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The Best TG cut-off Value for 18F-FDG PET/CT Imaging of De-Differentiated Thyroid Cancer with Elevated TG and Negative 131-RAI WBS

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ABSTRACT:

Objective: to determine the most appropriate threshold for thyroid cancer tumor indicators (TG and Anti-TG) among individuals who exhibited negative I^{131}-WBS and rising tumor marker levels during follow-up who have clinical suspicion of de-differentiation and are therefore referred for ^{18}F-FDG PET/CT scan. **Methods:** We looked at 31 patients' FDG PET/CT scans of thyroid carcinoma that was well-differentiated at the time of thyroidectomy, but their RAI WBS became negative during follow-up, in spite of rising TG or Anti-TG levels. **Results:** PET/CT revealed a positive finding in 21 patients (67.7%), and was negative in 10 patients (32.2%). The most common site of a PET/CT finding was the lymph nodes (n = 24, 77.4%), followed by distant metastases (n=8, 25.8%), and the least common site was the thyroid bed (n=7, 22.6%). Patients whose PET/CT results were positive had TG values that were significantly different from those whose results were negative (p=0.006), but there was no statistically significant difference in the Anti-TG values between those whose PET results were positive and those whose were negative (p=0.29). A solid predictor of a positive PET/CT result is serum TG level, according to ROC-curve study for the optimal TG level before PET/CT, which showed an area under the curve (AUC) of 81%. The ideal detection limit is 25 ng/ml, which provides a sensitivity of 81%, a specificity of 60% percent and an overall accuracy of 74 %. An area under the curve (AUC) of 63% was shown by the ROC-curve analysis for the optimal Anti-TG level before PET/CT, indicating that serum TG level is a poor predictor of a positive PET/CT outcome. An estimated 27 ng/ml was the ideal level for the Anti-TG, with a sensitivity of 58%, specificity of 67 %, and an overall accuracy of 61 %.

Conclusion: Patients with suspicion of de-differentiation during follow-up after an initial diagnosis of differentiated thyroid cancer might benefit from PET/CT as a diagnostic tool. Nevertheless, our findings suggest that the optimal TG level for PET/CT to reliably detect a positive result is more than 25 ng/ml.

Keywords: De-differentiated thyroid cancer, PET, Thyroglobulin.

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INTRODUCTION:

The prognosis is usually excellent for differentiated carcinoma of the thyroid (DTC), which is the most common endocrine malignancy. 10 year survival rate is reported to be higher than 90% [(1)]. Diagnosis of DTC often occurs incidentally due to the low aggressiveness of this malignancy, which often does not manifest clinically. The low aggressiveness of DTC at the cellular level is associated with the expression of the sodium iodide symporter (NIS), a protein required for iodine uptake by normal cells of the thyroid and is expressed across their plasma membrane. The use of RAI in the detection and treatment of thyroid cancer is based on the NIS-mediated iodine transportation across the cell membrane of DTC [(2)]. The lack of NIS expression on the neoplastic cells is responsible for the cellular de-differentiation in up to 5% of originally diagnosed DTC. This results in the loss of iodine uptake ability, leading to failure of diagnosis and treatment by RAI [(3)]. Clinical suspicion of de-differentiation takes place when the patient presents with rising titres of the tumor markers (TG and/or anti-TG), whereas diagnostic whole-body scanning (WBS) by RAI is negative. In these patients, an imaging tool other than RAI is necessary to localize the source of rising TG. Although ultrasonography of the neck may pinpoint the exact location of loco-regional persistent or recurrent disease (in the thyroid bed and cervical lymph nodes), it has its limitations as it is operator-dependent, which therefore results in reports of variable confidences in the clinical practice. It also cannot diagnose distant metastases [(4)]. For patients with thyroid carcinoma who are clinically thought to have de-differentiated, FDG PET/CT has recently gained acceptance as an appropriate whole body imaging approach. However, the ideal serum TG level linked with high FDG PET/CT

sensitivity is still up for discussion and investigation. There is currently no agreement on the optimal TG level prior to FDG PET/CT scan, despite variable suggested levels in literature [5, 6, 7]. Our research was set out to determine which TG cut-off level is most strongly linked to a positive PET/CT result at our institution in terms of sensitivity, specificity and overall accuracy.

