Role of Ultrasonography in Outcome Prediction in Subclinical Hypothyroid Patients Treated with Levothyroxine

Dong Yeob Shin[1], Eun-Kyung Kim[2] and Eun Jig Lee[1]

[1] *Endocrinology, Internal Medicine, Yonsei University College of Medicine, Seoul, Korea*
[2] *Department of Radiology, Yonsei University College of Medicine, Seoul, Korea*

Abstract. Progression to overt hypothyroidism and the associated adverse effects on lipid metabolism and the cardiovascular system are major concerns for patients diagnosed with subclinical hypothyroidism (SCH). No consensus regarding the clinical parameters associated with prognosis for this mild thyroid dysfunction has yet been established, although elevation of serum anti-thyroid peroxidase antibody (TPOAb) and decreased or heterogeneous echogenicity (diffuse thyroid disease, DT) on ultrasonography (US) are commonly observed. We investigated the value of ultrasonographic examination compared to the measurement of serum TPOAb and anti-thyroglobulin antibody (TgAb) for the evaluation of levothyroxine treatment on SCH. We analyzed 204 SCH patients who initially underwent thyroid ultrasonography and were given a low dose of levothyroxine for a mean of 6.94 months. Outcome was determined by the normalization or sustained elevation of serum TSH, and evaluated according to the presence of DT on subsequent US and serum TPOAb or TgAb. Sustained TSH elevation after levothyroxine replacement was more frequent in patients who initially showed DT on US, regardless of thyroid autoantibody level. Ultrasonographic morphology had a higher negative predictive value (81.8%) compared with the absence of TPOAb (73.9%) or TgAb (73.7%) and a similar positive predictive value (48.9%) to that of thyroid autoantibodies (46.8% for TPOAb and 50.0% for TgAb) in the outcome prediction of SCH. Thyroid US may provide valuable information on the course of SCH, and DT pattern can serve as a prognostic factor when combined with other known parameters.

Key words: Subclinical hypothyroidism, Ultrasonography, Thyroiditis, Thyroid microsomal antibodies

---

SUBCLINICAL HYPOTHYROIDISM (SCH) is defined as elevated serum levels of thyrotropin (TSH) combined with normal serum levels of free thyroxine (FT4) and total or free triiodothyronine (T3) [1]. In a recent population-based study in the United States, the prevalence of SCH was reported to be 12-15% in healthy people over 65 years of age, with a female predominance [2]. SCH is now a common medical problem diagnosed during routine health examinations, particularly in the elderly [3, 4].

The significance of SCH is largely due to its potential risk for developing into overt hypothyroidism.

Through several prospective studies, initial high serum TSH and high serum anti-thyroid peroxidase antibody (TPOAb) concentrations in patients with SCH have been accepted as independent predictors of the progression to overt hypothyroidism [5-9]. However, there have been no randomized clinical trials to provide a definite consensus on treatment guidelines or evaluation methods of SCH.

Decreased echogenicity or irregular heterogeneous echo pattern is a characteristic finding in diffuse thyroid disease (DT) examined with ultrasonography (US). These ultrasonographic features of the thyroid gland have also been demonstrated to be associated with both overt hypothyroidism and SCH status [10-13].

Although performing US in patients with SCH is not routinely recommended, we hypothesized that the existence of a DT pattern in US of patients with SCH could have a significant predictive value in de-

termining the prognosis. In this study, we retrospectively analyzed thyroid function test results after low dose levothyroxine replacement in patients with SCH and compared the treatment responses with respect to TPOAb and anti-thyroglobulin antibody (TgAb) status and the existence of DT on US examination.

