance to other HS-Tg assays, with functional sensitivity being reported as low as 0.05µg/L [5,7,8]. We have also demonstrated that the CV is excellent (11%) at just below 0.05 µg/L. In our patient cohort, when a Tg-ST cut-off value of either 1µg/L or 2µg/L was used as the 'reference' comparator based on the commonly used criteria [10], HS-Tg at a cut-off of 0.2µg/L was superior to that of 0.1µg/L with a 100% specificity and comparable sensitivity for ruling out clinical recurrence of thyroid carcinoma. Moreover, there was no clinical or biological evidence of recurrence at this cut-off indicating that true sensitivity may be as high as 100%; however, longer-term follow-up of these patients would be required to confirm that. Patients with HS-Tg above 0.2µg/L had correspondingly elevated Tg-ST and warranted clinical follow up.

Despite a small sample size and low prevalence of recurrent thyroid carcinoma in our cohort, this study verifies the analytical validity and clinical utility of the Roche Elecsys® Tg II assay in our tertiary care center. In our cohort, 54% of all patients had a HS-Tg value of <0.06µg/L using the Roche HS-Tg assay and could have been spared a Tg-ST thus reducing costs and improving efficiencies for patients and physicians.

However, there are considerable challenges as two patients who had a HS-Tg result between 0.06-0.2 µg/L had Tg-ST result of >2µg/L. Although both patients had no clinical or radiological evidence of recurrence over the follow-up period, it still created a clinical uncertainty. Therefore, we suggest that Hs-Tg results between 0.06-0.2 µg/L should perhaps be considered as a 'grey zone' and these patients should still undergo Tg-ST until long term outcomes of such patients can be established through longer prospective studies. With the steady advent of HS-Tg assays in many labs, prospective studies are imminently required to determine accurate cutoffs to avoid clinical and therapeutic uncertainties, which may provoke anxieties for DTC patients.

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