DIFFERENTIATED THYROID CANCER:
THE PREDICTIVE VALUE
OF STIMULATED THYROGLOBULIN
SIX MONTHS AFTER RADIOIODINE ABLATION

BY

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DECLARATION

I hereby declare that this research was sent to Unversiti Sains Malaysia (USM) for the degree of Master of Medicine (Nuclear Medicine). It has not been sent to other universities. With that, this research can be used for consultation and photocopied as a reference.

Sincerely,

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"Trust in the Lord with all thine heart; and lean not unto thine own understanding.
In all thy ways acknowledge Him and He shall direct thy paths."

–Bible, Proverbs 3:5-6

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ABBREVIATIONS

Abbreviations	Terms
DTC	: differentiated thyroid cancer
RAT	: radioiodine therapy
RRA	: remnant radioiodine ablation
TSH	: thyroid stimulating hormone
Tg	: thyroglobulin
sTg	: stimulated thyroglobulin
SED	: structural evidence of disease
NMDPH	: Nuclear Medicine Department Penang Hospital
^{18}F-FDG	: 2-deoxy-2-(18F) fluoro-D-glucose
PET	: positron emission tomography
131-I Dx WBS	: radioiodine-131 diagnostic whole body scan
131-I Rx WBS	: radioiodine-131 post therapy whole body scan
Anti-Tg Ab	: anti-thyroglobulin antibody
ELISA	: enzyme-linked immunosorbent assay
NPV	: negative predictive value
PPV	: positive predictive value

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ABSTRAK

Pengenalan dan Tujuan:

Kejadian kanser tiroid differentiated telah meningkat dengan ketara di seluruh dunia. Tiyroglobulin (Tg) serum dan imbasan seluruh badan selepas rawatan radioiodin (I-131 Dx WBS) adalah dua jenis siasatan susulan yang dijalani selepas rawatan kanser di peringkat awal (pembedahan pembuangan seluruh tiroid dan pemusnahan sisa tiroid dengan radioiodin). Kajian ini bertujuan untuk menilai korelasi dan nilai ramalan Tg terangsang (sTg) 6 bulan selepas pemusnahan sisa tiroid dengan radioiodin untuk menentukan kewujudan penyakit berstruktur (SED) dalam I-131 Dx WBS. Jika sTg mempunyai nilai ramalan yang sangat tinggi terhadap kewujudan penyakit, ia boleh menggantikan I-131 Dx WBS untuk menilai kewujudan penyakit. Nilai ulangan sTg juga ditentukan bagi dua sampel berlainan yang diambil selepas tempoh 6 bulan.

Metodologi:

sTg diambil 6 bulan selepas rawatan peringkat awal diikuti dengan I-131 Dx WBS pada masa yang sama. Korelasi, sensitiviti, spesifisiti, nilai ramalan negatif (NPV) dan nilai ramalan positif (PPV) sTg terhadap SED dalam I-131 Dx WBS ditentukan. Enam bulan kemudian, sTg diukur semula dengan menggunakan kit immunoassay ELISA yang sama tanpa sebarang rawatan tambahan diberi dalam tempoh enam bulan itu.

Keputusan:

sTg berkorelasi baik dengan SED dalam 131-I Dx WBS. Kedua-dua nilai potongan positif sTg 1.0 ug/L dan 2.0 ug/L mempunyai NPV yang baik iaitu 85.0% dan 85.7% masing-masing untuk pengecualian penyakit. Walaubagaimanapun, keputusan menunjukkan PPV adalah rendah apabila menggunakan tahap potongan positif 10.0 ug/L iaitu 41.7% untuk meramalkan kewujudan penyakit. Koefisien korelasi intraclass adalah setinggi 96.6% untuk dua sTg yang diambil dalam tempoh 6 bulan berbeza.

Kesimpulan:

Pengukuran sTg yang dilakukan di pusat kajian ini boleh dipercayai. sTg mempunyai nilai ramalan negatif yang baik untuk penyakit berstruktur, dengan ini boleh melengkapkan I-131 Dx WBS untuk menggecualikan kewujudan penyakit, tetapi sTg bukan penanda yang baik untuk menentukan kewujudan penyakit struktur.

ABSTRACT

Background and Aims:

The incidence of differentiated thyroid cancer (DTC) has increased substantially worldwide in the past four decades. Serum thyroglobulin (Tg) and radioiodine diagnostic whole body scan (131-I Dx WBS) are two main surveillance tools to investigate for persistent disease after initial therapy - total thyroidectomy followed by radioiodine remnant ablation (RRA). This study evaluates the correlation, predictive value and reliability of stimulated thyroglobulin (sTg) for detection of structural evidence of disease (SED) in 131-I Dx WBS 6 months after initial therapy in the Nuclear Medicine Department Penang Hospital (NMDPH).

