Serum negative autoimmune thyroiditis displays a milder clinical picture compared with classic Hashimoto's thyroiditis

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Abstract

Background: Despite high sensitivity of current assays for autoantibodies to thyroperoxidase (TPO) and to thyroglobulin (Tg), some hypothyroid patients still present with negative tests for circulating anti-thyroid Abs. These patients usually referred to as having seronegative autoimmune thyroiditis (seronegative CAT) have not been characterized, and definite proof that their clinical phenotype is similar to that of patients with classic chronic autoimmune thyroiditis (CAT) is lacking.
Objective: To compare the clinical phenotype of seronegative CAT (SN-CAT) and CAT as diagnosed according to a raised serum level of TSH with negative and positive tests for anti-thyroid Abs respectively.
Methods: A case–control retrospective study enrolling 55 patients with SN-CAT and 110 patients with CAT was performed. Serum free triiodothyronine (FT_3), free thyroxine (FT_4), TSH, Tg Abs, and TPO Abs were measured in all patients.
Results: Patients with SN-CAT displayed significantly lower mean levels of TSH (6.6 ± 3.4 vs 10.2 ± 9.8 μU/ml; $P=0.009$), higher mean FT_4 levels (1.1 ± 0.2 vs 0.9 ± 0.2 ng/dl; $P=0.0002$), and similar FT_3 levels when compared with CAT patients. Mean thyroid volume was significantly greater in patients with CAT when compared with SN-CAT patients (11.2 ± 6.5 vs 8.1 ± 3.7 ml; $P=0.001$). Logistic regression demonstrated that FT_4 (0.123 (0.019–0.775); ($P=0.026$)) and thyroid volume (1.243 (1.108–1.394); ($P=0.0002$)) were significantly and independently related to the diagnosis (CAT/SN-CAT). Patients with SN-CAT had a similar prevalence of thyroid nodules and female gender but a lower prevalence of overt hypothyroidism (5.4 vs 20.9%; $P=0.012$) as opposed to patients with CAT.
Conclusions: These results suggest an autoimmune etiology of SN-CAT, which, however, seems to have a milder clinical course when compared with CAT.

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Introduction

Chronic autoimmune thyroiditis (CAT) is the main cause of hypothyroidism in the general population (1). Auto-antibodies to thyroperoxidase (TPO) and to thyroglobulin (Tg) are the circulating hallmark of this autoimmune thyroid disease (1, 2). Positive tests for TPO Abs are present in ∼90% (TPO Abs) and 50% (Tg Abs) of hypothyroid patients with CAT (1, 3, 4). Their prevalence in the general population is highly variable, given the strong gender and age effect, but it may reach a rate of 12–15% in females in

their third to fourth decade of life (5, 6). Early studies supported the hypothesis that TPO Abs would play a pathogenic role in the development of autoimmune thyroiditis (7). Indeed, they fix complement and, at least *in vitro*, can produce antibody-dependent cell cytotoxicity (8). However, the high prevalence of positive tests for TPO Abs in euthyroid subjects and the observation that neonates born to mothers with circulating TPO Abs have a normal thyroid gland (9) have made it clear that TPO Abs

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are more likely markers and/or risk factors for the subsequent development of autoimmune thyroid disease rather than pathogenic factors (10).

In spite of the high sensitivity of modern assay methods for TPO Abs and TG Abs, a consistent percentage of hypothyroid patients present with negative tests for these thyroid autoantibodies (11). As most of these patients display a hypoechoic pattern of their thyroid at neck ultrasound (US) examination, the diagnosis of 'serum negative autoimmune thyroiditis' (seronegative CAT) is commonly used to define the underlying thyroid disorder. (12). Besides the description of some clinical cases, in which seronegative CAT (SN-CAT) was detected in association with type 1 diabetes mellitus (13) or rheumatoid arthritis (14, 15), no currently published study evaluated a large series of patients with SN-CAT. The prevalence of SN-CAT can be estimated indirectly at ~5% taking into account the rate of hypothyroid patients showing positive tests for thyroid autoantibodies in the published studies (11). The rather low prevalence of the condition and the non-routine, at least in previous years, use of thyroid US scan contributed to the opinion that these patients have a similar clinical phenotype when compared with those having classic CAT. In recent years, the routine use of ultrasensitive thyroid-stimulating hormone (TSH) measurements, which increased the detection of subclinical hypothyroidism in patients, contributed to raise the prevalence of SN-CAT, as it is known that the rates of positive thyroid Ab tests increase with increasing levels of TSH (6, 16).

