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Original scientific paper

Importance of Measurement of Thyroglobulin and Anti-Thyroglobulin Antibodies in Differentiated Thyroid Cancer

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A B S T R A C T

Differentiated thyroid cancers include papillary and follicular carcinomas, both originating from follicular epithelium. Treatment of choice is usually total or near total thyroidectomy, followed by ablative radioiodine ^{131}I treatment, and by the long term administration of thyroid hormone. Despite its excellent prognosis, recurrent disease does occur in approximately 20–40% of patients. Guidelines for the follow-up management of differentiated thyroid cancer are commonly based on circulating thyrogobulin measurement in the complete absence of eutopic thyroid tissue. A retrospective review was conducted on 116 patients (66 papillary and 50 follicular carcinoma, mean age 51.2 years) who had undergone total or near total thyroidectomy and radioactive iodine remnant ablation. Serum thyroglobulin (Tg) and anti-thyroglobulin antibodies (TgAb) levels were measured preoperatively, 1 month after thyroidectomy (before ^{131}I treatment) and 6 and 12 months after ablation therapy (Tg1, TgAb1 and Tg2, TgAb2, respectively). During one year of follow-up, in a total of 24 patients (21%) recurrent disease were confirmed by ultrasonography and whole-body-scanning, mostly. It was found significant correlation between serum Tg levels (measured preoperatively and postoperatively) and recurrent diseases ($p<0.05$), while serum TgAb levels did not have any statistical significance. However, in multivariate regression analysis only Tg levels measured 12 months after the therapy (Tg2) remained a significant predictor of recurrent disease ($p=0.008$). Although a high Tg level before surgery does not indicate that tumor is present, in the postoperative period and after ablative therapy Tg has proven predictive value because stimulated Tg levels above 10ng/ml confirmed that indicate residual or recurrent cancer, and its periodically measurements is recommended.

Key words: *differentiated thyroid cancer, thyroglobulin, anti-thyroglobulin antibody*

Introduction

Differentiated thyroid cancer (DTC), arising from thyroid follicular epithelial cells, accounts for approximately 90% of all thyroid cancer[1]. There are two major forms of DTC: papillary and follicular. Papillary cancer is the most common type of thyroid cancer (about 85%)[2], which outcome is variable. The lymph-node involvement is frequent and early in papillary cancer, with specific localization to lymph nodes next to the trachea or windpipe, called the paratracheal lymph nodes[3]. Vascular invasion is rare, and distant metastases are observed in less than 10% of cases[4–6]. Generally, the prognosis of papillary cancer is excellent. Follicular cancer is less frequent than papillary (5% to 15% of thyroid malignancies), and shows a major degree of aggressiveness with respect to papillary cancer[7]. In fact, follicular cancer has a greater rate of recurrence and is more frequently associated with extra-glandular extension and distant metastases, preferring to spread through the blood stream[8,4,5]. Follicular cancer outcome is generally less favorable than papillary prognosis.

Management of DTC

Initial management of DTC is surgical and includes near total thyroidectomy with/or without lymph node

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dissection, followed by radioiodine ^{131}I ablation of residual thyroid tissue[9]. Patients require thyroid hormone replacement; thyroxine is decreased serum thyroid stimulating hormone (TSH) until is <0.1 mU/L. Low TSH levels in the bloodstream reduce tumor growth rates and reduce recurrence rates of DTC – the greater the suppression of TSH the better the outcome[10].

Thyroglobulin – assessment tool for follow-up

Thyroglobulin (Tg) is a 670 kDa glycoprotein made up of two identical subunits[11] and acts as a scaffold for thyroid hormone synthesis. It is specific for follicular-derived thyroid cells[12]. More than 95% of papillary and follicular carcinomas are Tg positive, even those that are metastatic. Well-differentiated tumors generally express more Tg than poorly differentiated tumors[13]. The primary use of serum Tg measurement is, however, as tumor marker for patients affected by DTC[6, 14,16]. Serum Tg measurement is the best means of detecting normal and malignant thyroid tissue because there are no other sources to falsely elevate it. Its growing role as a biomarker with a very high reliability is due to the fact that, based on recently published data, the frequency of diagnosis of new thyroid cancer appears to be increasing, especially in women. Of high clinical interest is the issue of Tg interference by autoantibodies, which occurs with all immunometric methods (although radioimunoassays are somewhat more resistant)[16]. Approximately 25% of patients with DTC display anti-thyroglobulin antibodies (TgAb), compared to 10% of the general population[17]. These cause measured Tg levels to be falsely low, likely due to thyroglobulin-antibody complexes not being detected[18]. Persistence or rising thyroglobulin antibodies may be a marker for antigenic stimulation and thus ongoing disease[17].