Methodology:

sTg was measured 6 months after initial therapy with 131-I Dx WBS done in the same setting to evaluate SED. Correlation, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of sTg to diagnose SED in 131-I Dx WBS was analyzed. Six months later, repeatability of sTg are measured under similar condition using the similar immunoassay ELISA method to determine the reliability of sTg measurement.

Results:

sTg level correlates well with SED in 131-I Dx WBS. Both sTg cutoff positivity of 1.0 ug/L and 2.0 ug/L have good NPV of 85.0% and 85.7% respectively to exclude SED in I-131 Dx WBS. However, sTg positive cutoff level of 10.0 ug/L has poor PPV of 41.7% to predict the presence of structural disease in I-131 Dx WBS. The intraclass correlation coefficient for two different sTg taken 6 months apart is 96.6%.

Conclusion:

sTg measurement done in NMDPH is reliable and it correlates well with SED in 131-I Dx WBS. sTg have good negative predictive value for persistent structural disease and could complement I-131 Dx WBS to exclude the presence of disease but is not a good marker to predict persistent structural disease in NMDPH.

CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

1.1 DIFFERENTIATED THYROID CANCER

Differentiated thyroid cancer (DTC) is the most common endocrine malignancy. (Lebastchi and Callender, 2014). It is mostly diagnosed at young age with more than 60% of patients are below 55 years old when diagnosed with DTC (Davies and Welch, 2006). DTC has substantially increased in incidence worldwide in the past four decades and is mainly due to advancement of medical imaging techniques to detect small thyroid nodules (Chen *et al.*, 2009; Colonna *et al.*, 2015; Sierra *et al.*, 2016). Most DTC is diagnosed with only locoregional disease on presentation which carries a very low mortality rate and good prognosis with 90-95% of 10 years progression free survival. Increase in incidence and low mortality rate lead to exponential increase in prevalence of DTC and this renders long-term surveillance the major part of DTC management (Degroot *et al.*, 1990).

Other than uncommon familial syndromes such as familial adenomatous polyposis, Gardner's syndrome and Cowden's disease, radiation exposure is a known predisposing factor of DTC (Machens et al., 2002). Those with serum thyroid stimulating hormone (TSH) concentration above the mean, even within normal range, also found greater likelihood to develop DTC than populations with TSH below the mean (Haymart *et al.*, 2008). Currently, there are theories on various oncogenes and tumor suppressor gene like RET, ras, braf, trk, met and P53 playing roles in the signal transduction systems related to the development of thyroid cancer (Parameswaran et al., 2010). The most observed genetic alteration in papillary thyroid cancer is BRAFV600E mutation, which has gained considerable research interest in its prediction

and prognostication of papillary thyroid cancer. However, its clinical relevance is still unclear (Baldini et al., 2014).

DTC consists mainly of papillary thyroid carcinoma (83%), follicular thyroid carcinoma (15%) and Hurthle cell carcinoma (2%) (Sipos and Mazzaferri, 2010). Papillary, follicular and Hurthle cell thyroid cancer vary in histological appearance but there is no difference in functionality of iodine uptake (Baldini et al., 2014). This enables radioiodine to be an widely utilized therapeutic and diagnostic agent for DTC patients.

1.2 RISK STRATIFICATION AND MANAGEMENT OF DIFFERENTIATED THYROID CANCER

The management plan of DTC depends very much on the risk of recurrence in 10 years time. The American Joint Committee on Cancer (AJCC) on tumor-node-metastasis (TNM) cancer staging system emphasizes a few high risk factors for persistent/ recurrent disease in DTC. Age at the time of diagnosis, tumor size, high-risk histology, extent of tumor, vascular invasion, cervical lymph nodes involvement and distant metastasis are associated with higher risk of DTC (Edge and Compton, 2010). Patients diagnosed with DTC at age above 45 or below 10 years old carry higher risk of recurrence. A recent large scale study involving 9484 patients suggests using age 55 and above, instead of 45, as the cutoff age to differentiate between high and low risk age groups. This has downstaged 12% of patients from the high risk to the low risk group, with the downstaged group having 10-year disease specific survival of 97.6%.

(Nixon et al., 2016).

High risk histological findings (tall cell or columnar cell in papillary thyroid carcinoma, widely invasive follicular thyroid carcinoma, poorly differentiated and Hurthle cell carcinomas) are associated with a poorer outcome (Degroot *et al.*, 1990; Asanuma *et al.*, 2001). The extent of the tumor also affects the risk of developing recurrent disease. Gross extrathyroidal extension of tumor into adjacent soft tissue, incomplete tumor resection, >5 metastatic lymph nodes or any lymph nodes metastasis >3 cm, extensive vascular invasion in follicular thyroid cancer and distant metastasis are among the high risk features stated in American Thyroid Association guidelines 2015. Other than these mentioned features, patients are categorized to low and intermediate risk groups (Refer Appendix A).