Up to date, the clinical presentation of patients with SN-CAT was not systematically investigated; in particular, it is still unknown whether its clinical features are similar to those of classic CAT.

The aim of this study was to compare the clinical phenotype of patients who received a diagnosis of SN-CAT with that of patients with classic CAT.

Subjects and methods

Subjects

The study group encompassed 55 patients who received a diagnosis of SN-CAT in the Outpatient Clinic of the Unit of Internal Medicine and Endocrinology of the Fondazione S. Maugeri I.R.C.C.S. (Pavia, Italy) between 2008 and 2011. The diagnosis of SN-CAT was based on the following criteria: i) presence of subclinical and/or overt hypothyroidism, as assessed by a serum TSH level >4.0 μU/ml associated with either normal or low FT_4 levels

respectively; ii) negative tests for circulating Tg Abs and TPO Abs on at least two consecutive measurements; and iii) a US scan of the thyroid showing a hypoechoic pattern of its parenchyma. The study group encompassed seven males and 48 females. Their median (and range) age was 47.7 (17–80) years.

The control group was composed of a double number of patients (110; 12 males and 98 females) who had received a diagnosis of CAT. Control patients with CAT were consecutively and retrospectively selected using a computerized database. Inclusion criteria were positive tests for either TG Abs, TPO Abs, or both, accompanied by clinically overt or subclinical hypothyroidism.

In all patients and controls, the diagnosis of subclinical hypothyroidism was confirmed on at least two separate TSH measurements at time intervals ranging from 2 to 6 months. Common exclusion criteria were: i) pregnancy at diagnosis and/or within 1 year from study entry; ii) a history of irradiation of the neck; iii) current and/or previous L-thyroxine (T_4) treatment; iv) treatment with corticosteroids, amiodarone, lithium, oral contraceptives, or other thyroid interfering drugs; v) inter-current chronic illnesses; vi) a previous diagnosis of thyroid disease, in particular Graves' disease and subacute thyroiditis; and vii) presence of obesity (BMI level $\geq 30 \text{ kg/m}^2$).

The family history, as well as the co-morbidity for other allergic or autoimmune (allergic rhinitis or asthma, thyroid diseases, type 1 diabetes mellitus, psoriasis, vitiligo, alopecia, celiac disease, Crohn's disease, rheumatoid arthritis, atrophic gastritis, and lupus) and non-autoimmune (thyroid diseases) disorders, was recorded. The study was approved by the Local Ethics Committee.

Serum assays

Serum concentrations of free triiodothyronine (FT_3, normal range 1.5–4.1 pg/ml), free T_4 (FT_4, normal range 0.8–1.9 ng/dl), and TSH (normal range 0.4–4.0 mIU/l) were measured using immunochemiluminescent assays by an automated analyser (Immulite 2000, DPC Cirrus, Los Angeles, CA, USA) employing commercially available kits (all from Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay coefficient of variation (CV) values for these hormones ranged from 4.3 to 8.4% for FT_3, from 5.2 to 7.5% for FT_4, and from 5.1 to 12.5% for TSH. Inter-assay CV values ranged from 5.4 to 10.0% for FT_3, from 7.7 to 9.0% for FT_4, and from 6.4 to 12.5% for TSH. The analytical sensitivities were 1.0 pg/ml for FT_3, 0.3 ng/dl for FT_4, and 0.004 μIU/l for TSH (third-generation TSH assay). The serum

concentrations of Tg Abs (normal range <60 U/ml) and TPO Abs (normal range <60 U/ml) were measured using immunochemiluminescent assays employing commercially available kits (Brahams, Hennigsdorf, Germany). The sensitivity of the assay was 33 U/ml for TGAb and 50 U/ml for TPO Abs. The intra- and inter-assay CV values were 2.6 and 13% respectively for TG Abs and 3.9 and 8% respectively for TPO Abs. Samples were assayed in duplicate. Quality control pools at low, normal, and high concentrations for all parameters were present in each assay.

