The association of other autoimmune diseases

in patients with Graves' disease (with or without ophthalmopathy):

review of the literature and report of a large series.

Silvia Martina Ferrari[1], Poupak Fallahi[1], Ilaria Ruffilli[1],

Giusy Elia[1], Francesca Ragusa[1], Salvatore Benvenga[2-4], Alessandro Antonelli[1]

[1]Department of Clinical and Experimental Medicine, University of Pisa, Via Savi 10, 56126 Pisa, Italy; [2]Department of Clinical and Experimental Medicine, University of Messina, Italy; [3]Master Program on Childhood, Adolescent and Women's Endocrine Health, University of Messina, Italy; [4]Interdepartmental Program of Molecular and Clinical Endocrinology and Women's Endocrine Health, Azienda Ospedaliera Universitaria Policlinico 'G. Martino', I-98125 Messina, Italy.

Running Title: The association of other autoimmune diseases in patients with GD (with/without GO)

Corresponding Author

Alessandro Antonelli, MD

Director: Immuno-Endocrine Section of Internal Medicine

Professor of Medicine, Endocrinology, Clinical Pathology

Head, Laboratory of Primary Human Cells

Department of Clinical and Experimental Medicine

University of Pisa, School of Medicine

Via Savi, 10, I-56126, Pisa, Italy

Phone: +39-050-992318

Mobile: +39-335-8119294 or +39-335-344701

Fax: +39-050-993472 or +39-050-500841

e-mail: alessandro.antonelli@med.unipi.it

Abstract

Graves' disease (GD) and autoimmune thyroiditis (AT) are the two main clinical presentations of AITD, and their clinical hallmarks are thyrotoxicosis and hypothyroidism, respectively. GD, and AT, can be associated with other organ specific, or systemic autoimmune diseases in the same patient. However discordant results have been reported in the literature about the possible associations. Here, we review the association of GD and other autoimmune syndromes.

Furthermore, we report the results of our prospective study that investigated the prevalence of other autoimmune disorders in 3209 GD patients (984 with Graves' ophthalmopathy), with respect to 1069 healthy controls, or 1069 patients with AT, or 1069 with multinodular goiter (matched by age, gender, coming from the same area, with a similar iodine intake).

On the whole, 16.7% of GD patients had another associated autoimmune disease; and the most frequently observed were: vitiligo (2.6%), chronic autoimmune gastritis (2.4%), rheumatoid arthritis (1.9%), polymyalgia rheumatica (1.3%), multiple sclerosis (0.3%), celiac disease (1.1%), diabetes (type 1) (0.9%), systemic lupus erythematosus and sarcoidosis (<0.1%), Sjogren disease (0.8%). Moreover, 1.5% patients with GD had three associated autoimmune disorders.

Interestingly, patients with Graves' ophthalmopathy (GO) had another autoimmmune disorder more frequently (18.9%), with respect to GD patients without GO (15.6%). However the pattern of the associated autoimmune disorders in GD was not significantly different from that observed in AT patients.

In conclusion, we suggest GD patients who are still sick, or who develop new unspecific symptoms (even if during an appropriate treatment of hyperthyroidism) should be appropriately screened for the presence of other autoimmune disorders.

Keywords: Autoimmune thyroid diseases, Graves' diseases, Graves' ophthalmopathy, Autoimmune thyroiditis, vitiligo, chronic autoimmune gastritis, rheumatoid arthritis, polymyalgia

rheumatica, celiac disease, type 1 diabetes (type 1), Sjogren disease, multiple sclerosis, systemic lupus erythematosus, sarcoidosis.

Take-home messages:

- About 17% of Graves' diseases (GD) patients had another associated autoimmune disease; and the most frequently observed were: vitiligo, chronic autoimmune gastritis, rheumatoid arthritis, polymyalgia rheumatica, multiple sclerosis, celiac disease, diabetes (type 1), systemic lupus erythematosus and sarcoidosis, Sjogren disease. Moreover, 1.5% patients with GD had three associated autoimmune disorders. Interestingly, patients with Graves' ophthalmopathy (GO) had another autoimmmune disorder more frequently (18.9%), with respect to GD patients without GO (15.6%).
- GD patients who are still sick, or who develop new unspecific symptoms (even if during an appropriate treatment of hyperthyroidism) should be appropriately screened for other autoimmune disorders (not to delay the diagnosis), and treatment of these ones, overall when GO is present.