Materials and Methods

Subjects

We initially reviewed the medical records of a total of 312 Korean patients with SCH who were referred to our endocrinology clinic from January 2007 to January 2008 for evaluation of abnormally elevated serum TSH levels. All patients subsequently underwent thyroid US with color-flow Doppler initially for the purpose of screening for underlying thyroid disease. All of the subjects continued to have elevated baseline serum TSH levels with normal levels of serum FT4 and T3 on the second hormonal assay measured in the endocrinology laboratory of our hospital upon the initial visit to our clinic. We analyzed echogenicity patterns of thyroid US results and TPOAb and TgAb statuses from all subjects. We excluded subjects who had previous thyroid diseases or definite symptoms of hypothyroidism or large goiter from analyses. We further excluded subjects with increased intra-thyroidal vascularity based on Doppler examination to rule out the possibility of underlying Graves' disease, those with any record of medication history that could influence thyroid function, and those with other autoimmune diseases or any serious underlying systemic disease involving major organs, e.g., diabetes mellitus, cardiovascular disease, and pituitary or hypothalamic disorders. Further, all subjects with a history of pregnancy or delivery up to twelve months prior to visiting our clinic were excluded from our analysis. Ultimately, the medical records of 204 patients who were followed up after 6 – 12 months (mean 6.94 ± 1.85 months) of low dose levothyroxine (50 μg per day) replacement therapy were reviewed for reassessment of serum TSH concentrations following treatment. Adherence to levothyroxine was verified with verbal self-reports at each follow-up visit. We then evaluated the difference in the proportion of the subjects who maintained elevated serum TSH levels despite levothyroxine replacement in patients grouped by echo pattern on US and by

TPOAb and TgAb statuses. This retrospective study was approved by our Institutional Review Board, and informed consent was not required.

Measurement of thyroid hormones and anti-thyroid autoantibodies

Thyroid function was evaluated using venous blood samples. Serum was isolated from the blood samples, centrifuged immediately, and kept frozen at -70°C. Serum FT4 and TSH levels were measured by radioimmunoassay (Trinity Biotech, Co. Wicklow, Ireland), as was serum T3 (Diasorin, Saluggia, Italy), TPOAb (Brahms, Hennigsdorf/Berlin, Germany), and TgAb (Brahms, Hennigsdorf/Berlin, Germany). SCH was defined in this study as serum TSH > 3.1 μIU/mL (normal range 0.4~3.1) with FT4 between 0.73 to 1.95 ng/dL, considering the normal reference value of each radioimmunoassay kit. The existence of TPOAb and/or TgAb was defined as a serum concentration of each anti-thyroid autoantibody > 60 IU/L.

Ultrasonographic evaluation

Ultrasonographic evaluation of the thyroid gland was performed initially with an HDI 3000 or HDI 5000 system (Philips Medical Systems, Bothell, WA, USA) or an Acuson Sequoia 512 system (Siemens Medical Solutions, Mountain View, CA, USA) for evaluation of the thyroid gland and neck. One of three radiologists with four, six, and ten years of experience in thyroid imaging performed a real time sonographic exam and interpreted the results. Ultrasonographic features of DT were defined using the generally accepted standards of diffuse parenchymal hypoechogenicity or a heterogeneous echo pattern of the thyroid gland [13-15] (Fig. 1). We also reviewed color-flow Doppler examination results for all subjects in order to detect hypervascularity in the thyroid gland. The US results were grouped according to the existence of DT, irrespective of focal thyroid lesion that was not the focus of this study. Furthermore, all subjects were divided into four groups according to the presence of TPOAb or TgAb and DT based on US examination to compare the treatment response in each subject group.

Statistical analysis

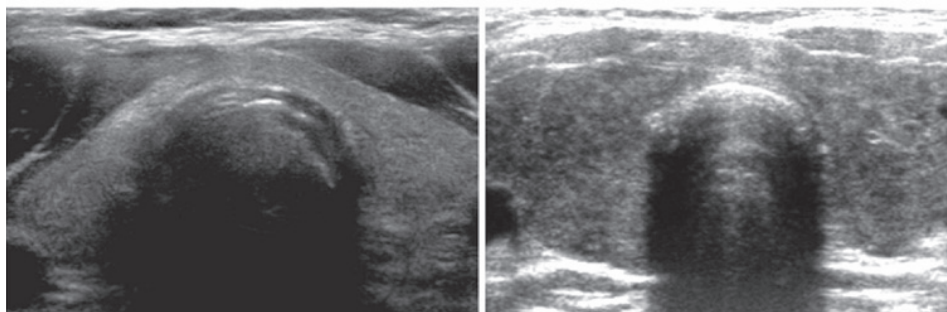Descriptive analyses of all clinical and biochemical

Fig. 1. Examples of thyroid ultrasonographic (US) findings showing normal homogeneous echogenicity (A) and diffuse heterogeneous echogenicity (B) compatible with diffuse thyroiditis.