Setting the reference limit for thyroid autoantibodies positivity

Considering that the manufacturer's cutoff (<60 UI/ml for both antibodies) might not be adequate, a control group of 55 age- and sex-matched subjects in whom thyroid disorders had been excluded by a complete thyroid work-up (history, physical examination, TSH, FT_3, FT_4, and thyroid US) was recruited. The results of samples found to be below the limit of detection (<10 UI/ml for both Tg Abs and TPO Abs) were arbitrarily estimated to be 10 UI/ml. In a control group, the mean $\pm$S.D. circulating levels of thyroid autoantibodies were 14.11 ± 9.76 UI/ml for Tg Abs and 14.74 ± 8.98 UI/ml for TPO Abs. On this basis, the cut-off for defining a positive test was chosen for Tg Abs and TPO Abs at >2 S.D. of the mean level found in the control group (>33.6 UI/ml for Tg Abs and >32.7 UI/ml for TPO Abs).

According to these in house-established reference limits, five out of 55 (9.1%) and one out of 55 (1.8%) patients with SN-CAT displayed above normal titers for Tg Abs and TPO Abs respectively. Statistical analysis performed after exclusion of these six patients confirmed the results obtained when the whole study group ($n=55$) was taken into account. Thus, we decided not to exclude the above-mentioned six patients.

Statistical analysis

Statistical analysis was performed using the SPSS Software (SPSS, Inc.). Between-groups comparisons were performed using the Student's t-test for unpaired data and the Mann–Whitney U-test according to a normal or a non-parametric distribution; comparisons were performed using the Student's t-test for paired data and the Wilcoxon's test according to a normal or a non-parametric distribution. Frequencies among groups were compared using the χ^2-test with Fisher's correction when appropriate. To test the effects of different variables independent of a

covariate, multivariate logistic regression analysis was used and partial correlation coefficients were computed. The multivariate model was constructed by entering the diagnosis (CAT and SN-CAT) as a dependent variable, while TSH, FT_4, and thyroid volume (all factors found to be significant at $P<0.05$ in the univariate analysis) were entered as covariates. A P value of <0.05 was considered statistically significant. Results are expressed as mean $\pm$S.D., unless otherwise stated.

Results

The clinical and anthropometric data of patients with SN-CAT and of those with classic CAT are shown in Table 1. The two groups had a similar M:F ratio (12:98 for CAT vs 7:48 for SN-CAT), age at first diagnosis (47.3 ± 14.7 vs 47.7 ± 16.6 years), height (161.0 ± 7.8 vs 161.2 ± 8.4 cm), weight (62.7 ± 10.8 vs 60.7 ± 10.4 kg), and BMI (24.1 ± 3.4 vs 23.2 ± 2.9 kg/m^2). As also shown in Fig. 1, patients with SN-CAT displayed significantly lower levels of serum TSH (6.6 ± 3.4 vs 10.2 ± 9.8 µU/l; $P=0.009$) and higher FT_4 levels (1.1 ± 0.2 vs 0.9 ± 0.2 ng/dl; $P=0.0002$) compared with those having CAT. The circulating concentrations of FT_3 were similar in the two groups (3.2 ± 0.7 vs 3.4 ± 0.9 pg/ml). The prevalence of overt hypothyroidism was significantly ($P=0.012$) higher in

Table 1 Anthropometric and clinical parameters in patients with CAT and SN-CAT. Data are shown as mean$\pm$S.D. unless otherwise stated.