1. Introduction

Autoimmune thyroid diseases (AITD) are the most frequent autoimmune disorders and result from an immune assault on the thyroid, and they are classified as "T cell-mediated organ-specific autoimmune disorders" [1]. AITD include two main clinical presentations: Autoimmune thyroiditis (AT) and Graves' disease (GD). The clinical hallmarks of GD and AT are thyrotoxicosis and hypothyroidism, respectively. The prevalence of AITD is about 5% [2]; however, the prevalence of antithyroid antibodies (ATA) may be even higher [3]: (1) the risk of women in comparison to men is more elevated (approximately 6/1); (2) hypothyroidism is more frequent in older people; (3) there is a great geographic variability, and the prevalences of AITD and thyroid antibodies differ with race; (4) the incidence of AITD is more elevated in subjects living in iodine-sufficient areas in comparison to those in iodine-deficient areas [3]. The incidence rate of AITD is increasing [4], even if it is not possible to know whether this is due to a higher accuracy of diagnostic procedures [5–8]. In many cases, AITD may be associated in the same patient with other organ-specific autoimmune attacks (such as in the case of type II autoimmune polyglandular sindrome); the most frequent association was between AITD and type I diabetes (T1D) [9-12].

Furthermore, ATA and thyroid function abnormalities have been frequently described in patients with systemic rheumatologic autoimmune diseases, such as Sjögren's syndrome (SS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) [13-17].

Recently it has been reported a high frequency (9.67%) of coexisting autoimmune disorders in 2791 GD patients from UK [18].

However other discordant results have been reported in the literature [19-22].

It has been suggested that, the small samples size, the use of retrospective recruitment of cases, and of control groups not matched by gender, age or geographic distribution, or iodine intake, could have hindered the data published by different papers. Furthermore, to eliminate bias in case

selection, prospective studies and a tight disease definition are needed [23].

Here, we review the association of GD and other autoimmune syndromes. Furthermore, we report the results obtained in our prospective study that investigated the prevalence of other autoimmune disorders in GD patients, with respect to healthy controls, or patients with AT, or with multinodular goiter.

2. Material and Methods

2.1 Study subjects

From 1993 to 2010 we have evaluated prospectively the prevalence of other autoimmune disorders in outpatient clinic in 3209 consecutive Caucasian patients with GD (**Table 1**), with or without ophthalmopathy (GO). The diagnosis of GD [24] was established from the clinical presentation (presence of a diffuse goiter, varying in size from normal to very large), thyroid hormones and thyroid autoantibodies measurements [presence of anti-TSH-receptor autoantobodies (TRAb), and/or thyroid ultrasonography (decreased, dyshomogeneous echogenicity, and diffuse goiter)]. The majority of these patients had goiter (63%), the others showed a normal thyroid volume. A minority of patients (7%) were submitted to fine-needle aspiration to exclude the presence of thyroid cancer or lymphoma; in these cases, cytology excluded the presence of a malignancy [24, 25].

The diagnosis of GO was established from the clinical presentation [26].

In GO patients, eye disease activity was assessed by the Clinical Activity Score (CAS) [27].

2.2. Control groups

The prevalence of autoimmune disorders was investigated in three age (±5 years), and gender, matched, control groups (3 patients/1 control) (**Table 1**), extracted from the same random sample of the general population, with similar iodine intake.

Control I (n = 1069) comprised people extracted ramdonly from the general population of the same geographic area [28]. A complete thyroid screening [history; physical examination;

ultrasonography; free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), anti-thyroperoxidase (AbTPO) and anti-thyroglobulin (AbTg) autoantibodies dosage] in this control group excluded the presence of thyroid disorders.

Control II was constituted by 1069 patients with autoimmune thyroiditis (AT) extracted from the same random sample of the general population. The diagnosis of AT [14,29] was established from the clinical presentation (presence of a firm goiter, varying in size from small to very large, with a lobulated surface), thyroid hormones and thyroid autoantibodies measurements, and/or thyroid ultrasonography (decreased, dyshomogeneous echogenicity) [28].

The majority of these patients had a normal thyroid volume, some showed goiter (9%) or atrophic thyroiditis (10%). A minority of patients (7%) were submitted to fine-needle aspiration to exclude the presence of thyroid cancer or lymphoma; in these cases, cytology confirmed the presence of a lymphocytic infiltration [14,28].