Table 1. Principal characteristics of the subjects

	TPOAb(+)		TPOAb(-)			
	DT(+)(47)	DT(-)(15)	DT(+)(47)	DT(-)(95)	Total(204)	P value*
Age (years)	46.0±11.4	54.4±9.2	51.5±12.1	50.7±14.3	50±13.0	0.069
Sex (M:F)	5:42	5:10	5:42	16:79	31:173	0.136**
T3 (ng/dL)	142.4±29.2	143.2±70.9	147.9±38.7	148.5±36.4	146.3±38.5	0.203
TgAb# (%)	48.9	53.3	34	5.3	25.5	<0.001
FT4 (ng/dL)	1.0±0.2	1.1±0.1	1.0±0.2	1.2±0.8	1.1±0.6	0.323
Initial TSH (μIU/mL)	5.8±3.0	5.0±2.2	5.1±1.9	4.9±1.7	5.1±2.2	0.153
Follow-up TSH (μIU/mL)	4.2±3.6	2.7±2.1	3.4±2.5	2.1±1.3	2.9±2.5	<0.001
Treatment period (months)	7.1±2.2	6.5±1.2	7.5±3.1	6.8±1.7	6.9±1.9	0.432
P-value##	<0.001	0.014	<0.001	<0.001	<0.001	

Data are mean ± standard deviation or number

TPOAb, anti-thyroid peroxidase autoantibody

DT, Diffuse thyroid disease on ultrasonography

*Mean values of each patient group were compared using ANOVA.

** Sex ratio of each patient group was compared using Pearson's chi-square test.

The prevalence of anti-thyroglobulin antibody (TgAb)

Differences in mean values of the initial and follow-up TSH levels for each patient group were validated by paired *t*-test.

parameters are presented as mean ± standard deviation (SD). The baseline characteristics and clinical parameters of each subject group according to US echo pattern and TPOAb or TgAb status were compared and differences between all groups were determined by ANOVA or Pearson's chi-square test. Initial mean serum TSH concentrations and those measured after levothyroxine replacement therapy were compared in each group and also in all subjects using the paired *t*-test. The proportion of patients in each subject group who maintained an elevated TSH level after levothyroxine replacement was compared using Pearson's chi-square test. We also used Pearson's chi-square test to evaluate differences in the frequencies of sustained TSH elevation according to TgAb positivity and DT on US. All statistical tests were two-tailed and *P* < 0.05 was considered statistically significant. Statistical data analyses were performed using the SPSS soft-

ware package for Windows (Version 12.0; SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of subjects

Serum TSH concentrations were analyzed from 204 (31 males and 173 females) subjects who were reassessed for thyroid function after levothyroxine replacement over 6 to 12 (mean 6.94 ± 1.85) months. Subjects were divided into four groups based on the presence of neither, either, or both TPOAb and DT on initial examination by laboratory assay and US, respectively. The principal clinical and biochemical characteristics of each group and all 204 patients are presented in Table 1. The mean values of age, sex ra-

Table 2. Changes of TSH level in patient groups according to different ultrasonographic findings and TPOAb statuses

	TPOAb(+)		TPOAb(-)		
	DT(+)	DT(-)	DT(+)	DT(-)	Total
Normal TSH	22	11	26	79	138
	(46.8%)	(73.3%)	(55.3%)	(83.2%)	(67.6%)
Elevated TSH	25	4	21	16	66
	(53.2%)	(26.7%)	(44.7%)	(16.8%)	(32.4%)
Total	47	15	47	95	204
	(100%)	(100%)	(100%)	(100%)	(100%)

TPOAb, anti-thyroid peroxidase autoantibody
DT, Diffuse thyroid disease on ultrasonography
Pearson's chi-square test ($P<0.001$)

tio, and T3, FT4, and TSH levels at initial presentation were not statistically different between the four subject groups based on TPOAb status or presence of DT on US. Each patient group showed a different prevalence for TgAb, which was higher in the group with positive TPOAb or DT on US. The group with neither of those findings had a very low prevalence of TgAb positivity (5.3%).

