Autoimmune comorbidities in Hashimoto's thyroiditis: different patterns of association in adulthood and childhood/adolescence

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Abstract

Objective: Hashimoto's thyroiditis (HT), the most common autoimmune thyroid disease at any age, is often associated with other autoimmune diseases. The present study was aimed to describe the type and frequency of non-thyroidal autoimmune diseases (NTADs) in HT patients and to delineate the clinical pattern of diseases clustering in pediatric/adolescent and adult age.

Design: Cross-sectional study.

Methods: 1053 newly diagnosed HT patients (500 adults (467 F, mean age 40.2 ± 13.7 years) and 553 children/adolescents (449 F, mean age 11.1 ± 3.0 years)) were evaluated for common NTADs by means of careful recording of medical history, physical examination and assessment of selected autoantibody profiles.

Results: The prevalence of associated NTADs was significantly higher in adults than that in pediatric/adolescent HT patients ($P < .0001$). In addition, the number of adult patients suffering from two or more associated NTADs was significantly higher than that of children/adolescent ($P < 0.0001$). A female prevalence was evident in both cohorts, but was significant in the adults ($P < 0.0001$). The epidemiological distribution of NTADs was strongly different in the two cohorts, the most frequent associated diseases being arthropathies and connective tissue diseases in adults and type 1 diabetes and coeliac disease in children/adolescents. Skin diseases were represented with similar prevalence in both cohorts, vitiligo being the most common.

Conclusions: Age at HT presentation may influence autoimmune diseases clustering, favoring the association of specific NTADs in different ages of life. Moreover, the association between HT and NTADs increases with age and occurs most frequently in adults.

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Introduction

Hashimoto's thyroiditis (HT) is worldwide the most prevalent autoimmune thyroid disease at any age, and its incidence has been increased in the last decades (1, 2, 3). It represents a prototypical organ-specific autoimmune disorder, in which environmental and existential factors trigger the development of the immune response against thyroid antigens in genetically susceptible individuals (4, 5, 6, 7). The complex interaction between the genetic background

and existential/environmental factors also influences the outcome of the disease, that generally proceeds from an underlying autoimmune diathesis, featured by anti-thyroid autoantibodies production, toward subclinical and then overt hypothyroidism, although its presentation can be even with transient thyrotoxicosis (1, 3, 8, 9).

HT is known to cluster with other autoimmune disorders, and the concept of an autoimmune diathesis

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is widely accepted (4, 5, 7, 10, 11). Previous studies have reported an increased prevalence and relative risk of other autoimmune diseases in subjects with HT and their relatives, mainly highlighting the importance of a shared genetic predisposition (11, 12, 13). A wide spectrum of autoimmune disorders ranging from either endocrine or non-endocrine organ-specific diseases to systemic diseases has been variously described in association with HT (11, 12, 13, 14, 15, 16, 17, 18, 19, 20). Such an association has been firmly established for some disorders (namely pernicious anemia), to the extent that screening recommendations can be made (11), whereas it has been reported in variable percentages for others, including various rheumatological and cutaneous conditions (12, 13, 14, 15, 16, 17, 18, 19, 20). However, most studies evaluating such associations have examined the prevalence of HT in non-thyroidal autoimmune diseases rather than the reverse (10, 14, 15, 16, 17, 18, 19, 20). Also, this estimate indicates that beside the shared genetic predisposition, environmental and existential factors could be critical in determining the diversity of autoimmune manifestations in predisposed individuals (11).

The present study was aimed to investigate the clustering of non-thyroidal autoimmune diseases (NTADs) in two cohorts of patients, children/adolescents and adults, who were all affected by HT. The aim of our study was to ascertain, for the first time, if the age at HT presentation might *per se* influence the aggregation of NTADs in patients with the same autoimmune disorder (namely HT) by conditioning a different clustering in pediatric and adult age.

Subjects and methods

Patients

In this cross-sectional study, we enrolled 1053 newly diagnosed HT patients (500 adults aged 19–80 years and 553 children/adolescents aged 3–18 years) who consecutively referred to the outpatient clinic of the Endocrinology and Paediatrics' Division respectively, of our University Hospital from September 2012 to September 2015. Subjects affected by any syndromic disease (i.e. Trisomy 21, Turner syndrome, Klinefelter syndrome, autoimmune polyglandular syndromes type 1 and type 2) were excluded, as well as were related subjects, to avoid selection bias.

HT was diagnosed according to the currently accepted laboratory and ultrasonographic criteria

(serum anti-thyroid antibodies positivity and heterogeneous echo-structure with diffuse or patchy hypoechogenicity at US) (1). Concerning the functional status, individuals with a TSH >5 μ IU/mL at the time of diagnosis were defined hypothyroid and further subdivided into subclinical and overt hypothyroid according to the TSH value being below or above 10 μ IU/mL respectively, whereas thyrotoxicosis was defined as undetectable (<0.01 μ IU/mL) or low (<0.4 μ IU/mL) TSH with elevated free thyroid hormones (21).