Control III comprised 1069 patients with non-toxic multinodular goiter (MNG) extracted from the same random sample of the general population. The majority of these patients had a normal thyroid volume, some showed goiter (35%). All MNG patients were submitted to thyroid ultrasonography and fine-needle aspiration, if necessary; cytology confirmed the absence of a malignancy.

In all patients and controls, a blood sample was collected in the morning, after overnight fasting, and serum was kept frozen until thyroid hormones, and thyroid autoantibodies measurements.

All study subjects gave their informed consent to the study, which was approved by the local Ethical Committee of the University of Pisa.

Thyroid blood flow was evaluated using color-flow doppler in all patients, and controls [14,28].

2.3. Laboratory tests

Thyroidal function and autoantibodies were evaluated as earlier described [29]. Serum FT3 and FT4 were evaluated by radio-immuno assay (RIA) (AMERLEX-MAB FT3/FT4 Kit; Amersham, UK), while TSH (DiaSorin, USA), AbTPO and AbTg (ICN Pharmaceuticals, USA) by IRMA. Positivity for AbTg or AbTPO, was defined as > 50, and >10 IU/mL, respectively. TRAb (anti-TSH-receptor

autoantibodies) were measured in patients with the use of a radioreceptor assay (Radim, Italy) (normal range 0-1 UI/ml).

2.4. Evaluation of other autoimmune diseases

During the study enrollment a specialist physician interviewed the patients, and a clearly defined questionnaire was completed by controls and patients in order to evaluate the presence of other frequent autoimmune disorders. The diagnosis of other autoimmune diseases was done recalling patients, cross-checking with present records and medications by the recruiting physician. Moreover a specific specialist (i.e. rheumatologist, dermatologist, gastroenterologist or internal medicine physician) verified the diagnoses and confirmed or not it according to the criteria reported by the scientific societies [30].

A patient was defined as "positive", only in the presence of a clear evidence of coexisting autoimmune diseases.

2.5. Data analysis

For normally distributed variables, values are expressed as mean ± SD. For normally distributed variables one-way analysis of variance (ANOVA) or the Mann-Whitney U test was used to compare mean group values. $\chi 2$ test was used to compare proportions and Bonferroni-Dunn test for post-hoc comparisons on normally distributed variables.

3. Results

Table 1 reports thyroid evaluation in GD, AT, and MNG patients vs. controls. The four groups were not different, in gender or age distribution because of the matching procedure. Thyroid volume was significantly larger in GD, and MNG patients. In GD, and AT, a hypoechoeic (58%, and 64%, respectively) and hypervascular (64%, and 29%, respectively) thyroid was present. All GD patients were hyperthyroid, while 24% of AT patients were hypothyroid; no thyroid dysfunction were present in MNG patients and controls. In GD, and AT, AbTPO (65%, and 84%, respectively), or

AbTg (54%, and 75%, respectively) autoantibodies were present. TRAb were present only in GD patients (87%).

In GD, or AT patients a significantly higher prevalence (**Table 2**) of autoimmune diseases (vs. controls or MNG patients) was observed for the following: T1D; celiac disease; chronic autoimmune gastritis (CAG); vitiligo (Vit); RA; SSc; SS; SLE; sarcoidosis; hepatitis C virus (HCV)-related mixed cryoglobulinemia (MC); polymyalgia rheumatica (Polym); multiple scleosis. For psoriatic arthritis and alopecia, a statistical evaluation closed to the significance was reported, while no significance was observed for the other diseases. On the whole the prevalence of another autoimmune disease was significantly higher in GD, or AT patients, with respect to MNG, or controls (16.7%, 18.5%, 3.5%, and 3.3%; respectively). The most commonly reported autoimmune diseases in GD patients were: Vit (2.6%), CAG (2.4%), RA (1.9%), Polym (1.3%), celiac disease (1.1%), T1D (0.9%), SS (0.8%), multiple sclerosis (0.3%), SLE and sarcoidosis (<0.1%). The pattern of the associated autoimmune disorders in GD was not significantly different from that observed in AT patients.

The association of three autoimmune disorders was reported in 48 (1.5%) GD patients, in 15 (1.4%) AT patients, in only 1 control subject, and in none MNG patient (**Table 3**). The most common associations in GD were GD+CAG+Polym and GD+CAG+Vit. The pattern of the three associated autoimmune diseases in GD was not significantly different from that observed in AT patients.