PATIENTS and METHODS:

This retrospective study was performed at the Nuclear Medicine Department, Faculty of Medicine, Cairo University. The research ethics commission at the institution gave its approval. The research comprised 31 individuals diagnosed with differentiated thyroid carcinoma, all of which had papillary thyroid carcinoma as confirmed by histopathology. Each patient had a complete thyroidectomy followed by 131RAI ablation. They showed up with elevated or increasing TG or Anti-TG levels during follow-up, despite the fact that 131RAI WBS came out negative. The institutional protocol was followed by all patients undergoing FDG PET/CT scans. This protocol includes ensuring that blood sugar levels were less than 100 mg/dl before the scan, fasting for at least 6 hours, injecting 0.1 mCi/kg FDG, waiting 45 to 90 minutes for uptake, and finally scanning from the vertex to the mid-thigh. Low-dose CT for attenuation correction was performed first, followed by multi-slice high-dose CT to pinpoint anatomical locations, and finally PET was performed to evaluate FDG uptake. Two experienced nuclear medicine doctors evaluated the PET/CT scans. The visual assessment of FDG absorption was supplemented by semi-quantitative measurements of the SUV of the lesions that were visually observed.

Statistical Analysis:

When applicable, data were statistically reported using median and range, or frequencies (number of instances) and percentages, or mean ± standard deviation (± SD). The normal assumption was checked for numerical data using the Kolmogorov Smirnov test. In order to compare TG and anti-TG levels among the research groups, the Mann Whitney U test for independent samples was used. Precision was denoted by the words specificity and sensitivity. The optimal cutoff value for TG and anti-TG levels in predicting positive PET/CT findings was determined using receiver operator characteristic (ROC) analysis. To be

deemed statistically significant, two-sided p-values have to be less than 0.05. All statistical analyses were conducted using Windows version 22 of IBM SPSS (Statistical Package for the Social Science), which is developed by IBM Corp. and is available for Microsoft Windows.

RESULTS:

Our research cohort had an average age of 44.4 years (standard deviation = 17), with a range of 16–76 years. Males made for 32.3% (n=10) of the patients investigated, while females accounted for 67.7% (n=21). In 21 cases (67.7%), PET/CT showed a positive result, whereas in 10 individuals (32.3%), it showed a negative result Table 1. Among the sites where PET/CT scans were detected, lymph nodes (n=24, 77.4%), distant metastases (n=8, 25.8%), and the thyroid bed (n=7, 22.6%), in that order, were the most prevalent. The cervical lymph nodes (LNs) were the most prevalent location for nodal illness, but the lung was the most common location for distant metastases. Table 2 provides further information on the location of the PET/CT results.

The average time interval between first diagnosis of differentiated papillary thyroid cancer (date of thyroidectomy), and the date of clinical suspicion of de-differentiation (date of PET/CT) was 42.9 months (SD= 36.9), with a minimum time interval of 6.5 months and a maximum time interval of 173.5 months Table 3.

Table 1 Patient characteristics data

		Count	Percent
Gender	Male	10	32,2%
	Female	21	67.7%
PET/CT result	Positive	21	67.7%
	Negative	10	32.3%

Table 2 Site of PET CT findings

		Number	Percentage
Thyroid bed		**7**	**22.6**
LNs		**24**	**77.4**
	➢ **Cervical** (either unilateral or bilateral, either isolated or accompanied with the affection of other LN groups)	**21**	
	➢ **Para-pharyngeal**	**1**	
	➢ **Para- tracheal**	**3**	
	➢ **Multiple widespread**	**4**	
Distant metastases		**8**	**25.8**
	➢ **Bone**	**1**	**3.2**
	➢ **Lungs**	**6**	**19.4**
	➢ **Pancreatic head**	**1**	**3.2**

Table 3 Time interval between first diagnosis of thyroid cancer (date of thyroidectomy) and the date of clinical suspicion of de-differentiation (date of PET/CT)

	Range (in months)	Average (in months)	SD
Operation – PET/CT interval	6.5 – 173.5	42.9	36.9

Residual/recurrence thyroid bed lesions averaged 1.2 cm in size with an average SUV of 7.9, nodal lesions averaged 1.5 cm in size with an average SUV of 5.0, while distant metastatic lesions averaged 1.3 cm in size with an average SUV of 6.1. Table 4 provides more information on the size and SUVs of the PET/CT scan findings.