TSH concentration after levothyroxine replacement

The mean serum TSH concentration after levothyroxine therapy was found to be significantly lower than the initial serum TSH level in all patient groups and all subjects when analyzed using the data from the 204 subjects who were followed up after levothyroxine treatment (Table 1). Furthermore, post-treatment TSH concentration was highest in the group of subjects that initially showed both elevated serum TPOAb and DT on US examination, followed by the groups showing only one of the features in the initial evaluation. It was lowest in the group of patients who had neither of those findings. Interestingly, the mean serum TSH level of the subjects who showed DT on US without serum TPOAb was even higher than that of patients who had positive TPOAb and whose thyroid gland showed normal homogeneous echogenicity.

Change of TSH level after levothyroxine replacement

We examined changes in patterns of serum TSH concentration after levothyroxine replacement in each patient depending on whether TPOAb or DT on US was detected initially. The proportion of patients who maintained elevated serum TSH level despite levothyroxine replacement was compared among subject groups (Table 2). As expected, the patient group initially showing both TPOAb and DT on US had the highest (53.2%) and the group showing none of these features had the lowest (16.8%) proportion of subjects with continued elevated TSH concentration after levothyroxine therapy. The proportion of patients with sustained serum TSH elevation in the subject groups with either TPOAb or DT on US fell in between, although more patients with DT without TPOAb on the initial US examination had continued elevated serum TSH level after levothyroxine therapy (44.7%) than those who had TPOAb without DT (26.7%). Differences between all four patient groups were statistically significant.

TgAb and US findings and responses to levothyroxine treatment

We further evaluated the correlation of TgAb positivity to the change in serum TSH in response to levothyroxine replacement as well as to the US finding. TgAb was positive in 52 out of 204 patients (25.5%). Followed-up TSH level also showed significant difference among patients groups according to TgAb positivity and US finding while baseline characteristics including mean age, sex ratio, initial T3, FT4, TSH levels and treatment period did not (Table 3). We also found that more patients had continued elevated TSH levels after levothyroxine treatment when TgAb and/or DT on US was present, much like that with TPOAb combined with US (Table 4).

Positive and negative predictive values

As the initial mean serum TSH concentrations were not different between subject groups, we evaluated the

Table 3. Principal characteristics of each patient group according to TgAb positivity and DT on US

	TgAb(+)		TgAb(-)			
	DT(+)(39)	DT(-)(13)	DT(+)(55)	DT(-)(97)	Total(204)	P value*
Age (years)	46.1±12.0	53.1±11.5	50.1±11.7	51.0±14.0	50±13.0	0.155
Sex (M:F)	6:33	1:12	4:51	20:77	31:173	0.686**
T3 (ng/dL)	146.1±36.8	127.5±48.3	142.1±33.5	149.6±38.8	146.3±38.5	0.404
FT4 (ng/dL)	1.0±0.2	1.1±0.1	1.0±0.2	1.2±0.8	1.1±0.6	0.292
Initial TSH (μIU/mL)	5.7±3.0	4.8±2.3	5.3±2.1	4.9±1.7	5.1±2.2	0.364
Follow-up TSH (μIU/mL)	4.0±3.0	2.6±2.2	3.6±3.2	2.1±1.4	2.9±2.5	<0.001
Treatment period (months)	7.2±2.2	6.5±1.0	7.1±2.0	6.8±1.7	6.9±1.9	0.379
P-value#	0.019	0.007	<0.001	<0.001	<0.001	

Data are mean ± standard deviation or number

TPOAb, anti-thyroid peroxidase autoantibody

DT, Diffuse thyroid disease on ultrasonography

*Mean values of each patient group were compared using ANOVA.