The fundamental management approach for all DTC includes surgical resection, with or without radioiodine remnant ablation (RRA), followed by long term thyroid stimulating hormone (TSH) suppressions therapy with oral L-Thyroxine. Types of surgical resection, either lobectomy or total thyroidectomy, with or without prophylactic central compartment neck dissection, depends on the size of the primary tumor, intra-operative findings and histological findings (Beenken *et al.*, 2000). The surgical approach of DTC with tumor size larger than 1.0 cm is total thyroidectomy with optional RRA (Chow *et al.*, 2003; Moo and Fahey III, 2011). Subsequently, radioiodine-131 post therapy whole body scan (131-I Rx WBS) day 4-7 post RRA will be performed for restaging (Pacini *et al.*, 2010; National Comprehensive Cancer Network, 2013; Haugen *et al.*, 2016). Papillary thyroid cancer ≤1.0 cm and minimally invasive

follicular thyroid cancer are considered to be in low risk group, lobectomy without RRA is recommended. However, features of multifocality and cervical lymph nodes metastasis carry higher risk of persistent/ recurrent disease and similar initial therapy regime (total thyroidectomy followed by RRA) would be adopted (Chow *et al.*, 2003).

Recent large prospective cohort studies on risk stratification done 6-12 months after initial therapy, based on therapy response, is superior to previously adopted risk stratification done during initial therapy. The attempt to re-stratify patients based on response to initial therapy helps prevent over-treatment of patients with low-risk disease while providing a more comprehensive approach to those remaining in the high risk group (Castagna et al., 2011; Tuttle and Sabra, 2013).

The latest American Thyroid Association guideline 2015 has categorized the response to initial therapy into four groups: excellent response, biochemical incomplete response, structural incomplete response and indeterminate response (Table 1). Response assessment shall be done within 2 years after initial therapy. The subsequent management approach for different groups of patients differs according to the risk of recurrent disease in future and disease specific death. It is stated that patients who achieves excellent response to initial therapy (in both structural and biochemical remission) have only 1% to 4% disease specific death rate. On the other hand, patients who have persistent locoregional structural disease have disease specific death rate of 11% while distant metastasis carries up to 50% of disease specific death rate (Haugen *et al.*, 2016). As such, the treating physician must attempt to restage all patients after total thyroidectomy and RRA with serum thyroglobulin (Tg)

and structural imaging after initial therapy due to clinical implications as shown in Table 1 below.

Table 1 Clinical Implications of Response to Therapy Reclassification in Patients with Differentiated Thyroid Cancer Treated with Total Thyroidectomy and Radioiodine Remnant Ablation, adapted from (Haugen et al., 2016)

Category	*Definitions*[a]	*Clinical outcomes*	*Management implications*
Excellent response	Negative imaging ***and either*** Suppressed Tg <0.2 ng/mL[b] ***or*** TSH-stimulated Tg <1 ng/mL[b]	1%–4% recurrence[c] <1% disease specific death[c]	An excellent response to therapy should lead to an early decrease in the intensity and frequency of follow up and the degree of TSH suppression
Biochemical incomplete response	Negative imaging ***and*** Suppressed Tg ≥1 ng/mL[b] ***or*** Stimulated Tg ≥10 ng/mL[b] ***or*** Rising anti-Tg antibody levels	At least 30% spontaneously evolve to NED[d] 20% achieve NED after additional therapy[a] 20% develop structural disease[a] <1% disease specific death[a]	If associated with stable or declining serum Tg values, a biochemical incomplete response should lead to continued observation with ongoing TSH suppression in most patients. Rising Tg or anti-Tg antibody values should prompt additional investigations and potentially additional therapies.
Structural incomplete response	Structural or functional evidence of disease With any Tg level With or without anti-Tg antibodies	50%–85% continue to have persistent disease despite additional therapy[c] Disease specific death rates as high as 11% with loco-regional metastases and 50% with structural distant metastases[a]	A structural incomplete response may lead to additional treatments or ongoing observation depending on multiple clinico-pathologic factors including the size, location, rate of growth, RAI avidity, ^{18}FDG avidity, and specific pathology of the structural lesions.
Indeterminate response	Nonspecific findings on imaging studies Faint uptake in thyroid bed on RAI scanning Nonstimulated Tg detectable, but <1 ng/mL Stimulated Tg detectable, but <10 ng/mL ***or*** Anti-Tg antibodies stable or declining in the absence of structural or functional disease	15%–20% will have structural disease identified during follow-up[a] In the remainder, the nonspecific changes are either stable, or resolve[a] <1% disease specific death[a]	An indeterminate response should lead to continued observation with appropriate serial imaging of the nonspecific lesions and serum Tg monitoring. Nonspecific findings that become suspicious over time can be further evaluated with additional imaging or biopsy.