Long-term follow-up

Since DTC may recur at any time for years and thyroxine therapy is life-long, a long term follow up by serum Tg measurements and ultrasonography (US) and/or whole-body-scanning (WBS) is necessary[19,20]. Tg measurement is then part of routinely surveillance and now it is considered the elected biological marker for papillary and follicular thyroid cancer, together referred to DTC[21]. TgAbs should thus be quantitatively measured with each serum Tg request, and, when present, usually invalidate the serum Tg result.

The aim of the present study was to assess the prognostic value of Tg and TgAb in patients with differentiated thyroid cancer.

Patients and Methods

The study included 116 adult patients (mean age 51.2 years, range 20–81) treated for a well-differentiated thyroid carcinoma between 2000 and 2010. Data were retrospectively collected from medical records at Clinic of Nuclear Medicine, Clinical Center University of Sarajevo. All patients underwent total or near total thyroidectomy at Clinic of Oto-Rhino-Laryngology and Clinic of Oncology and Glandular surgery, Clinical Center University of Sarajevo. In all patients ablation with radioiodine ^{131}I followed postoperatively, and all patients received suppressive treatment with thyroxin to maintain thyroid stimulating hormone (TSH) below 0.05mU/l. Serum thyroglobulin (Tg) and anti-thyroglobulin antibodies (TgAb) levels were measured preoperatively, 1 month after thyroidectomy (before ^{131}I treatment) and 6 (Tg1, TgAb1) and 12 months (Tg2, TgAb2) after ablation therapy. In detection of carcinoma recurrence or disease-free status, follow-up protocol included also ultrasonography (US) and whole-body-scanning (WBS). Other imaging studies such as computed tomography (CT) and magnetic resonance imaging (MRI) were performed when metastasis outside the neck was clinically suspected.

Serum levels of Tg and TgAb were assessed using the electrochemiluminescence immunoassay (Elecsys and cobase immunoassay analyzers), with functional sensitivity of at least 0.1 ng/ml for Tg and 10 IU/ml for TgAb. Normal range of Tg by this method is 1.4–78 ng/ml, and normal range of TgAb is <115 IU/ml. Stimulated Tg levels after thyreoidectomy and radioiodine ablation above 5 to 10 ng/mL were considered to indicate residual or recurrent tumor, regardless of the WBS finding[22], so as threshold level of Tg for high risk for residual or recurrent disease we used value of 10 ng/ml.

Statistical analysis

Statistical analyses were performed using SPSS statistical software system (version 16.0, SPSS Inc, Chicago, IL, USA). Data are presented as mean±S.E.M. Normal distribution was tested using the Kolmogorov–Smirnov test. Difference between groups was tested using the t-test, while the difference in serial values were tested with repeated measures one-way ANOVA. To test the predictor for the recurrent disease the following variables were entered in the model: age, gender, TNM classification, tumor histology diagnosis, Tg and TgAb levels and ^{131}I doses received. Two-tailed p values <0.05 were accepted as statistically significant.

Results

Baseline characteristics of the patients included in the study are shown in Table 1. In all patients ablation with radioiodine ^{131}I followed thyroidectomy, a mean dose of 81.4mCi.

Recurrent disease was confirmed in 24 patients (21%), among them 10 papillary (42%) and 14 follicular cancers (58%), as it is shown in Table 2.

In our study serum Tg levels measured preoperatively and Tg levels measured before ^{131}I therapy introduction were significantly higher in patients with recurrent disease compared to the patients without recurrent disease ($p<0.05$, Table 3). Serum Tg levels were also significantly higher in patients with recurrent diseases measured 6 and 12 months after ablation therapy in compari-

TABLE 1
CHARACTERISTICS OF THE PATIENTS

	N	(%)
Mean age (yrs)	51.20 (20–81)	
Gender		
Female	89	(77)
Male	27	(23)
Histology		
Papillary cancer	66	(57)
Follicular cancer	50	(43)
TNM-classification		
T1–T3	107	(92)
T4	9	(8)
N0	67	(58)
N1a	21	(18)
N1b	15	(13)
Nx	13	(11)
M0	104	(90)
Mx	12	(10)
Total	116	(100)