** Sex ratio of each patient group was compared using Pearson's chi-square test.

Differences in mean values of the initial and follow-up TSH levels for each patient group were validated by paired *t*-test.

Table 4. Changes of TSH level in patient groups according to different ultrasonographic findings and TgAb statuses

	TgAb(+)		TgAb(-)		
	DT(+)	DT(-)	DT(+)	DT(-)	Total
Normal TSH	17	9	31	81	138
	(43.6%)	(69.2%)	(56.4%)	(83.5%)	(67.6%)
Elevated TSH	22	4	24	16	66
	(56.4%)	(30.8%)	(43.6%)	(16.5%)	(32.4%)
Total	39	13	55	97	204
	(100%)	(100%)	(100%)	(100%)	(100%)

TgAb, anti-thyroglobulin antibody

DT, Diffuse thyroid disease on ultrasonography

Pearson's chi-square test (*P*<0.001)

possible value of US findings with DT for predicting levothyroxine replacement in SCH patients by comparing US with standard predictive factors for SCH prognosis. We calculated the positive (PPV) and negative predictive values (NPV) for DT on initial US, with TPOAb, TgAb, and high TSH level as independent parameters (Table 5). The PPV of DT on initial US examination for constantly elevated TSH after levothyroxine replacement was lower than that of high initial serum TSH concentration (≥ 10 μIU/mL), but higher than the PPV for the presence of TPOAb. Furthermore, DT on US had a better NPV than any of other factors. In other words, if a patient with SCH showed DT on their initial US examination, the PPV of US for sustained elevation of serum TSH after levothyroxine replacement was 48.9%, much higher than the PPV of TPOAb. In addition, the NPV of an initial DT-negative thyroid US in an SCH patient for

Table 5. Predictive value of each parameter for levothyroxine treatment response

	DT	TPOAb	TgAb	High TSH*
PPV (%)	48.9	46.8	50	77.8
NPV (%)	81.8	73.9	73.7	69.7

* High TSH: TSH serum concentration greater than 10 μIU/mL

PPV, positive predictive value

NPV, negative predictive value

TPOAb, anti-thyroid peroxidase autoantibody

TgAb, anti-thyroglobulin antibody

DT, Diffuse thyroid disease on ultrasonography

sustained elevated serum TSH level was also higher than that of TPOAb absence, and even higher than that of an absence of high initial serum TSH level (≥ 10 μIU/mL). TgAb showed a higher PPV for sustained elevation of serum TSH after levothyroxine treatment than did either TPOAb or US finding of DT, but the

NPV of no DT on US was still higher than the absence of TgAb.

Discussion

While SCH and overt hypothyroidism share almost the same etiology, the clinical symptoms of SCH are vague and signs compatible to hypothyroidism are typically absent [16]. Consequently, diagnosis of SCH is solely dependent on a thyroid function test. After excluding cases with previously existing overt hyper- or hypothyroidism, otherwise healthy patients with SCH merely show incidental laboratory findings of slightly elevated TSH concentrations. Although SCH is a rather benign abnormality compared to other diseases of the thyroid gland, its overall significance in the absence of specific, related symptoms and whether treatment is required remain challenging issues to both physicians and patients in daily clinical practice. Problems regarding the diagnosis and treatment of SCH are becoming even more important as human life expectancies increase, as thyroid dysfunction, including SCH, is found more often in the elderly [2, 8, 9, 17, 18].

Although there have been no randomized clinical trials assessing the long-term effects of early treatment of SCH until recently, the strategy of levothyroxine replacement for SCH has raised concerns due to the adverse cardiovascular effects of SCH by influencing the lipid profile and endothelial dysfunction of the patient, the possibility of progression to overt hypothyroidism, and maternal hypothyroidism that may have disastrous effects on fetal brain development in pregnant women with SCH [19-23]. So while the initiation of levothyroxine replacement seems to be supported in most patients with SCH, the necessity for large, controlled clinical trials still exists [3, 17].