NED denotes a patient as having no evidence of disease at final follow-up.

1.3 IMAGING MODALITY IN DIFFERENTIATED THYROID CANCER FOLLOW UP

Radioiodine-131 diagnosis whole body scan (I-131 Dx WBS) has been a procedure of choice since a few decades ago to determine persistent/ recurrent disease in the first 2 years after initial therapy for ATA low and intermediate risk group patients. DTC preserves functional integrity of sodium iodide symporter, thus able to concentrate iodide, enabling I-131 Dx WBS to be an useful tool to detect micrometastasis and macrometastasis disease (Galligan et al., 1982; Van Sorge-Van Boxtel et al., 1993).

British Thyroid Association guideline recommends the use of ultrasound neck and serum Tg in the follow up of low risk DTC patients (Perros *et al.*, 2014). One study suggests that when sTg <3 ug/L, I-131 Dx WBS may be avoided in the follow-up of DTC patients and only neck ultrasound is warranted to monitor persistent/ recurrent disease (Pacini et al., 2002). Nevertheless, ultrasound neck has limitation to pin point micrometastasis or lesion smaller than 2 mm and is not suitable to visualize deep cervical or mediastinal regions. Thus most centers are still adopting I-131 Dx WBS as the main modality for assessment of structural disease.(Lee et al., 2013).

For high risk patients, computed tomographic (CT), magnetic resonance imaging (MRI) or 18-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) are the modalities of choice for further investigation for metastatic disease, depending on the clinical presentation (Lamartina et al., 2016). Suspicious of non-iodine avid disease should be raised in cases of elevation and rising trend of serum Tg but no structural evidence (SED) in I-131 Dx WBS. For this condition,

18F-FDG PET/CT proved to be a useful diagnostic tool to detect and localize locoregional recurrences as well as distant metastases in non-iodine avid disease (Kim et al., 2009; Dennis et al., 2012; Hamed et al., 2014). High 18F-FDG PET/CT uptake in non-iodine avid lesions suggests lost of sodium-iodide symporter activity and aggressive cell growth with enhanced glucose transporter genes expression in DTC (Dong et al., 2009).

However, interestingly in some cases of raised serum Tg levels but negative findings in both I-131 Dx WBS and 18F-FDG PET/CT, good evolution is noted on follow-up serum Tg with descending Tg trend despite without additional treatment. A significant percentage of them even reach normal Tg levels on follow-up. As such, the predictive values of sTg for persistent/ recurrent disease and the optimum positive cutoff level of sTg to predict persistent/ recurrent disease is essential to avoid over- or under- investigation into this group of patients with raised serum Tg and negative I-131 Dx WBS (Pachon-Garrudo *et al.*, 2012).

1.4 THYROGLOBULIN IN DIFFERENTIATED THYROID CANCER FOLLOW-UP

Tg is a thyroid specific glycoprotein produced in thyroid follicular cells prompted by TSH stimulation. Tg stays mainly intrathyroidal under normal physiological conditions and acts as the precursor of thyroid hormone synthesis. A small proportion is iodized as active thyroid hormone and released into the circulation through the process of exocytosis (Shlossberg et al., 1979; Rivolta and Targovnik, 2006). DTC preserves its Tg synthesis function and enables Tg to be a useful biomarker

in DTC (Evans *et al.*, 2015). Tg taken under TSH stimulation, either by withdrawal of thyroxine or human recombinant TSH, is called stimulated Tg (sTg). Serum TSH >30 mU/L is essential not only for adequate activation of thyroid follicular cells to take up radioiodine but also to ensure the synthesis and release of serum Tg which improves the detectability of serum Tg (Duntas and Biondi, 2007).

1.0 ug/L serum Tg concentration indicates presence of approximately one gram of normal thyroid tissue in the body. Post total thyroidectomy and successful RRA, when there is no more thyroid tissue left in the body, serum Tg should be undetectable (Francis and Schlumberger, 2008). Thus, in DTC patients after initial therapy, serum Tg taken at least 3 months after completion of therapy, becomes a very useful marker to exclude persistent disease (Schlumberger and Baudin, 1998). Any patient post total thyroidectomy and RRA with detectable serum Tg must be regarded as having persistent disease and subsequent work-up to localize Tg producing lesion is required (Pacini and Pinchera, 1999). A pooled data from a meta analysis demonstrated sTg taken within 2 years after initial therapy had NPV of 97% for persistent/ recurrent disease when using positive cutoff level of 1.0 ug/L while cutoff level of 2.0 ug/L achieved NPV of 99% (Giovanella *et al.*, 2013). On top of diagnostic value, sTg level post initial therapy also has prognostic significance with higher levels implying higher risk of future cancer recurrence (Pelttari *et al.*, 2010).