The presence of GO was observed in 984 (30.6%) of GD patients; while 2225 (69.4%) had no sign of GO. The pattern of the associated autoimmune disorders in GD, with or without GO, was not significantly different (**Table 4**). However, on the whole the prevalence of another autoimmune disorder was signficantly higher in GO (18.9%), than in GD patients without GO (15.6%).

4. Discussion

This is, as far as we know, the largest prospective study that have evaluated the association of other autoimmune diseases in patients with GD. On the whole 16.7% of GD patients had another

associated autoimmune disease; and the most frequently observed were: Vit (2.6%), CAG (2.4%), RA (1.9%), Polym (1.3%), celiac disease (1.1%), T1D (0.9%), SS (0.8%), multiple sclerosis (0.3%), SLE and sarcoidosis (<0.1%). Moreover, 1.5% patients with GD had three associated autoimmune disorders. This study first shows that GO patients had another autoimmmune disorder more frequently (18.9%), with respect to GD patients without GO (15.6%). However the pattern of the associated autoimmune disorders in GD was not significantly different from that observed in AT patients.

These results extend and complete the findings of other studies. For example the results are only in part in agreement with those reported recently in a cross-sectional retrospective multicenter study of 2791 Caucasian subjects with GD in UK. All subjects completed a structured questionnaire seeking a personal history of common autoimmune disorder. In GD the frequency of another autoimmune disorder was 9.67%. RA was the most frequent coexisting autoimmune disorder (in 3.15% of GD). In GD relative risks of other autoimmune diseases were significantly increased (for pernicious anemia, SLE, Addison's disease, celiac disease, and Vit) [18]. However, for each autoimmune disease the relative risk was calculated dividing the observed prevalence by the best estimate of UK population prevalence according to the present literature, but an appropriate internal control group was lacking.

Our paper has some important strength points in comparison to the above reported paper: 1) it is a prospective study; 2) the definition of GD, AT, MNG, and healthy subjects has been performed by a complete thyroid screening; 3) a specialist (i.e. rheumatologist, dermatologist, gastroenterologist or internal medicine physician) verified the presence of other autoimmune diseases and confirmed or not it, according to criteria reported by the scientific societies (not on the basis of a questionnaire); 4) our study reports three age- and gender-matched control groups (in fact, age and gender, are a determinant risk factor for thyroid autoimmunity), excluding the influence of these parameters on the last results; 5) the three controls were extracted from a sample of the general population, with similar iodine intake; 6) a control group of healthy subjects (without thyroid autoimmune disorders)

has permitted to emphasize the differences with GD patients; 7) a control group of MNG patients without thyroid autoimmunity has permitted to rule out the possible effect of non-autoimmune thyroid pathologies on the results; 8) a control group of AT patients has permitted to compare the coexistence of autoimmune diseases, with respect to GD; 9) GD patients were evaluted for the presence/absence of GO, making possible the comparions of the associated autoimmune diseases in GO, vs. GD patients without GO; 10) a larger number of patients and controls has been evaluated; 11) a larger number of autoimmune diseases have been evaluated.

A significant high prevalence of autoimmune disorders was reported in GD patients for: T1D; CAG; celiac disease; Vit; Polym; RA; SSc; SS; SLE; sarcoidosis; MC; multiple scleosis. For psoriatic arthritis and alopecia, a statistical evaluation closed to the significance was reported, while no significance was observed for the other diseases. A larger series of patients is necessary to confirm or not the associations for some of the other autoimmune diseases, as the statistical significance is difficult to be reached, owing to the low prevalence of these disorders (i.e. dermatomyositis, or primary biliary cirrhosis, etc).

Of note, the association of 3 autoimmune diseases was reported nearly only in GD and AT patients, and the most common associations in GD were GD+CAG+Vit and GD+CAG+Polym. The pattern of the three associated autoimmune diseases in GD was not significantly different from that observed in AT patients.

The exact pathogenetic mechanisms underlying the above reported associations are not known. The influence of genetic suceptibility on the association of distinct autoimmune disorders has been reported, as: a) significant clustering of AITD is shown within families (as about 40-50% of AT patients report another family member with AT) [31]; b) evidence comes from twin studies for GD [32] and AT [33] with concordance rates of 30–40% in monozygotic twins and 0–7% in dizygotic twins. Moreover, new recent insights in genome-wide association studies (GWAS) about

autoimmune and immune-mediated diseases have increased the knowledge of the pathogenesis underlying these disorders [20], suggesting a common genetic susceptibility [20,34].