Table 4 Size and SUV max of the PET/CT findings

		Range	Mean	±SD
Thyroid bed	Size (in cm)	0.9 – 1.5	1.2	0.3
	SUV	2.6 – 17.6	7.9	6.7
LNs	Size (in cm)	0.8 -4.0	1.5	0.7
	SUV	1.6 – 11.0	5.0	3.0
Distant metastases	Size (in cm)	0.6 – 2.8	1.3	0.9
	SUV	2.6 – 9.9	6.1	3.4

In patients who had a positive PET/CT result, the average TG level prior to PET/CT was 206.3 ng/ml (SD= 240.4) with a range of 5.0 to 907 ng/ml; while in patients who had a negative PET/CT result, the average TG level was 36.7 ng/ml (SD= 37.5) with a range of 0.5 to 101 ng/ml Table 5.

Table 5 TG levels prior to PET/CT

	Range (ng/ml)	Average (ng/ml)	SD
PET result negative	0.5 – 101	36.7	37.5
PET result positive	5.0 – 907	206.3	240.4

The average Anti-TG level prior to PET/CT in PET positive patients was 114.9 ng/ml (SD=247.7) with a range of 0 -988 ng/ml, while in PET negative patients it was 45.2 ng/ml (SD= 33.67), with a range of 13- 100 ng/ml Table 6.

Our study found that the likelihood of a positive PET/CT result was 40% for TG levels ranging from 1 to 10 ng/ml; 57% for TG levels ranging from 10 to 100 ng/ml; and 92% for TG levels over 100 ng/ml Table 7.

Table 6 Anti –TG levels prior to PET/CT

	Range (ng/ml)	**Average (ng/ml)**	**SD**
PET result negative	13 – 100	45.2	33.67
PET result positive	0 – 988	114.9	247.7

Table 7 Likelihood of a positive PET/CT findings according to TG level range

	Likelihood of a positive PET/CT findings
TG level range 1 – 10 ng/ml	**40%**
TG level range 10 – 100 ng/ml	**57%**
TG level range over 100 ng/ml	**92%**

Patients whose PET/CT results were positive had TG values that were significantly higher from those whose PET/CT results were negative (p=0.006),

but there was a statistically insignificant difference in the Anti-TG values between those whose PET results were positive and those whose PET results were negative (p=0.29).

The serum TG levels was a solid predictor of a positive PET/CT result, according to ROC-curve study for the optimal TG level before PET/CT, which showed an area under the curve (AUC) of 81%. The ideal cut-off level for TG was determined to be 25 ng/ml Figure 1, which gives a sensitivity of 81%, a specificity of 60% and an overall accuracy of 74%.

ROC curve for the Anti-TG showed an area under the curve (AUC) of 63%, indicating that serum TG level is a poor predictor of a positive PET/CT outcome. With an estimated sensitivity of 58%, specificity of 67%, and overall accuracy of 61%, the ideal cut-off level for Anti-TG was determined to be 27 ng/ml Figure 2. Table 8 provides an overview of the results of the ROC curve analysis for TG and Anti-TG.

Table 8 Results of the ROC curve analysis for the best TG and Anti-TG cut-off values for the prediction of a positive PET/CT finding

	Optimal cut-off value	AUC	Sensitivity	Specificity	Overall accuracy
ROC curve for TG	25 ng/ml	81%	81%	60%	74%
ROC curve for Anti-TG	27 ng/ml	63%	58%	67%	61%

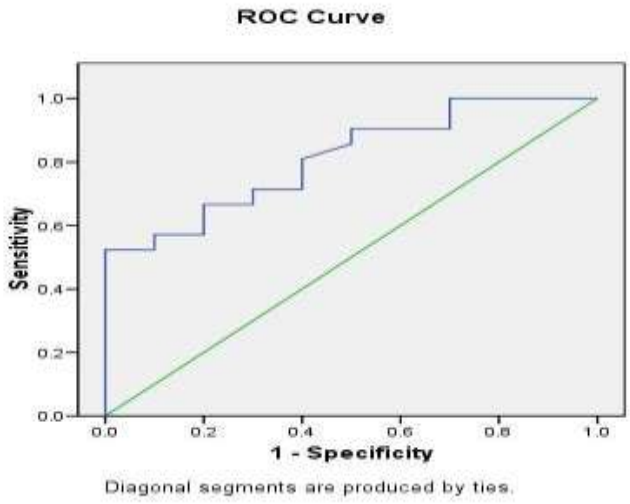

Figure 1: ROC curve analysis for the optimal serum TG level for positive PET/CT finding.