A few studies established positive relationship between serum Tg concentration and tumor burden but the exact amount of cancer cells required to increase serum Tg levels is unknown. Serum Tg levels integrates 3 factors: the mass of

thyroid tissue present, the degree of TSH receptor stimulation and the tumor's intrinsic ability to synthesize and secrete Tg (Spencer *et al.*, 1999). In DTC patients post total thyroidectomy without RRA, sTg has excellent correlation with distant metastases (Ashcraft and Van Herle, 1981; Ramanna *et al.*, 1985). On the contrary, for those post total thyroidectomy and RRA patients, the detection rates of remnant or residual DTC tissue are 80% when sTg ≥10 ug/L, 97% with sTg ≥5 ug/L and 100% with sTg ≥2 ug/L (Fatourechi and Hay, 2000). An observational study of 366 DTC patients found that sTg obtained 6 months after initial therapy, using positive cutoff level of 10.0 ug/L has a sensitivity of 100.0%, specificity of 93.1% and positive predictive value (PPV) of 77% for recurrent disease, while sTg positive cutoff level of 2.0 ug/L has similar sensitivity but lower specificity (82%) and low PPV (54%) for recurrent disease. (Heemstra *et al.*, 2007a).

Unfortunately, anti-Tg antibody (anti-Tg Ab) interference could alter serum Tg concentration in the samples causing falsely low negative serum Tg. This happens in up to 20% of DTC patients (Pacini and Pinchera, 1999). All available immunoassays to measure Tg have similar limitations and in the presence of anti-Tg Ab it becomes technically challenging to ensure the accuracy of serum Tg measurement (Tate and Ward, 2004). Thus, Tg is only valid as a diagnostic or surveillance tool in the absence of anti-Tg Ab. sTg measurements from the washout of the needle used in fine-needle aspiration cytology in metastatic lymph nodes are not affected by circulating anti-Tg Ab and overcomes the pitfall to correctly diagnose early DTC recurrence. However, this method is not yet widely adopted into clinical practice (Cappelli *et al.*, 2013).

For the reasons mentioned above, the latest American Thyroid Association guideline (2015) emphasizes the role of Tg in the follow-up of patients after initial therapy in DTC. The Tg level has significant impact on the management of patients, especially those who have no SED in imaging (Table 1). Patients who exhibited no SED in imaging and Tg <1 ug/L off L-Thyroxine or Tg <0.2 ug/L on L-Thyroxine were considered having excellent response to therapy and needing less degree of TSH suppression and less intensity of follow-up. On the other hand, after initial therapy, when there is no SED, sTg ≥10.0 ug/L off L-Thyroxine or sTg ≥1.0 ug/L on L-Thyroxine or rising trend of serum Tg should prompt additional investigations and potential additional therapies might be needed. The reason that being with the latter mentioned Tg level, despite absence of SED, patients have 20% risk of developing structural disease later (Haugen et al., 2016).

1.5 MANAGEMENT OF DIFFERENTIATED THYROID CANCER IN MALAYSIA

Malaysia National Cancer Registry Report 2007-2011 showed that thyroid cancer is the tenth commonest cancers in Malaysia with average incidence of 110 new cases in male and 345 new cases in female per year (AbM *et al.*, 2016). This study was conducted in Nuclear Medicine Department Penang Hospital, the third largest center in Malaysia offering RRA for DTC patients. Malaysian Consensus Guidelines on Well Differentiated Thyroid Cancer has similar recommendation to ATA guidelines in terms of surgical management, RRA, TSH suppression therapy and follow up on DTC patients.

Patients who have tumors greater than 1 cm will undergo total thyroidectomy

followed by RRA with a day 4-5 post therapy whole body scan (I-131 Rx WBS) for cancer restaging. For those having iodine uptake in neck only and not elsewhere in the body, a radioiodine diagnostic whole body scan (I-131 Dx WBS) will be done 6 months later for assessment. On the other hand, for patients with distant metastasis, subsequent high dose radioiodine therapy (RAT) will be employed.

As mentioned earlier, 131-I Dx WBS was the imaging of choice to assess disease status 6 months after initial therapy for DTC patients in Malaysia. For those who have achieved complete structural response to therapy (I-131 Dx WBS shows no SED in the neck and elsewhere), regardless of sTg level, a second 131-I Dx WBS will be repeated 6 months later as confirmatory study. On the contrary, if there is persistent structural disease 6 months after RRA, high dose RAT will be given to patients. Patients who have two consecutive I-131 Dx WBS showing no SED but noted raised sTg and the trend is rising with few consecutive readings would be further investigated with 18F-FDG PET/CT to look for non-iodine avid disease.