The risk of progression to overt hypothyroidism in SCH patients is known to be higher in patients with underlying thyroid disease [3]. With the exception of patients with a previously documented history of overt thyroid dysfunctions, however, predicting the natural course of the disease and assessing the risk of progression to a more severe status of thyroid dysfunction is not easy in patients with a first-time diagnosis of SCH. The presence of anti-thyroid antibodies such as TPOAb has been used most commonly as a parameter for estimating the existence of underlying autoimmune thyroid disease and for initially predicting the

clinical course of SCH [6, 7]. Levothyroxine therapy for SCH is highly indicated in subjects who test positive for anti-thyroid autoantibody or in patients with sufficiently high serum TSH levels (≥ 10 μIU/mL) [24]. Another supplementary tool for differentiating cases with a higher risk of progression to overt hypothyroidism or predicting treatment effects in SCH is still needed, however, because serum TSH concentrations are rather mildly elevated in most patients with SCH [25].

While it is well known that decreased echogenicity or an irregular echo pattern on thyroid US represent various forms of autoimmune thyroiditis, and that these findings are closely associated with thyroid dysfunctions including overt and subclinical hypothyroidism [10-13, 26], there is no consensus regarding the significance of thyroid US in the prognosis of SCH. Recently, Nys *et al*. analyzed the results of thyroid US examination in 1,845 cases of SCH, finding that thyroid US could enhance diagnosis for some patients that were negative for thyroid antibodies, allowing detection of autoimmune thyroiditis [27]. Rosario *et al*. evaluated the three-year natural history of mild SCH in 117 patients and reported that TPOAb-positive patients and patients with negative TPOAb but an US with hypoechogenicity had similar clinical courses, suggesting that US may be a good way to evaluate prognosis in mild SCH [28].

In this study, we showed that patients with mild SCH and the same mean level of serum TSH concentration had different responses to levothyroxine treatment according to their TPOAb status and thyroid US echo patterns. The follow-up period of our study was shorter than that of other previous studies investigating the natural history of SCH because we focused on the effects of short-term levothyroxine treatment instead of progression to overt hypothyroidism. Accordingly, most of the subjects with sustained elevated TSH level at follow-up did not progress to overt hypothyroidism, but retained a subclinical hypothyroid status despite low-dose levothyroxine replacement. Such patients required a dose escalation of levothyroxine to maintain euthyroid status after the mean follow-up period, and they were considered to have declining thyroid function.

Thyroid US findings compatible to DT displayed a superior predictive value for sustained serum TSH elevation after levothyroxine therapy compared to TPOAb positivity. Further, considering that thyroid US had the highest NPV in our study, normal homogeneous echogenicity on the initial thyroid US in a

patient with SCH would be a better prognostic factor for the effect of levothyroxine treatment than either TPOAb negativity or mildly elevated TSH (< 10 μIU/mL). Furthermore, in our study, patients who were both TPOAb positive and showed DT on US were more likely to experience sustained TSH elevation after levothyroxine therapy than were patients with only one finding (TPOAb positivity or DT on thyroid US). In contrast, in the patient group with neither TPOAb positivity nor DT on US, the probability of sustained TSH was lower than that of any other patient group. Thyroid US used in conjunction with the TPOAb assay during the initial work up of a patient with SCH appears to be more helpful than TPOAb alone in the prediction of clinical course and establishment of a treatment plan, especially in cases with mild elevation of serum TSH concentration.