TABLE 2
CHARACTERISTICS OF THE PATIENTS WITH RECURRENT DISEASE

	N	(%)
Mean age (yrs)	54.75 (27–81)	
Gender		
Female	20	(83)
Male	4	(17)
Histology		
Papillary cancer	10	(42)
Follicular cancer	14	(58)
Total	24	(100)

TABLE 3
VALUES OF THYROGLOBULIN AND ANTI-THYROGLOBULIN--ANTIBODIES BEFORE SURGERY AND BEFORE ^{131}I TREATMENT, AND ITS CORELATION BETWEEN PATIENTS WITH NO EVIDENCE OF DISEASE AND PATIENTS WITH RECURRENCE

		N	$\overline{X}$	SEM	p-value
Tg presurgical	NED	67	160.8	35.2	0.037*
	REC	20	381.8	94.0	
TgAb presurgical	NED	49	193.6	80.2	NS
	REC	16	181.2	59.2	
Tg before ^{131}I	NED	91	9.3	3.1	0.023*
	REC	22	190.8	73.8	
TgAb before ^{131}I	NED	85	69.5	26.2	NS
	REC	20	31.3	9.7	

Tg – thyroglobulin (ng/ml), TgAb – anti-thyroglobulin antibody (IU/ml), ^{131}I – radioiodine treatment, NED – no evidence of disease, REC – recurrence, SEM – standard error of the mean, NS – no significant, *significant correlation ($p<0.05$)

son with the patients without recurrent disease ($p<0.05$, Table 4). However, serum TgAb levels were similar in patients with or without recurrent disease, with no statistical significance.

TABLE 4
VALUES OF THYROGLOBULIN AND ANTI-THYROGLOBULIN--ANTIBODIES 6 AND 12 MONTHS AFTER ABLATION, AND ITS CORELATION BETWEEN PATIENTS WITH NO EVIDENCE OF DISEASE AND PATIENTS WITH RECURRENCE

		N	$\overline{X}$	SEM	p-value
Tg1	NED	88	6.06	1.3	
	REC	19	336.4	92.6	0.002
TgAb1	NED	65	111.0	39.3	
	REC	15	129.3	41.7	NS
Tg2	NED	83	3.2	0.9	
	REC	16	369.0	111.3	0.005
TgAb2	NED	68	77.5	38.7	
	REC	14	51.7	20.9	NS

Tg1 – thyroglobulin 6 months after radioiodine remnant ablation, TgAb1 – anti-thyroglobulin antibody 6 months after radioiodine remnant ablation, Tg2 – thyroglobulin 12 months after radioiodine remnant ablation, TgAb2 – anti-thyroglobulin antibody 12 months after radioiodine remnant ablation, NED – no evidence of disease, REC – recurrence, SEM – standard error of the mean, NS – no significant, *significant correlation ($p<0.05$)

As threshold level for Tg measured 6 and 12 months after radioiodine remnant ablation was used value of 10ng/ml. According to Tg threshold level (above/below) and disease recurrence (yes/no), all patients were grouped in 4 groups: group 1. (patients with recurrent disease and Tg above threshold level), group 2. (patients with recurrent disease and Tg below threshold level), group 3. (patients with no evidence of disease and Tg above threshold level) and group 4. (patients with no evidence of disease and Tg below threshold). Mean values of Tg measured before thyroidectomy and before ^{131}I therapy compared with mean values of Tg measured 6 and 12 month after, showed significant correlation within the groups 1, 3 and 4 ($p<0.05$, Table 5), while mean values of TgAb did not show any significant correlation within the same groups (Table 6).

A significant association between serum Tg levels (measured preoperatively and postoperatively) and recurrent diseases, as well as between ^{131}I doses received and recurrent diseases was observed in our study. However, in multivariate regression analysis only Tg levels measured 12 months after the therapy (Tg2) remained a significant predictor of recurrent disease (Exp(B)=1.165, p=0.008, Table 7).