Even though serum Tg and 131-I Dx WBS are considered two fundamental tools in assessing DTC status after initial therapy in Malaysia, there has been no study done to determine the correlation and independent predictive value of serum Tg for the presence of persistent SED in 131-I Dx WBS in DTC patients. In routine clinical practice, all samples are sent to outsource laboratory, Institute of Medical Research Malaysia, for measurement. The reliability of Tg measurement used would be of great concern as it has significant impact for the course follow-up on DTC patients in NMDPH. This study helps to gain perspective into the above mentioned areas of interest.

CHAPTER 2

STUDY OBJECTIVES & BENEFITS

2.1 OBJECTIVES OF THE STUDY

General Objective

To determine the correlation, positive predictive value and negative predictive value of sTg for SED in I-131 Dx WBS 6 months after RRA.

Specific Objective

To determine the repeatability of sTg in the same patient measured 6 months apart in the same laboratory using the same method.

2.2 BENEFITS OF THE STUDY

If the NPV and PPV of sTg for persistent disease are very high, it may be considered as a substitute for I-131 Dx WBS to detect persistent disease.

2.3 STUDY HYPOTHESIS

1. sTg positive cutoff level 1.0 ug/L and 2.0 ug/L has NPV 95% to exclude SED in I-131 Dx WBS.
2. sTg ≥10 ug/L has PPV >70% to detect SED in I-131 Dx WBS.

CHAPTER 3

MATERIALS & METHODS OF THE STUDY

3.1 STUDY BACKGROUND

This study was conducted among ATA low and intermediate risk DTC patients treated in NMDPH for the duration of 18 months.

This study has the approval of Medical Research and Ethics Committee, Ministry of Health Malaysia with reference number NMRRM–15–1324-25854 [Appendix L] and The Research Ethics Committee (Human), school of Medical Sciences, Universiti Sains Malaysia with certificate number USM/JEPeM/16020051 [Appendix M].

3.2 SUBJECT RECRUITMENT

All eligible patients aged 18 and above with histologically proven differentiated thyroid carcinoma who came for I-131 Dx WBS 6 months after RRA within the study period in NMDPH were recruited.

The eligibility criteria included prior total thyroidectomy followed by radioiodine remnant ablation 6 months earlier, where a day 4-5 post I-131 treatment whole body scan showed uptake in the neck only (those with locally advanced cancer or distance metastasis were excluded from the study as they were given high dose RAT instead of doing a I-131 Dx WBS); adequate TSH stimulation with level >30 mU/L; and without the interference of anti-Tg antibody in the serum.

A total of 46 patients were recruited for this study and, of whom 4 were dropped from the study due to inadequate TSH stimulation and the presence of anti-Tg antibody in the serum.

3.3 STEPS AND INSTRUMENTS OF THE STUDY

Patients who came for 131-I Dx WBS were prepared as per our usual protocol (withheld oral L-thyroxine for 4 weeks and on low iodine diet for 2 weeks). After taking consent, medical officers in NMDPH took the relevant history and performed physical examination. The indication of I-131 Dx WBS and radiation safety precautions were explained to patients.

Venous blood was taken for serum Tg, anti-Tg antibody, T4 and TSH prior to radioiodine administration. All serum Tg was sent to Institute of Medical Research (IMR) Malaysia for testing using the ELISA kit produced by Dialab. All serum anti-Tg antibody was taken from the similar syringe and was tested. Patients with the presence of serum anti-Tg antibody were later excluded from this study.

Image acquisition of the whole body was performed on day 4-5 post 5 mCi of radioiodine I-131 administration, using gamma camera according to protocol (Refer appendix B). Scan findings were interpreted by a nuclear medicine specialist in NMDPH.

The subsequent serum TSH, Tg and anti-Tg antibody were taken 6 months later (12 months post RRA) using the same preparation protocol. Patients with the presence of serum anti-Tg antibody were excluded from this study.

3.4 STUDY TERMINOLOGIES

Radioiodine Remnant Ablation (RRA)

Destruction of remnant thyroid tissue or microscopic disease in the thyroid bed after total or near-total thyroidectomy with administration of 80-120 mCi radioiodine-131.

Radioiodine Therapy (RAT)

Destruction of microscopic and macroscopic thyroid neoplastic cells with administration of 100-150 mCi radioiodine-131.

Post Therapy Whole Body Scan (I-131 Rx WBS)

Whole body scan with gamma camera after 4/5 days of high dose radioiodine-131 ingestion.

Diagnostic Whole Body Scan (I-131 Dx WBS)

Whole body scan with gamma camera after 4/5 days of low dose 5 mCi radioiodine-131 ingestion.

Structural Evidence of Disease (SED)

Presence of normal thyroid tissue, microscopic disease in the thyroid bed or metastatic regional cervical lymph nodes detected by imaging.