	CAT (Ab+)	**SN-CAT** (Ab−)	**P value**
No. of patients	110	55	
Sex (M/F)	12/98	7/48	0.730
Age at diagnosis (years)	47.3 ± 14.7	47.7 ± 16.6	0.869
Weight (kg)	62.7 ± 10.8	60.7 ± 10.4	0.261
Height (cm)	161.0 ± 7.8	161.2 ± 8.4	0.875
BMI (kg/m^2)	24.1 ± 3.4	23.2 ± 2.9	0.101
FT_3 (pg/ml)	3.2 ± 0.7	3.4 ± 0.9	0.097
FT_4 (ng/dl)	0.9 ± 0.2	1.1 ± 0.2	**0.0002**
TSH (µU/ml)	10.2 ± 9.8	6.6 ± 3.4	**0.009**
TSH (µU/ml) (median and range)	**6.8 (4.2–58.0)**	**5.8 (4.1–27.0)**	**0.0007**
Overt/subclinical hypothyroidism	**23/87 (20.9%)**	**3/52 (5.4%)**	**0.012**
Thyroid volume (ml)	**11.2$\pm$6.5**	**8.1$\pm$3.7**	**0.001**
Thyroid volume (ml) (median and range)	**10 (3–62)**	**8 (2–20)**	**0.00003**
Nodule (yes/no)	25/85 (22.7%)	14/41 (25.4%)	0.697
Goiter >20 ml	3 (2.7%)	0 (0%)	0.551

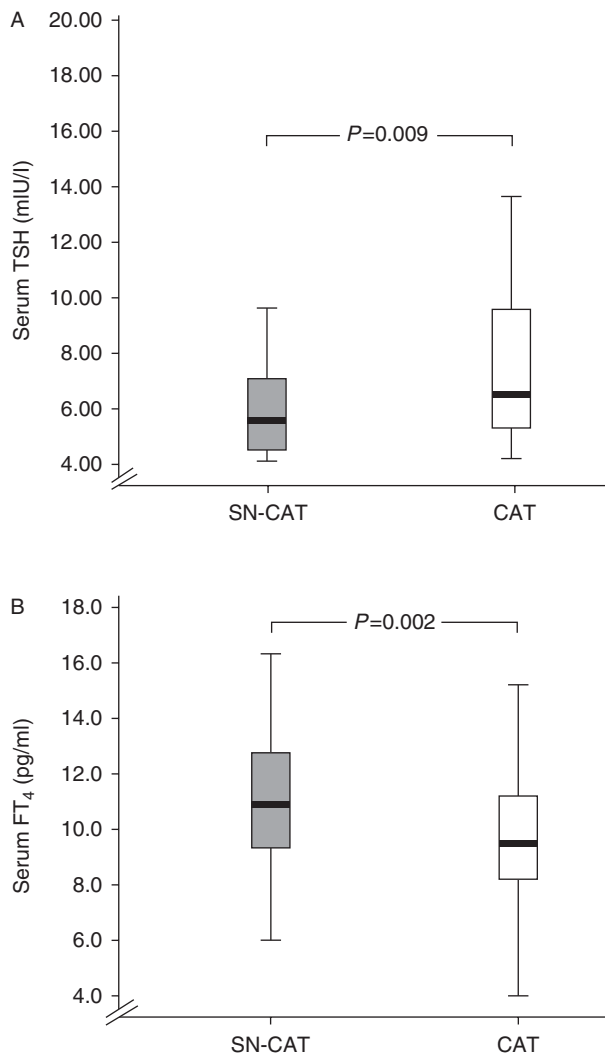Bold characters indicate significant differences.

Figure 1

(A) Distribution of the serum levels of TSH in patients with SN-CAT and CAT. (B) Distribution of the serum levels of FT$_4$ in patients with SN-CAT and CAT. The data are expressed as median and 25th and 75th percentiles in boxes and 5th and 95th percentiles as whiskers.

patients with CAT (23/110, 20.9%) as opposed to those with SN-CAT (3/55; 5.4%). Mean thyroid volume, as measured by US, was significantly greater in patients with CAT when compared with those having SN-CAT (11.2 ± 6.5 vs 8.1 ± 3.7 ml; $P=0.001$). Although the statistical significance was not reached, three patients with CAT and none with SN-CAT had goiter (diagnosed when the thyroid volume was >20 ml). A similar prevalence of thyroid nodules was found in patients with CAT (22.7%) when compared with those having SN-CAT (25.4%).

To test the independent effect of each variable, a logistic regression model was constructed with the diagnosis (CAT/SN-CAT) as a dependent variable and all factors showing significant differences in the univariate analysis (TSH, FT$_4$, and thyroid volume) entered as covariates. The results shown in Table 2 demonstrated that only FT$_4$ and thyroid volume were significantly and independently related to the diagnosis of CAT and SN-CAT.