However, also environmental factors are determinant for the appearance of AITD in susceptible subjects [35,36]. Increased iodine intake, selenium and vitamin D deficiency, exposure to radiation (from nuclear fallout, or due to medical radiation) are environmental factors increasing AITD prevalence [37].

Cigarette smoking is associated with GD and GO, while it decreases the risk of hypothyroidism and thyroid autoimmunity. Viral infections are important environmental factors in the pathogenesis of AITD, too, particularly human parvovirus B19 (EVB19) and HCV. Regarding the various existing chemical contaminants, pesticides and halogenated organochlorines differently disrupt thyroid function. Polychlorinated biphenyls and their metabolites and polybrominated diethyl ethers bind to thyroid transport proteins (i.e. transthyretin), displace thyroxine, and disrupt thyroid function. Considering drugs, interferon- and iodine-containing drugs have been associated with AITD [35].

A prevalent Th1 immune pattern has been shown in patients with AT, GD, GO [38-40], T1D, SLE, SSc, RA, MC, and others, in the initial phase of these disorders. Furthermore, in GD, GO, SLE, MC (and others) a Th1 prevalence has been shown in the active phase, that switches to a Th2 profile in the inactive phase [28, 29, 38, 41-43] of the disease. So it has been hypothesized that the influence of genetic and environmental factors could determinate autoimmune phenomena in different organs in the same subject [1], characterized by predominance of a Th1 immune pattern at the initial, or active, phase of these disorders.

In conclusion, it has been demonstrated a significantly more elevated risk of other autoimmune diseases in GD patients. Since about 17% of GD patients can have another associated autoimmune disease, GD patients who are still sick, or who develop new unspecific symptoms (even if during an appropriate treatment of hyperthyroidism) should be appropriately screened for other autoimmune

disorders (not to delay the diagnosis), and treatment of these ones, overall when GO is present.

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1) Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmun Rev 2015;14:174–80.
2) Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clinical Endocrinol (Oxford) 1995;43:55–68.
3) McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity Endocrine 2012;2:252–65.
4) Pedersen IB, Laurberg P, Knudsen N, Jørgensen T, Perrild H, Ovesen L, et al. An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. J Clin Endocrinol Metab 2007;92:3122–7.
5) Rizzo M, Rossi RT, Bonaffini O, Scisca C, Altavilla G, Calbo L, et al. Increased annual frequency of Hashimoto's thyroiditis between years 1988 and 2007 at a cytological unit of Sicily. Ann Endocrinol (Paris) 2010;71:525–34.
6) Benvenga S1, Trimarchi F, Robbins J. Circulating thyroid hormone autoantibodies. J Endocrinol Invest 1987;10:605–19.
7) Latina A, Gullo D, Trimarchi F, Benvenga S. Hashimoto's thyroiditis: similar and dissimilar characteristics in neighboring areas. Possible implications for the epidemiology of thyroid cancer PloS One 2013;8:e55450.
8) Rizzo M, Sindoni A, Talamo Rossi R, Bonaffini O, Panetta S, Scisca C, et al. Annual increase in the frequency of papillary thyroid carcinoma as diagnosed by fine-needle aspiration at a cytology unit in Sicily. Hormones (Athens) 2013;12:46–57.
9) Dittmar M, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. J Clin Endocrinol Metab 2003;88:2983–92.

10) Antonelli A, Tuomi T, Nannipieri M, Fallahi P, Nesti C, Okamoto H, et al. Autoimmunity to CD38 and GAD in Type I and Type II diabetes: CD38 and HLA genotypes and clinical phenotypes. Diabetologia 2002;45:1298–306.

11) Trimarchi F, De Luca F, Vanelli M, Benvenga S, Siracusano MF, Volta C, et al. Circulating thyroid antibodies and thyroid function studies in children and adolescents with insulin-dependent diabetes mellitus. Eur J Pediatr 1984;142:253–6.

12) Pupilli C, Giannini S, Marchetti P, Lupi R, Antonelli A, Malavasi F, et al. Autoantibodies to CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) in Caucasian patients with diabetes: effects on insulin release from human islets. Diabetes 1999;48:2309-15.

13) Fallahi P, Ferrari SM, Ruffilli I, Elia G, Biricotti M, Vita R, et al. The association of other autoimmune diseases in patients with autoimmune thyroiditis: Review of the literature and report of a large series of patients. Autoimmun Rev 2016;15:1125–8.