Initial therapy

Total thyroidectomy followed by radioiodine remnant ablation with high dose radioiodine (80 to 120 mCi) for DTC patients.

Persistent disease

Persistent normal thyroid tissue, microscopic disease in the thyroid or metastatic regional cervical lymph nodes 6 months after initial therapy.

Recurrent disease

The return of thyroid cancer in any part of the body after successful treatment.

Thyroglobulin (Tg)

Thyroglobulin is a large, iodinated, glycosylated protein with a molecular mass of 660 kDa, produced by thyroid follicular cells. Its level is measured by immunoassay.

Unstimulated Thyroglobulin (sTg)

Serum thyroglobulin level measured when patients are on TSH suppression therapy with Tab L-Thyroxine.

Stimulated Thyroglobulin (sTg)

Serum thyroglobulin level measured after 4 weeks of TSH suppression therapy (Thyroxine) withdrawal with TSH level of >30 mU/L.

Sensitivity

Sensitivity in this study denotes the ability of sTg to detect SED in I-131 Dx WBS.

Specificity

Specificity in this study denotes the ability of sTg to exclude SED in I-131 Dx WBS.

Negative Predictive Value (NPV)

The probability of patients who have a negative sTg test result actually having no SED in I-131 Dx WBS. It is the true negative rate among the negative test results.

Positive Predictive Value (PPV)

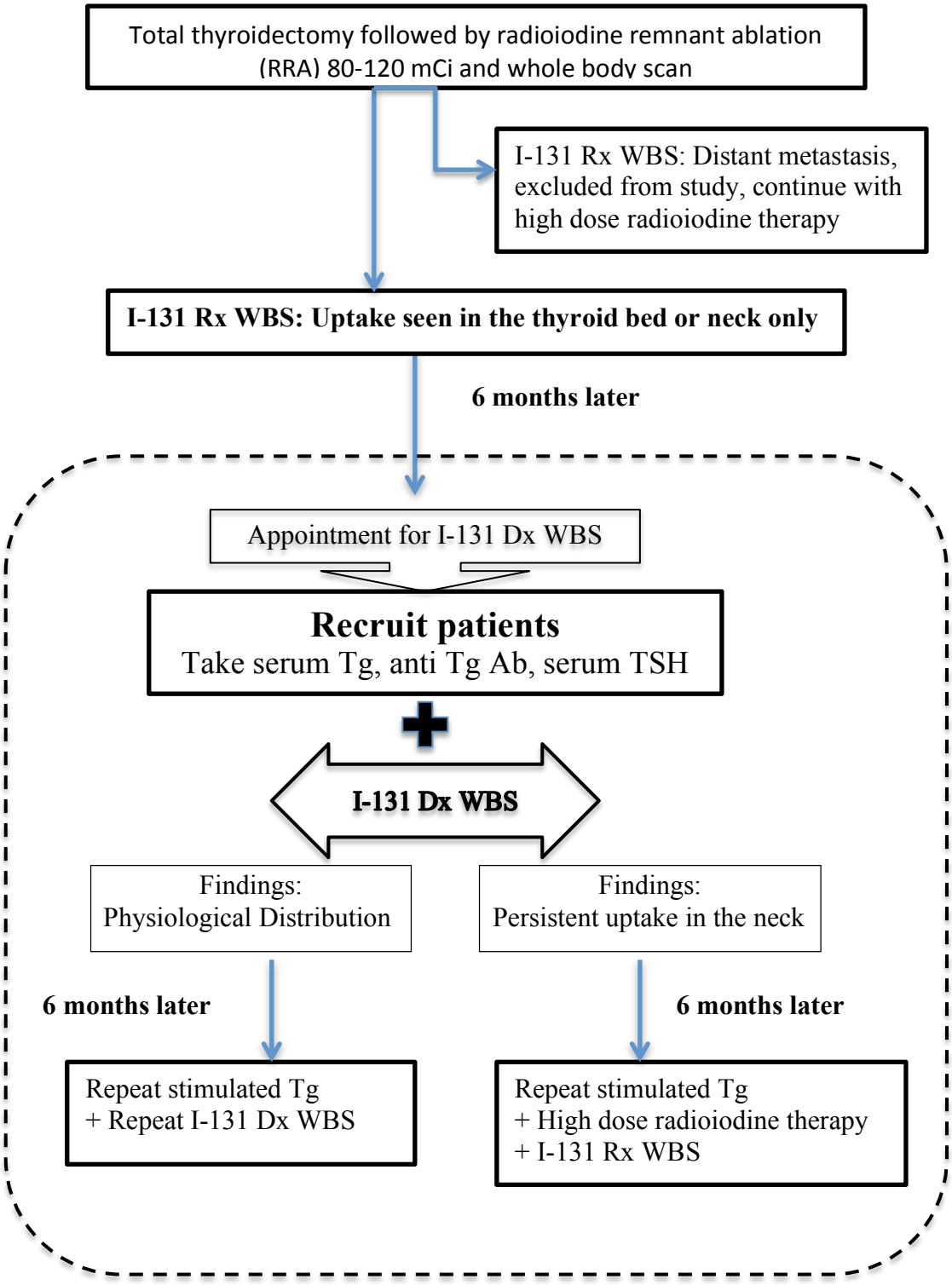
The probability of patients who have a positive sTg test result actually is having SED in I-131 Dx WBS. It is the true positive rate among the positive test result.