Along with TPOAb, TgAb is an important antithyroid autoantibody detected in the majority of patients with autoimmune thyroid diseases, including Hashimoto's thyroiditis [29]. The function of TgAb in the pathogenesis of autoimmune thyroid diseases is not yet fully understood, and there has been no prospective data evaluating the predictive value of TgAb for the development of overt hypothyroidism in patients with SCH [7]. We found that the outcome of short-term levothyroxine treatment in SCH varied significantly according to TgAb status and US finding, and that TgAb also had relatively high a PPV and NPV for sustained TSH elevation after levothyroxine treatment in SCH. TgAb is known to be associated with the histological diagnosis of Hashimoto's thyroiditis and development of hypothyroidism related to high iodine intake [29-31]. Accordingly, we proposed that thyroid US and thyroid autoantibodies, including both TPOAb and TgAb, appear to be better predictive factors for treatment outcome in SCH when used collectively. Further prospective studies are needed to validate the significance of TgAb in SCH.

Because this study has inherent limitations as a retrospective analysis, a more controlled, randomized trial is needed to determine the precise significance of thyroid US in predicting the overall prognosis of SCH. Differences in other clinical factors that influence thyroid function such as iodine intake, for example, could affect the results of our study as confounding factors. We believe that the impact of variable iodine intake was probably minimal in our study, as long as the subjects maintained sufficient iodine levels during treatment periods, which is typical in the average Korean diet. Variable adherence of patients to the recommendation of iodine restriction and actual changes in iodine intake also are possible factors that could influence the levothyroxine treatment effect. Further studies measuring serial urinary iodide excretion in SCH patients during the treatment period would be informative. In addition, we were unable to gather and analyze quantitative and comparative US thyroid size data for all groups, since not all US images contained thyroid size data.

In conclusion, we found that thyroid US in SCH shows a significant predictive value for levothyroxine treatment outcome comparable to other well-known prognostic parameters such as TPOAb positivity or high serum TSH levels. We also validated the possible role of TgAb as a prognostic parameter for SCH. Furthermore, combining the TPOAb or TgAb assay and thyroid US examination during initial evaluation may be a better strategy to guide treatment, especially in patients with SCH showing relatively mild TSH elevation (< 10 μIU/mL).

References

1. Evered DC, Ormston BJ, Smith PA, Hall R, Bird T (1973) Grades of hypothyroidism. *Br Med J* 1: 657-662.
2. Karlin NJ, Weintraub N, Chopra IJ (2004) Current controversies in endocrinology: screening of asymptomatic elderly for subclinical hypothyroidism. *J Am Med Dir Assoc* 5: 333-336.
3. Arrigo T, Wasniewska M, Crisafulli G, Lombardo F, Messina MF, Rulli I, *et al.* (2008) Subclinical hypothyroidism: the state of the art. *J Endocrinol Invest* 31: 79-84.
4. Sawin CT, Chopra D, Azizi F, Mannix JE, Bacharach P (1979) The aging thyroid. Increased prevalence of elevated serum thyrotropin levels in the elderly. *JAMA* 242: 247-250.
5. Diez JJ, Iglesias P (2004) Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. *J Clin Endocrinol Metab* 89: 4890-4897.
6. Diez JJ, Iglesias P, Burman KD (2005) Spontaneous

normalization of thyrotropin concentrations in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab* 90: 4124-4127.

7. Huber G, Staub JJ, Meier C, Mitrache C, Guglielmetti M, Huber P, *et al.* (2002) Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. *J Clin Endocrinol Metab* 87: 3221-3226.

8. Rosenthal MJ, Hunt WC, Garry PJ, Goodwin JS (1987) Thyroid failure in the elderly. Microsomal antibodies as discriminant for therapy. *JAMA* 258: 209-213.

9. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, *et al.* (1995) The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol (Oxf)* 43: 55-68.

10. Loy M, Cianchetti ME, Cardia F, Melis A, Boi F, Mariotti S (2004) Correlation of computerized grayscale sonographic findings with thyroid function and thyroid autoimmune activity in patients with Hashimoto's thyroiditis. *J Clin Ultrasound* 32: 136-140.

11. Rago T, Chiovato L, Grasso L, Pinchera A, Vitti P (2001) Thyroid ultrasonography as a tool for detecting thyroid autoimmune diseases and predicting thyroid dsfunction in apparently healthy subjects. *J Endocrinol Invest* 24: 763-769.