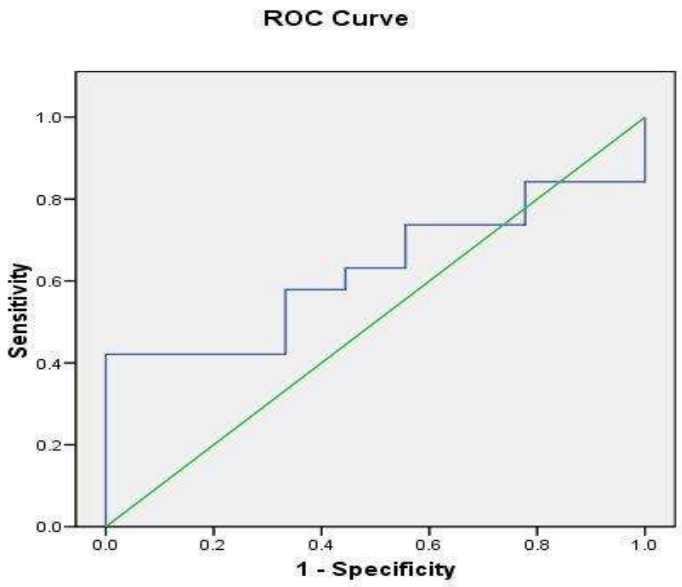

Figure 2: ROC curve analysis for the optimal serum Anti-TG level for a positive PET/CT finding.

DISCUSSION:

A de-differentiation clinical scenario would be a patient with an initially diagnosed differentiated thyroid cancer whose RAI WBS scan comes out negative despite persistently high or increasing TG levels during follow-up. It is expected that around 5% of patients with initially differentiated thyroid cancer may develop de-differentiation, which is linked to a poorer prognosis and worse overall survival rates [3]. In order to treat these patients effectively, identifying the cause of their increasing TG levels is the first and most critical step in their ongoing care. In all standard follow-up examinations of thyroid cancer patients, the neck ultrasound is used as the primary imaging modality in conjunction with RAI WBS. Still, although ultrasonography of the neck is quite accurate at picking up on recurring or persistent disease in the neck, it has its limitations. Firstly, it sometimes provides equivocal results that are indecisive about the nature of the detected tissue at the operative bed or at the cervical lymph nodes. Secondly its results can vary according to the experience of the operator, the machine or the technique used, which may influence the clinical interpretation and cause false

conclusions. Thirdly, it cannot detect disease outside of the neck [4]. F-18 FDG PET/CT has recently been an integral part of the management for this subset of thyroid cancer patients. Multiple investigations have shown that the likelihood of PET/CT positive results increases proportionately with rising TG levels, a finding that is linked to the fact that TG levels are proportional to the amount of neoplastic thyroid tissue [8]. However, the most accurate exact TG cut-off level that should be used to refer the patient for PET/CT is still not agreed upon and is an open question in the clinical setting and in the research literature. Scanning patients that have a TG level that is not high enough will result in PET/CT results that are falsely negative, i.e. decreased sensitivity of PET/CT. Variable TG cut-offs for referring thyroid cancer patients for PET/CT scan were proposed in literature. According to **Döner et al.,** a TG cut-off of 10.4 ng/ml is most closely associated with a positive FDG PET/CT result; **Bertange et al.** demonstrated that patients with TG levels above 12 ng/ml achieved the best FDG PET/CT accuracy; while Choi et al. proposed a TG cut-off of 21.5 ng/ml for that purpose (5,6,7). However, some authors took a different approach, and analyzed the optimal TG level using a range rather than a specific cut-off figure. As an example, **Shammas et al.** found that PET/CT had a sensitivity of 60% for serum TG levels below 5, 63% for TG values between 5 and 10, and 72% for TG levels beyond 10 ng/ml [9]. Comparatively, Na et al. demonstrated that PET sensitivity was 29 % in the 2–5 ng/ml TG range group, 57% in the 5–10 ng/ml range group, and 60% in the 10–20 ng/ml range group. They demonstrated a high sensitivity of 75%, 75%, and 100% for the 20-40, 40-80, and >80 ng/ml subgroups TG, respectively [10]. We found that serum TG level is a significant predictor of a positive PET/CT outcome; ROC-curve analysis for the optimal TG level before PET/CT showed an area under the curve (AUC) of 81%. The most accurate TG cut-off level was determined to be 25 ng/ml, which yields a sensitivity of 81%, a specificity of 60% and an overall accuracy of 74%. Our research found that the likelihood of a positive PET/CT result increases in direct proportion to increased TG levels, when we used the range approach. The trade-off between the expression of the NA-I symporter and the GLUT 1 glucose transporter on the cellular membrane of thyroid cancer cells provides the molecular basis for the inverse connection between RAI WBS and FDG PET/CT results. Normal thyroid cells have the Na-I symporter expressed on their cell membrane, which allows them to take up iodine. If thyroid malignant cells maintain their Na-I