14) Antonelli A, Fallahi P, Mosca M, Ferrari SM, Ruffilli I, Corti A, et al. Prevalence of thyroid dysfunctions in systemic lupus erythematosus. Metabolism 2010;59:896–900.

15) Antonelli A, Mosca M, Fallahi P, Neri R, Ferrari SM, D'Ascanio, et al. Thyroid cancer in systemic lupus erythematosus: a case-control study. J Clin Endocrinol Metab 2010;95:314–8.

16) Han TY, Lee JH, Noh TK, Choi MW, Yun JS, Lee KH, et al. Alopecia areata and overt thyroid diseases: A nationwide population-based study. J Dermatol 2018;45:1411-1417.

17) Nisihara R, Pigosso YG, Prado N, Utiyama SRR, De Carvalho GA, Skare TL. Rheumatic Disease Autoantibodies in Patients with Autoimmune Thyroid Diseases. Med Princ Pract 2018;27:332-336.

18) Boelaert K, Newby PR, Simmonds MJ, Holder RL, Carr-Smith JD, Heward JM, et al. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. Am J Med. 2010;123:183.e1–9.

19) Miyadera H, Tokunaga K. Associations of human leukocyte antigens with autoimmune diseases: challenges in identifying the mechanism. J Hum Genet 2015;60:697–702.

20) Cho JH, Feldman M. Heterogeneity of autoimmune diseases: pathophysiologic insights from genetics and implications for new therapies. Nat Med 2015;21:730–8.

21) Robazzi TC, Adan LF. Autoimmune thyroid disease in patients with rheumatic diseases. Rev Bras Reumatol 2012;52:417–30.

22) Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. Autoimmun Rev 2003;2:119–25.

23) Jenkins RC, Weetman AP. Disease associations with autoimmune thyroid disease. Thyroid 2002;12:977–88.

24) Antonelli A, Fallahi P, Nesti C, Pupilli C, Marchetti P, Takasawa S, et al. Anti-CD38 autoimmunity in patients with chronic autoimmune thyroiditis or Graves' disease. Clin Exp Immunol. 2001;126:426–31.

25) Miccoli P, Antonelli A, Spinelli C, Ferdeghini M, Fallahi P, Baschieri L. Completion total thyroidectomy in children with thyroid cancer secondary to the Chernobyl accident. Arch Surg 1998;133:89–93.

26) Baschieri L, Antonelli A, Nardi S, Alberti B, Lepri A, Canapicchi R, et al. Intravenous immunoglobulin versus corticosteroid in treatment of Graves' ophthalmopathy. Thyroid 1997;7:579–85.

27) Mourits MP, Koornneef L, Wiersinga WM, Prummel MF, Berghout A, van der Gaag R. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. Brit J Ophthalmol 1989;73:639–644.

28) Antonelli A, Fallahi P, Delle Sedie A, Ferrari SM, Maccheroni M, Bombardieri S, et al. High values of Th1 (CXCL10) and Th2 (CCL2) chemokines in patients with psoriatic arthtritis. Clin Exp Rheumatol 2009;27:22–7.

29) Antonelli A, Ferri C, Fallahi P, Ferrari SM, Frascerra S, Sebastiani M, et al. High values of CXCL10 serum levels in patients with hepatitis C associated mixed cryoglobulinemia in presence or absence of autoimmune thyroiditis. Cytokine 2008;42:137–43.

30) Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J Autoimmun 2009;33:197–207.

31) Brix TH, Christensen K, Holm NV, Harvald B, Hegedus L. A population-based study of Graves' disease in Danish twins. Clin Endocrinol (Oxf) 1998;48:397–400.

32) Ringold DA, Nicoloff JT, Kesler M, Davis H, Hamilton A, Mack T. Further evidence for a strong genetic influence on the development of autoimmune thyroid disease: the California twin study. Thyroid 2002;12:647–53.

33) Brix TH, Kyvik KO, Hegedus L. A population-based study of chronic autoimmune hypothyroidism in Danish twins. J Clin Endocrinol Metab 2000;85:536–9.

34) Su H, Rei N, Zhang L, Cheng J. Meta-analyses of IL1A polymorphisms and the risk of several autoimmune diseases published in databases. PLoS One 2018;13:e0198693.

35) Ferrari SM, Fallahi P, Antonelli A, Benvenga S. Environmental Issues in Thyroid Diseases. Front Endocrinol (Lausanne) 2017;8:50.