Discussion and Conclusion

The study found that DTC had highest incidence in women (3.3:1), as it confirmed Haselkorn et al[23]. Follicular cancer is less frequent than papillary type, but it

TABLE 5
CORELATION BETWEEN VALUES OF THYROGLOBULIN MEASURED BEFORE AND AFTER SURGERY, WITHIN DIFFERENT GROUPS

group	N	Tg presurgical ($\bar{X}$±SEM)	Tg before ^{131}I ($\bar{X}$±SEM)	Tg1 ($\bar{X}$±SEM)	Tg2 ($\bar{X}$±SEM)	p-value
1.	23	401.7±96.86	199.9±76.8	354.8±95.9	393.6±116.0	<0.05*
2.	1	–	–	–	–	–
3.	15	189.6±95.7	33.3±17.5	25.6±4.9	13.9±4.2	<0.05*
4.	77	154.5±37.8	4.6±0.64	2.1±0.35	0.85±0.19	<0.05*

Tg – thyroglobulin (ng/ml), ^{131}I – radioiodine treatment, Tg1 – thyroglobulin 6 months after radioiodine remnant ablation, Tg2 – thyroglobulin 12 months after radioiodine remnant ablation, *significant correlation ($p<0.05$)

TABLE 6
CORELATION BETWEEN VALUES OF ANTI-THYROGLOBULIN ANTIBODIES MEASURED BEFORE AND AFTER SURGERY, WITHIN DIFFERENT GROUPS

group	N	TgAb presurgical ($\bar{X}$±SEM)	TgAb before ^{131}I ($\bar{X}$±SEM)	TgAb1 ($\bar{X}$±SEM)	TgAb2 ($\bar{X}$±SEM)	p-value
1.	23	181.2±59.17	31.2±10.2	130.8±44.7	53.6±22.5	NS
2.	1	–	–	–	–	–
3.	15	16.4±3.1	13.3±0.9	39.3±20.9	21.3±5.6	NS
4.	77	223.1±92.9	78.7±30.3	125.6±46.9	88.3±46.1	NS

TgAb – anti-thyroglobulin antibody (IU/ml), ^{131}I – radioiodine treatment, TgAb1 – anti-thyroglobulin antibody 6 months after radioiodine remnant ablation, TgAb2 – anti-thyroglobulin antibody 12 months after radioiodine remnant ablation, NS – no significant

TABLE 7
MULTIVARIATE REGRESSION ANALYSIS OF ASSOCIATION BETWEEN THE VARIOUS PARAMETERS (AGE, TUMOR HISTOLOGY, ^{131}I DOSE, SERUM THYROGLOBULIN BEFORE AND AFTER SURGERY) AND RECURRENT DISEASE

	p-value	Exp(B)
Age	0.342	0.948
Histology		
Papillary cancer	0.738	0.588
Follicular cancer	1.000	0.000
Tg presurgical	0.792	1.001
Tg before ^{131}I	0.381	0.941
I^{131} dose (mCi)	0.340	1.029
Tg1	0.458	1.039
Tg2	0.008*	1.165
Constant	0.250	0.018

Tg – thyroglobulin (ng/ml), ^{131}I – radioiodine treatment, Tg1 – thyroglobulin 6 months after radioiodine remnant ablation, Tg2 – thyroglobulin 12 months after radioiodine remnant ablation, *significant correlation ($p<0.05$)

also has generally less favorable outcome[5], as the study found higher incidence of recurrent disease in patients with follicular cancer. We investigated whether serum Tg determination before surgery for DTC may have any prognostic value, and found that serum Tg levels measured preoperatively were significantly higher in patients with recurrent disease compared to the patients without recurrent disease ($p<0.05$). Gibelli[24] et al found that pre-operative assessment of serum Tg may identify patients who might present recurrence without increased Tg, and in whom standard follow-up by monitoring Tg serum levels is inadequate. However, a high Tg level before surgery does not indicate that a tumor is present[25,26], a high serum Tg concentrations may be due to an abnormally large thyroid, excessive thyroid stimulation, or physical damage to the thyroid[24], and this study failed to show any prognostic value of presurgical serum Tg determination that consequently should not be measured. All patients underwent surgical procedure which includes total or near total thyroidectomy with/or without neck lymph node dissection, followed by radioiodine ^{131}I ablation of residual thyroid tissue, as it is estimated as treatment of choice in management of DTC[9,10,27]. Although DTC is characterized by an excellent prognosis after thyroidectomy and radioiodine ablation, recurrences occur often even many years after this initial therapy[10,28], recurrent disease does occur in approximately 20–40% of patients[29]. During one year of follow-up in our study, in a total of 24 patients (21%) recurrent disease were confirmed by ultrasonography and whole-body-scanning, mostly, and follicular cancers showed a major degree of aggressiveness with respect to papillary cancer, as it confirmed by Pacini et al[7]. The clinical and economical relevance of optimal follow-up with accurate diagnostic testing is crucial considering the large population of patients in life long follow-up for DTC world-wide. Traditionally, the cornerstone of follow-up is serum Tg measurement. Optimal sensitivity is reached when Tg is measured during thyrotropin (TSH) stimulation because TSH promotes Tg synthesis[28]. Changes in