A positive family history for thyroid diseases was present in 33.6% of patients with CAT and 43.6% of those with SN-CAT ($P=0.21$). However, patients with CAT displayed a significantly higher prevalence of positive family history for autoimmune diseases when compared with patients having SN-CAT (44.5 vs 25.4%; $P=0.017$).

The thyroid disease-free control group also served to assess whether patients with SN-CAT had a higher than normal frequency of autoimmune diseases (including autoimmune thyroid diseases) or non-autoimmune thyroid diseases in their family. A positive family history of autoimmune diseases was present in 14.5% of thyroid disease-free control subjects. The correspondent figure for a positive family history of non-autoimmune thyroid diseases was 10.9%. When these family histories were compared with those observed in SN-CAT patients, a strong significant difference (10.9 vs 43.6%; $P=0.00012$) emerged for a positive family history of non-autoimmune thyroid diseases. On the other hand, despite an ~ 1.5-fold increase, the rate of positive family history for autoimmune diseases in patients with SN-CAT did not reach significance compared with controls (25.4 vs 14.5%; $P=0.153$).

As a further step, co-morbidities were taken into account. Patients with SN-CAT showed a significantly lower rate of co-morbidities for non-thyroid autoimmune diseases compared with those having CAT (21.8 vs 44.5%; $P=0.004$).

Table 2 Results of logistic regression analysis using the diagnosis (SN-CAT and CAT) as a dichotomous dependent variable.

			95% CI	
	P value	Exp(β)	Lower	Upper
Serum FT$_4$ (ng/dl)	0.026	0.123	0.019	0.775
Serum TSH (μU/ml)	0.085	1.102	0.987	1.231
Thyroid volume (ml)	0.0002	1.243	1.108	1.394

Covariates were chosen on the basis of *P* values (<0.05) as a result of the univariate analysis.

Discussion

The results of this study, which investigated the so far largest series of patients with SN-CAT, indicate significant differences in the clinical presentation of patients with SN-CAT compared with those having classic CAT.

First, a prevalence of female gender was observed both in patients with SN-CAT and in those with CAT. This is a strong argument pointing toward an autoimmune etiology of SN-CAT. Indeed, in conditions such as morbid obesity, in which the raised serum levels of TSH are not sustained by an autoimmune process, no gender effect is observed.

Secondly, the age at presentation was similar in patients with SN-CAT and those with CAT. This finding would contrast with the common belief that a hypoechoic pattern of the thyroid at US anticipates the appearance of circulating thyroid Abs [17, 18, 19, 20].

Thirdly, lower serum TSH levels and higher serum FT_4 levels are found in patients with SN-CAT when compared with patients having classic CAT, even if the results of the multivariate analysis clearly indicate that the effect should be mainly ascribed to FT_4. Although the cross-sectional design of this study does not allow drawing conclusions as to the natural history of SN-CAT, given that the age at diagnosis was similar in the two groups, it could be speculated that the course of SN-CAT is less aggressive than that of CAT. This hypothesis would be further supported by the significantly lower rate of overt hypothyroidism observed among patients with SN-CAT. The absence of goiter in patients with SN-CAT and the observation that their thyroid volume was smaller might suggest that the autoimmune inflammatory infiltrate is less severe. Indeed, in the classical description of auto-immune thyroiditis by Hashimoto [21], most patients had goiter.

Fourthly, patients with SN-CAT had the same prevalence of thyroid nodules when compared with patients having CAT. This observation implies that, compared with CAT, no nodule-dependent selection bias affected the recruitment patients with SN-CAT.

The last finding to be discussed is the lower prevalence of a positive family history for autoimmune diseases and associated autoimmune conditions in patients with SN-CAT as opposed to those with classic CAT. Given that autoimmune diseases result from the interplay between genetic and environmental factors, it could be speculated that patients developing SN-CAT would have a weaker genetic predisposition, while environmental factors play a more relevant role in their pathogenesis [22, 23]. In this regard, it is also important to recall that two previously described cases of patients with SN-CAT were found to be associated with serum negative rheumatoid arthritis [14, 15]. Thus, it could be speculated that patients with SN-CAT might be more prone to develop other serum negative autoimmune diseases, the diagnosis of which might be more cumbersome.