36) Perricone C, Versini M, Ben-Ami D, Gertel S, Watad A, Segel MJ, Ceccarelli F, Conti F, Cantarini L, Bogdanos DP, Antonelli A, Amital H, Valesini G, Shoenfeld Y. Smoke and autoimmunity: The fire behind the disease. Autoimmun Rev. 2016;15:354-74.

37) Martino E, Macchia E, Aghini-Lombardi F, Antonelli A, Lenziardi M, Concetti R, et al. Is humoral thyroid autoimmunity relevant in amiodarone iodine-induced thyrotoxicosis (AIIT)? Clin Endocrinol (Oxf) 1986;24:627–33.

38) Antonelli A, Ferrari SM, Frascerra S, Pupilli C, Mancusi C, Metelli MR, et al. CXCL9 and CXCL11 chemokines modulation by peroxisome proliferator-activated receptoralpha

agonists secretion in Graves' and normal thyrocytes. J Clin Endocrinol Metab 2010;95:413–20.

39) Antonelli A, Ferrari SM, Corrado A, Ferrannini E, Fallahi P. CXCR3, CXCL10 and type 1 diabetes. Cytokine Growth Factor Rev 2014;25:57–65.

40) Zhao J, Lin B, Deng H, Zhi X, Li Y, Liu Y, Bible PW, Li Q, Xu B, Wei L, Yang H, Huang D. Decreased Expression of TIM-3 on Th17 Cells Associated with Ophthalmopathy in Patients with Graves' Disease. Curr Mol Med. 2018;18:83-90.

41) Zignego AL. Enhanced TH1 cytokine production in hepatitis C virus-infected patients with mixed cryoglobulinemia: understanding the pathological issues. J Hepatol 2004;41:1045–9.

42) Antonelli A, Ferrari SM, Giuggioli D, Ferrannini E, Ferri C, Fallahi P. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. Autoimmun Rev 2014;13(3):272–80.

43) Fallahi P, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. Clin Dev Immunol. 2012;2012:468107.

Table 1. Thyroid status of control subjects, and patients with autoimmune thyroiditis, or euthyroid multinodular goiter, or Graves' disease.

	controls I	*thyroiditis*	*multinodular goiter*	*Graves' disease*	*P*
n	1069	1069	1069	3209	
Age (years)	44 ± 16	45 ± 17	46 ± 9	44 ± 15	ns
Gender (M/F)%	18	18	18	18	ns
Thyroid volume (ml)	10 ± 6	13 ± 10	19 ± 18*	21±16*	0.0001
Hypoechoic (%)	0	64	0	58	0.0001
Hypervascular (%)	0	29	0	64	0.0001
Serum TSH (mcU/ml)	1.5 ± 0.9	2.8± 3.0	1.2 ± 0.8	0.1 ± 0.2§	0.001
TRAb positivity (%)	0	0	0	87§	0.0001
AbTPO positivity (%)	0	84	0	65	0.0001
AbTg positivity (%)	0	75	0	54	0.0001

Antithyroperoxidase antibody = AbTPO
Antithyroglobulin antibody = AbTg
Thyroid-stimulating hormone = TSH
Antithyrotropin-receptor antibody=TRAb
* p <0.05 or less vs. controls or vs. autoimmune thyroiditis
§ p<0.05 or less vs. controls, vs. autoimumune thyroiditis and vs. multinodular goiters

Table 2. Distribution of autoimmune diseases in GD patients, controls, AT, and MNG.