3.5 DATA COLLECTION AND ANALYSIS

Main demographic details of patients such as age, gender, histopathological findings (size of tumor, papillary or follicular; metastasis to cervical lymph nodes) were taken. Date of RRA, radioiodine dose administered, pre-RRA sTg, anti-Tg antibody, T4 and TSH levels as well as findings of 131-I Rx WBS were recorded. 131-I Dx WBS findings during patients recruitment were interpreted by a nuclear medicine specialist and grouped into two groups: complete structural response or structural evidence of disease (SED). For patients with 131-I Dx WBS showing abnormal uptake in the neck, high dose radioiodine therapy (RAT) 100-150 mCi was given 6 months later, followed by 131-I Rx WBS (using the same image acquisition protocol) to confirm the previous 131-I Dx WBS findings. On the other hand, patients for whom 131-I Dx WBS showed physiological uptake, another I-131 Dx WBS was performed 6 months later (using the same image acquisition protocol) for second confirmation.

sTg was taken at two different intervals: 6 months after RRA (when patients were recruited for this study) and 12 months after RRA. Serum anti-Tg antibody and TSH were taken together each time taking serum Tg. The collected data was analyzed using IBM Statistic SPSS Version 22. The correlation of sTg at 6 months post RRA with SED in 131-I Dx WBS was determined using simple logistic regression; sensitivity, specificity, negative predictive value and positive predictive value of sTg were determined using two by two tables and the repeatability of sTg taken during recruitment and 6 months later was determined using intraclass correlation coefficient.

3.6 STUDY ALGORITHM



CHAPTER 4

RESULTS

4.1 DEMOGRAPHIC DATA

Table 2 Demographic Data In terms of Age, Gender and Clinical Characteristics

Demographic Data and Clinical Characteristics			
		Number	Percent (%)
Age of Patients	<55	30	71.4
	≥55	12	28.6
Gender of Patients	Male	8	19.0
	Female	34	81.0
Types of Thyroid Cancer	Papillary	33	78.6
	Follicular	9	21.4
Tumor Size (cm)	0.1-1.0	6	14.3
	1.1-2.0	16	38.0
	2.1-4.0	15	35.7
	≥4.1	5	11.9
Cervical Lymph Nodes Metastasis	Yes	10	23.8
	No	32	76.2

Table 3 Stimulated Tg and Diagnostic Whole Body Scan Findings 6 months after Initial Therapy

Summary of Collected Data			
Stimulated Thyroglobulin 6 months after radioiodine remnant ablation (RRA)	<1.0	20	47.6
	1.0 - <2.0	1	2.4
	2.0 - <10.0	9	21.4
	≥ 10.0	12	28.5
Diagnostic whole body scan 6 months after RRA	SED in Neck	9	21.4
	No SED	33	78.6

(Notes: Please refer to Appendix E for details on each patients, clinical characteristics and data collected).

Table 4 Stimulated Thyroglobulin (sTg) in Patients With and Without Structural Evidence of Disease (SED) in Diagnostic Whole Body Scan (I-131 Dx WBS)

Persistent SED in I-131 Dx WBS in the neck	**Number of Patients**	**Mean sTg (ug/L)**
Yes	9	180.5
No	33	14.8

4.2 STIMULATED THYROGLOBULIN TO PREDICT STRUCTURAL DISEASE

Correlation of sTg and SED in I-131 WBS

Correlation between stimulated thyroglobulin 6 months after radioiodine remnant ablation and structural evidence of disease in I-131 WBS was determined using simple logistic regression SPSS:

Table 5 The correlation of sTg at 6 Months Post RRA with SED in 131-I Dx WBS.

	Unstandardized Coefficients		Standardized Coefficients		Sig.
Model	B	Std. Error	Beta	t	
1 (Constant)	.136	.060		2.276	.028
TG6	.003	.001	.511	3.756	.001

Simple logistic regression revealed significant correlation of sTg 6 months after RRA with SED in 131-I Dx WBS ($p<0.05$). This also implied the odd of having SED increased with every unit of increased in sTg. Subsequent analysis of the sensitivity, specificity, negative Predictive Value and Positive Predictive Value of sTg 6 months after RRA for SED in I-131 Dx WBS was determined using two by two table