The retrospective design of this study does not allow grading the echogenicity of the thyroid parenchyma at US, and this can be viewed as a limitation of this study. Therefore, it is impossible to compare the degree of hypoechogenicity in patients with SN-CAT with those with CAT. Future specifically designed studies will be required to address this issue.

The design of this study does not allow drawing firm conclusions about the prevalence of SN-CAT, but the following consideration could be proposed. The prevalence of SN-CAT can so far be estimated indirectly at $\sim$5% according to the rate of positive tests for thyroid Abs reported by previous clinical studies in patients with hypothyroidism [3, 4, 11]. However, these early studies enrolling patients with raised serum levels of TSH did not take into account the presence of clinical conditions associated with an increase in circulating TSH levels recently proven not to be indicative of thyroid failure. In particular, morbid obesity has recently been identified to cause an increase in the serum levels of TSH often associated with the presence of a hypoechoic pattern of the thyroid at US, which do not imply that hypothyroidism is present [24, 25, 26, 27]. Taking into account the above concepts, it would be reasonable to assume that the prevalence of SN-CAT might have been overestimated by earlier studies.

In conclusion, the results of the current study, performed in the largest series of patients with SN-CAT investigated so far, suggest an autoimmune etiology of the disease. Although SN-CAT displays similar clinical features when compared with CAT, serum negative patients appear to have a less aggressive disease.

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References

1 Pearce EN, Farwell AP & Braverman LE. Thyroiditis. *New England Journal of Medicine* 2003 **348** 2646–2655. (doi:10.1056/NEJMra021194)

2 Dayan CM & Daniels GH. Chronic autoimmune thyroiditis. *New England Journal of Medicine* 1996 **335** 99–107. (doi:10.1056/NEJM199607113350206)

3 Mariotti S, Anelli S, Ruf J, Bechi R, Czarnocka B, Lombardi A, Carayon P & Pinchera A. Comparison of serum thyroid microsomal and thyroid peroxidase autoantibodies in thyroid diseases. *Journal of Clinical Endocrinology and Metabolism* 1987 **65** 987–993. (doi:10.1210/jcem-65-5-987)

4 Mariotti S, Caturegli P, Piccolo P, Barbesino G & Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. *Journal of Clinical Endocrinology and Metabolism* 1990 **71** 661–669. (doi:10.1210/jcem-71-3-661)

5 Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA & Braverman LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 489–499. (doi:10.1210/jcem.87.2.8182)

6 O'Leary PC, Feddema PH, Michelangeli VP, Leedman PJ, Chew GT, Knuiman M, Kaye J & Walsh JP. Investigations of thyroid hormones and antibodies based on a community health survey: the Busselton thyroid study. *Clinical Endocrinology* 2006 **64** 97–104. (doi:10.1111/j.1365-2265.2005.02424.x)

7 Weetman AP. Autoimmune thyroid disease: propagation and progression. *European Journal of Endocrinology* 2003 **148** 1–9. (doi:10.1530/eje.0.1480001)

8 Chiovato L, Bassi P, Santini F, Mammoli C, Lapi P, Carayon P & Pinchera A. Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 1700–1705. (doi:10.1210/jcem.77.6.7903315)

9 Dussault JH, Letarte J, Guyda H & Laberge C. Lack of influence of thyroid antibodies on thyroid function in the newborn infant and on a mass screening program for congenital hypothyroidism. *Journal of Pediatrics* 1980 **96** 385–389. (doi:10.1016/S0022-3476(80)80677-9)

10 Weetman AP. Cellular immune responses in autoimmune thyroid disease. *Clinical Endocrinology* 2004 **61** 405–413. (doi:10.1111/j.1365-2265.2004.02085.x)

11 Takamatsu J, Yoshida S, Yokozawa T, Hirai K, Kuma K, Ohsawa N & Hosoya T. Correlation of antithyroglobulin and antithyroid-peroxidase antibody profiles with clinical and ultrasound characteristics of chronic thyroiditis. *Thyroid* 1998 **8** 1101–1106. (doi:10.1089/thy.1998.8.1101)

12 Baker JR Jr, Saunders NB, Wartofsky L, Tseng YC & Burman KD. Seronegative Hashimoto thyroiditis with thyroid autoantibody production localized to the thyroid. *Annals of Internal Medicine* 1988 **108** 26–30. (doi:10.7326/0003-4819-108-1-26)

13 Zhang DM, Zhou ZG, Zhang C, Huang G, Jin P, Wang JP, Wei JL & Hu BY. Subclassification of seronegative type 1 diabetic subjects with HLA-DQ genotypes. *Zhonghua Nei Ke Za Zhi* 2004 **43** 174–178.