	Controls **n = 1069**	**AT** **n = 1069**	**MNG** **n = 1069**	***GD*** **n = 3209**	***P*** $\chi 2$
Diabetes (type 1)	1	10	0	28	0.0008
Addison disease	0	2	0	4	0.3427
Chronic autoimmune gastritis	5	27	10	78	<0.0001
Celiac disease	3	14	4	34	0.0105
Crohn disease	0	3	2	10	0.3157
Ulcerative colitis	2	6	1	13	0.2070
Vitiligo	6	29	3	83	< 0.0001
Alopecia	0	4	0	9	0.0765
Psoriasis	3	5	3	16	0.6772
Psoriatic arthritis	1	6	0	11	0.0503
Myasthenia gravis	0	1	0	4	0.4574
Polymyalgia rheumatica	5	14	6	41	0.0388
Polymyositisy/dermatomyositis	0	2	0	7	0.2071
Primary biliary cirrhosis	0	1	0	2	0.6442
Chronic autoimmune hepatitis	0	1	0	2	0.6442
Rheumatoid arthritis	5	26	4	64	< 0.0001
Systemic sclerosis (scleroderma)	0	6	0	15	0.0132
Sjogren disease	1	10	0	27	0.0009
Systemic lupus erythematosus	0	8	0	21	0.0021
Sarcoidosis	1	7	0	17	0.0173
HCV-related cryoglobulinemia	0	6	0	14	0.0153
Glomerulonephritis Primary IgA	0	0	0	1	0.8014
Multiple sclerosis	2	7	2	26	0.0409
Uveitis (iridocyclitis)	1	3	3	10	0.6885
Total	36 (3.3%)	198 (18.5%)	38 (3.5%)	536 (16.7%)	<0.0001

P>0.05 AT, vs. GD, for all comparisons

Table 3. Patients with 3 associated autoimmune disorders in GD patients, controls, AT, and MNG.

	Controls n = 1069	**AT n = 1069**	**MNG n = 1069**	**GD n = 3209**	***P*** **$\chi 2$**
AT+CAG+Vit	1	4	0	13	ns
AT+CAG+Polym	0	2	0	7	ns
AT+CAG+Alo	0	1	0	3	ns
AT+CAG+T1D	0	0	0	2	ns
AT+CAG+SLE	0	1	0	2	ns
AT+CAG+Sarc	0	1	0	1	ns
AT+CAG+SSc	0	0	0	1	ns
AT+CAG+Sjog	0	1	0	4	ns
AT+Vit+Sjog	0	1	0	3	ns
AT+Vit+Polym	0	1	0	4	ns
AT+T1D+CelDis	0	1	0	3	ns
AT+RA+Vit	0	1	0	2	ns
AT+Sarc+Vit	0	0	0	1	ns
AT+Crohn+Vit	0	0	0	1	ns
AT+Sarc+Vit	0	1	0	1	ns
Total	1	15	0	48	< 0.0001

Alopecia (Alo); autoimmune thyroiditis (AT); celiac disease (CelDis); chronic autoimmune gastritis (CAG); Crohn disease (Crohn); polymyalgia rheumatica (Polym); rheumatoid arthritis (RA); sarcoidosis (Sarc); sjögren's syndrome (Sjog); systemic lupus erythematosus (SLE); systemic sclerosis (SSc); type 1 diabetes (T1D); ulcerative colitis (UlcCol); vitiligo (Vit).

Table 4. Distribution of autoimmune diseases in GD patients, with or without GO.

	No GO n = 2225	**With GO n = 984 (30.6%)**	**GD n = 3209**	***P*** **$\chi 2$**
Diabetes (type 1)	18	10	28	ns
Addison disease	3	1	4	ns
Chronic autoimmune gastritis	51	27	78	ns
Celiac disease	21	13	34	ns
Crohn disease	6	4	10	ns
Ulcerative colitis	9	4	13	ns
Vitiligo	50	33	83	ns
Alopecia	5	4	9	ns
Psoriasis	11	5	16	ns
Psoriatic arthritis	8	3	11	ns
Myasthenia gravis	3	1	4	ns
Polymyalgia rheumatica	28	13	41	ns
Polymyositisy/dermatomyositis	5	2	7	ns
Primary biliary cirrhosis	2	0	2	ns
Chronic autoimmune hepatitis	1	1	2	ns
Rheumatoid arthritis	40	24	64	ns
Systemic sclerosis (scleroderma)	10	5	15	ns
Sjogren disease	17	10	27	ns
Systemic lupus erythematosus	15	6	21	ns
Sarcoidosis	13	4	17	ns
HCV-related cryoglobulinemia	10	4	14	ns
Glomerulonephritis Primary IgA	1	0	1	ns
Multiple sclerosis	17	7	25	ns
Uveitis (iridocyclitis)	5	5	10	ns
Total	349 (15.6%)	187 (18.9%)	536 (16.7%)	0.0201

Highlights

- About 17% of GD patients had another associated autoimmune disease
- The association of 3 autoimmune diseases is present in 1.5 % of GD patients
- GO patients had another autoimmmune disorder more frequently vs. GD without GO
- The pattern of associated autoimmune disorders in GD is not different than in AT
- GD developing new unspecific symptoms should be screened for autoimmune disorders