12. Schiemann U, Avenhaus W, Konturek JW, Gellner R, Hengst K, Gross M (2003) Relationship of clinical features and laboratory parameters to thyroid echogenicity measured by standardized grey scale ultrasonography in patients with Hashimoto's thyroiditis. *Med Sci Monit* 9: MT13-17.

13. Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Pedersen IB, Rasmussen LB, *et al.* (2006) The association between hypoechogenicity or irregular echo pattern at thyroid ultrasonography and thyroid function in the general population. *Eur J Endocrinol* 155: 547-552.

14. Langer JE, Khan A, Nisenbaum HL, Baloch ZW, Horii SC, Coleman BG, *et al.* (2001) Sonographic appearance of focal thyroiditis. *AJR Am J Roentgenol* 176: 751-754.

15. Pedersen OM, Aardal NP, Larssen TB, Varhaug JE, Myking O, Vik-Mo H (2000) The value of ultrasonography in predicting autoimmune thyroid disease. *Thyroid* 10: 251-259.

16. Bemben DA, Hamm RM, Morgan L, Winn P, Davis A, Barton E (1994) Thyroid disease in the elderly. Part 2. Predictability of subclinical hypothyroidism. *J Fam Pract* 38: 583-588.

17. Feldt-Rasmussen U (2009) Is the treatment of subclinical hypothyroidism beneficial? *Nat Clin Pract Endocrinol Metab* 5: 86-87.

18. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC (1991) Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. *Clin Endocrinol (Oxf)* 34: 77-83.

19. Karmisholt J, Andersen S, Laurberg P (2008) Interval between tests and thyroxine estimation method influence outcome of monitoring of subclinical hypothyroidism. *J Clin Endocrinol Metab* 93: 1634-1640.

20. Rodondi N, Newman AB, Vittinghoff E, de Rekeneire N, Satterfield S, Harris TB, *et al.* (2005) Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. *Arch Intern Med* 165: 2460-2466.

21. Singh S, Duggal J, Molnar J, Maldonado F, Barsano CP, Arora R (2008) Impact of subclinical thyroid disorders on coronary heart disease, cardiovascular and all-cause mortality: a meta-analysis. *Int J Cardiol* 125: 41-48.

22. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, *et al.* (2004) Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA* 291: 228-238.

23. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, *et al.* (2005) Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Arch Intern Med* 165: 2467-2472.

24. Biondi B, Cooper DS (2008) The clinical significance of subclinical thyroid dysfunction. *Endocr Rev* 29: 76-131.

25. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC (2000) The Colorado thyroid disease prevalence study. *Arch Intern Med* 160: 526-534.

26. Raber W, Gessl A, Nowotny P, Vierhapper H (2002) Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. *Thyroid* 12: 725-731.

27. Nys P, Cordray JP, Merceron RE (2009) Etiologic discussion and clinical relevance of thyroid ultrasonography in subclinical hypothyroidism. A retrospective study in 1845 patients. *Ann Endocrinol (Paris)*.

28. Rosario PW, Bessa B, Valadao MM, Purisch S (2009) Natural history of mild subclinical hypothyroidism: prognostic value of ultrasound. *Thyroid* 19: 9-12.

29. Li Y, Teng D, Shan Z, Teng X, Guan H, Yu X, *et al.* (2008) Antithyroperoxidase and antithyroglobulin antibodies in a five-year follow-up survey of populations with different iodine intakes. *J Clin Endocrinol Metab* 93:1751-1757.

30. Kasagi K, Kousaka T, Higuchi K, Iida Y, Misaki T, Alam MS, *et al.* (1996) Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: comparison with histological findings. *Thyroid* 6:445-450.

31. Arai T, Kurashima C, Utsuyama M, Sawabe M, Ito H. (2000) Measurement of anti-thyroglobulin and anti-thyroid peroxidase antibodies using highly sensitive radioimmunoassay: an effective method for detecting asymptomatic focal lymphocytic thyroiditis in the elderly. *Endocr J* 47:575-582.