14 Spina MP, Cerri A, Piacentini V, Stringa A & Visca U. Seronegative hashitoxicosis in patient with rheumatoid arthritis. *Minerva Endocrinologica* 1990 **15** 173–176.

15 Punzi L, Schiavon F, Ramonda R, Cavasin F, Ruffatti A & Todesco S. Anti-thyroid microsomal antibody in synovial fluid as a revealing feature of seronegative autoimmune thyroiditis. *Clinical Rheumatology* 1991 **10** 181–183. (doi:10.1007/BF02207661)

16 Bülow Pedersen I, Laurberg P, Knudsen N, Jørgensen T, Perrild H, Ovesen L & Rasmussen LB. A population study of the association between thyroid autoantibodies in serum and abnormalities in thyroid function and structure. *Clinical Endocrinology* 2005 **62** 713–720. (doi:10.1111/j.1365-2265.2005.02284.x)

17 Marcocci C, Vitti P, Cetani F, Catalano F, Concetti R & Pinchera A. Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 209–213. (doi:10.1210/jcem-72-1-209)

18 Rago T, Chiovato L, Grasso L, Pinchera A & Vitti P. Thyroid ultrasonography as a tool for detecting thyroid autoimmune diseases and predicting thyroid dysfunction in apparently healthy subjects. *Journal of Endocrinological Investigation* 2001 **24** 763–769. (doi:10.1007/BF03343925)

19 Raber W, Gessl A, Nowotny P & Vierhapper H. Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. *Thyroid* 2002 **12** 725–731. (doi:10.1089/105072502760258712)

20 Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Pedersen IB, Rasmussen LB, Ovesen L & Jørgensen T. The association between hypoechogenicity or irregular echo pattern at thyroid ultrasonography and thyroid function in the general population. *European Journal of Endocrinology* 2006 **155** 547–552. (doi:10.1530/eje.1.02255)

21 Takami HE, Miyabe R & Kameyama K. Hashimoto's thyroiditis. *World Journal of Surgery* 2008 **32** 688–692. (doi:10.1007/s00268-008-9485-0)

22 Weetman AP. Autoimmune thyroid disease. *Autoimmunity* 2004 **37** 337–340. (doi:10.1080/08916930410001705394)

23 Weetman AP. Diseases associated with thyroid autoimmunity: explanations for the expanding spectrum. *Clinical Endocrinology* 2011 **74** 411–418. (doi:10.1111/j.1365-2265.2010.03855.x)

24 Radetti G, Kleon W, Buzi F, Crivellaro C, Pappalardo L, di Iorgi N & Maghnie M. Thyroid function and structure are affected in childhood obesity. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4749–4754. (doi:10.1210/jc.2008-0823)

25 Rotondi M, Leporati P, La Manna A, Pirali B, Mondello T, Fonte R, Magri F & Chiovato L. Raised serum TSH levels in patients with morbid obesity: is it enough to diagnose subclinical hypothyroidism? *European Journal of Endocrinology* 2009 **160** 403–408. (doi:10.1530/EJE-08-0734)

26 Rotondi M, Cappelli C, Leporati P, Chytiris S, Zerbini F, Fonte R, Magri F, Castellano M & Chiovato L. A hypoechoic pattern of the thyroid at ultrasound does not indicate autoimmune thyroid diseases in patients with morbid obesity. *European Journal of Endocrinology* 2010 **163** 105–109. (doi:10.1530/EJE-10-0288)

27 Rotondi M, Magri F & Chiovato L. Thyroid and obesity: not a one-way interaction. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 344–346. (doi:10.1210/jc.2010-2515)