the Tg level over time (six months or yearly intervals) are more important than any one Tg result. After surgery, blood samples are usually taken for Tg measurement while the patient is taking their daily dose of thyroxine medication (TSH low)[26]. Current clinical guidelines are based on immunometric assays with a functional sensitivity of 1ng/mL[27]. Using assays of this sensitivity, a detectable Tg on suppressive therapy reliably indicates ongoing disease, although a negative result does not. Stimulation of latent thyroid cancer cells to produce and secrete Tg (so-called stimulated Tg) can therefore alert the clinician to the need for further diagnostic evaluation or empiric treatment. Stimulated Tg levels above 5 to 10ng/mL were considered to indicate residual or recurrent tumor, regardless of the WBS finding[22,30–32], and our study found significant correlation between higher values of Tg1 and Tg2 (values above than 10ng/ml measured 6 and 12 months after ablation therapy, respectively) and recidives of cancer ($p<0.05$), in comparison with patients with no evidence of disease. However, in multivariate regression analysis between the various parameters (age, gender, tumor histology, dose of ^{131}I, Tg and TgAb measured before and after treatment), only Tg levels measured 12 months after the therapy (Tg2) remained a significant predictor of recurrent disease ($p= 0.008$). On the other hand, serum TgAb levels did not have any statistical significance. For patients with a negative stimulated Tg one year after initial treatment, the chance of a subsequent positive result during follow-up is low – thus one stimulated test may be adequate in this group[33]. In those with detectable stimulated Tg, serial evaluation may be useful[27,33].

In conclusion, although presurgical serum Tg level is no specific for DTC and could not have significant prognostic value, serum Tg level after surgery is an excellent biological marker of persistent or recurrent DTC during first year of follow-up. In fact, detection of stimulated Tg levels above threshold after total thyroid ablation suggests recurrent disease, and can therefore alert the clinician to the need for further diagnostic evaluation or empiric treatment.

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VAŽNOST ODREĐIVANJA TIREOGLOBULINA I ANTITIREOGLOBULINSKIH PROTUTIJELA KOD DIFERENCIRANOG CARCINOMA ŠTITNJAČE

S A Ž E T A K

Difencirani karcinom štitnjače uključuje papilarni i folikularni. Liječenje izbora je najčešće totoalna ili subtotalna tiroidektomija, nakon koje slijedi radiojodna terapija i dugotrajna supstitucija hormona. Usprkos odličnoj prognozi bolest se ponavlja u 20–40% bolesnika. Praćenje bolesnika se obično bazira na mjerenju cirkulirajućeg tireoglobulina. Retrospektivna studija je provedena na 116 bolesnika (66 papilarnih i 50 folikularnih carcinoma, srednja dob 51.2 godine) kod kojih je učinjena totalna ili skoro totalna tiroidektomija i radiojodna terapija. Preoperativno su mjereni tireoglobulin i anti-tiroglobulinska protutijela (TgAb), zatim 1 mjesec nakon tiroidektomije (prije radiojoda) te 6 i 12 mj. nakon ablacije. Kroz praćenje tijekom 1 godine kod ukupno 24 bolesnika se pojavila rekurentna bolest (21%), potvrđena ultrazvukom i scintigrafijom. Nađena je značajna korerlacija između serumskog tireoglobulina (preoperativno i postoperativno) i rekurentne bolesti ($p<0–05$), dok razina TbAg nije pokazala statistički značaj. Međutim, razina Tg nakon 12 mjeseci (Tg2) je ostala značajan pokazatelj rekurentne bolesti ($p=0.008$). Iako visok Tg prije kirurgije ne pokazuje da je tumor prisutan, u postoperativnom periodu i nakon ablativne terapije Tg ima prediktivnu vrijednost i preporučljivo je njegovo povremeno mjerenje.