symporter expression, they are categorized as well-differentiated and can therefore be treated by RAI and have a generally good prognosis. However, if the cells lose their expression of NA-I symporter, they concomitantly exhibit high expression of the non-specific GLUT-1 glucose transporter, and become categorized as de-differentiated. Glut-1 transporter is responsible for the facilitated transport of glucose through the cell membrane in proliferating cells, and is over-expressed in neoplastic cells. This is responsible for the high uptake of 18F-FDG in the PET/CT. In clinical practice, the decrease of RAI uptake in association with an increase in FDG uptake is known as "flip-flop phenomena" [(11)]. The ATA 2015 guidelines categorized RAI therapy response in thyroid cancer patients into 3 classes according to the laboratory and imaging findings using neck ultrasound and RAI WBS. A patient is considered to have a "excellent response" if no signs of disease are detected; a patient is considered to have a "structural incomplete response" if signs of disease are detected on conventional imaging modalities; and a patient is considered to have a "biochemical incomplete response" if there is a high TG level and no structural evidence of disease [(12)]. While patients with structural incomplete response, i.e. residual disease detected by neck ultrasound or RAI WBS, are treated by surgery and/or further RAI doses, patients with biochemical incomplete response represent a treatment dilemma. Scanning this group of patients with PET/CT and localizing the source of high TG provides a solution for this situation by localizing the source of persistent disease. Localizing the source of disease in a timely manner is of utmost importance as it diverts the patients to the most appropriate treatment which, when done at the proper and early time, improves the disease outcome, the quality of live as well as the overall survival of the patients. A recent systemic review by Bang et al showed that therapy was changed after FDG PET/CT imaging in 40% of thyroid cancer patients [(13)].

Re-differentiation of iodine refractory thyroid tumors is the most established line of treatment of de-differentiated thyroid cancer patients. The concept of the re-differentiation therapy is the restoring of the NIS expression, and thus restoring of the radioiodine sensitivity, rendering the patients manageable by RAI. The first reported method for re-differentiation therapy used retinoic acids for the regulation of NIS transcription. It has been shown that Retinoids, which are chemical substances linked to vitamin A, control the expression of the NIS gene. The breakthrough trial reported by Simon et al in 1996 demonstrated an increase in RAI absorption

after treatment with Retinoids, however, the tumor response in the study group was not particularly striking [14]. Similar findings were found in a large number of additional investigations. The poor efficacy of this treatment strategy was confirmed in a recent meta-analysis on Retinoids in radioiodine resistant thyroid cancer, which indicated a pooled impact of only 27.6% in the increase in RAI uptake and only 17% in the tumor response by RECIST criteria [15]. Other novel treatment options include modulation of the NIS signaling pathways, targeting the NIS trafficking to the plasma or the combinations of both [16, 17,18].

Our data revealed that there was no statistically significant difference in the anti-TG antibody levels between the PET-positive and PET-negative groups, even though the anti-TG level was greater in the former. Anti-TG was also shown to be an unreliable predictor of PET/CT results (61% accuracy) according to ROC curve analysis. This suggests that anti-TG levels should only be taken into account in cases when TG levels are not highly elevated, rather than being seen as an independent tumor marker. According to **Dekker et al.,** TG anti-bodies have no association with tumor features or risk factors, hence being positive for these antibodies should not be regarded as an independent risk factor for thyroid cancer patients [19]. Although anti-TG is used to verify that the TG level is not affected by TG antibody interference, **Spencer et al.** proposed that TG should be regarded as the principal tumor marker for thyroid cancer patients' monitoring, while anti-TG should only be regarded as a surrogate tumor marker [20]. Ultimately, our study's findings suggest that treating physicians should use serum TG levels as a benchmark for determining whether to send patients suspected of having de-differentiated thyroid cancer for an F-18 FDG PET/CT scan. A TG cut-off between 20 and 25 ng/ml is recommended by the majority of research published on a global scale, which is consistent with our proposed cut-off. There is hope for a better prognosis and longer life for these patients once FDG PET/CT localizes the source of persisting illness. Re-differentiation therapy is among the most studied treatment options, however variable other and different emerging therapy modalities are being investigated.

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