All HT patients included in the study were evaluated for non-thyroidal autoimmune diseases (NTADs) by means of careful recording of medical history, physical examination and assessment of selected autoantibody profiles. A number of patients have already been diagnosed for the NTAD prior to HT, but both diseases were diagnosed in the same period of life (adulthood or childhood/adolescence). Patients in whom HT was discovered in adulthood, whereas T1DM or CD, for instance, had been diagnosed in childhood were excluded. All the other subjects were screened for the following non-organ-specific autoantibodies, commonly utilized in rheumatologic practice: antinuclear (ANA), double-stranded (native) DNA (nDNA) and extractable nuclear antigens (ENA) antibodies. Additional specific autoantibodies (i.e. anti-centromere, anti-mitochondrial (AMA), liver–kidney microsomal (LKM), antibodies) were searched when appropriate. Rheumatic disorders, such as arthropathies and connective tissue diseases, were diagnosed on the basis of both clinical and laboratory evidence, according to the currently accepted criteria (22, 23, 24). Also, we assayed sera of each cohort for the following organ-specific autoantibodies: anti-gastric mucosa (anti-PCA), anti-adrenal cortex (ACA) and anti-21-hydroxylase (21-OHAb), anti-endomysium (EMA) and anti-tissue transglutaminase IgA (aTTG). Diagnosis of coeliac disease (CD) in patients who tested positive for the specific autoantibodies was confirmed by the small intestine biopsy (25). Liver biopsy was critical for the diagnosis of autoimmune hepatitis, as it was mucosal biopsy during colonoscopy for ulcerative colitis (26). Type 1 diabetes (T1DM) screening included determination of anti-glutamic acid decarboxylase (GADA), anti-insulin (IAA) and anti-islet cell (ICA) autoantibodies, as well as glycosylated hemoglobin. Oral glucose tolerance test was performed in patients with autoantibody-positive values (27). Diagnosis of dermatological diseases was based only on clinical features (28, 29, 30).

The study design was approved by the Ethics Committee of our hospital, and all the adult patients and the children's parents gave informed written consent to participate.

Methods

Serum TSH and free thyroxine (FT4) concentrations, as well as anti-thyroglobulin (TgAb) and anti-thyroid peroxidase antibodies (TPOAb), were measured by electrochemiluminescence immunoassay (ECLIA), using commercial kits for Elecsys 1010/2010 e modular analytics E170 supplied by Roche Diagnostics. Anti-TSH receptor antibodies (TRAbs) were measured by radioimmunoassay (Brahms Trak Human RIA, supplied by Dasit SpA). Normal values in our laboratory were TSH, 0.27–4.2 µIU/L; FT4, 12.0–22.0 pmol/L; TgAb, 0–115 IU/mL; TPOAb, 0–34 IU/mL; TRAb, <1 IU/L. However, it should be considered that the pediatric reference intervals for TSH are wider than those in the adult population with an upper limit up to 6.26 U/L (3). The aforementioned non-organ- and organ-specific autoantibodies were routinely measured by radioimmunoassay or by immunofluorescence or fluoro-enzyme immunoassay with commercial kits, according to the manufacturer's instructions. All samples were processed centrally in the laboratory of our University Hospital of Messina. For all assays, the intra- and the inter-assay CVs were <5% and <10% respectively.

Thyroid ultrasonography (US) was performed in all patients at the first evaluation using a real-time 2D apparatus (General Electric Healthcare, UK), with a 7.5–10 MHz linear transducer at the Radiology Unit of our University Hospital of Messina.

Statistical analysis

Numerical data are expressed as mean and standard deviation (s.d.), and categorical variables as number and percentage.

To assess the association between cohorts (adults or children/adolescents) and other categorical variables, such as sex, type of comorbidity and functional status (euthyroidism, subclinical and overt hypothyroidism and thyrotoxicosis), we estimated chi-square test and the relative significance. The same test was applied to compare patients with and without associated NTADs for all the examined categorical variables, for adults and children/adolescents, separately and, also, to compare the global prevalence of comorbidities in both cohorts.

For NTADs affecting at least 6 patients, we could calculate median age at diagnosis and relative interquartile range. We compared age among the 12 selected NTADs using Kruskal–Wallis test; as it resulted to be highly significant, we performed pairwise comparisons between the NTADs for age using Mann–Whitney test. For these multiple comparisons, we had to apply Bonferroni's correction, for which the significance alpha level .050 has to be divided by the number of

Table 1 General characteristics of the study population. Data are expressed as mean $\pm$ s.d., median in *italics*. Normal values are specified under 'Materials and methods' section.

Parameters	Adults (n=500)	Children/adolescents (n=553)	P value
Age (year)			
Mean $\pm$ s.d. (range)	40.22 $\pm$ 13.72 (19–80)	11.11 $\pm$ 2.96 (3–18)	–
Sex			
Female	467 (93.4%)	449 (81.2%)	<0.0001
Male	33 (6.6%)	104 (18.8%)	
F:M ratio	14.5:1	4.3:1	
Functional status (n (%))			
Euthyroidism	285 (57%)	300 (54.3%)	0.413
Hypothyroidism	215 (43%)	217 (39.2%)	0.234
Subclinical	146 (29.2%)	95 (17.20%)	<0.0001
Overt	69 (13.8%)	122 (22%)	
Hyperthyroidism	0	36 (6.5%)	–
TSH (µIU/L)	5.75 $\pm$ 13.03, 2.48	13.02 $\pm$ 29.07, 4.0	<0.0001
FT4 (pmol/L)	13.87 $\pm$ 3.55, 14.0	13.85 $\pm$ 6.53, 13.3	0.952
Tg-Ab (IU/L)	899.95 $\pm$ 3448.25, 165.0	826.86 $\pm$ 2041.40, 237.0	0.673
TPO-Ab (IU/L)	7444.85 $\pm$ 92176.49, 216.50	1237.71 $\pm$ 2622.60, 554.0	0.114

P-Values typed in bold are significant ($P \leq 0.05$).

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the possible seventy-eight pairwise comparisons, so the new 'adjusted' significance level for this analysis is equal to $.050/78 = .0006$.

Moreover, to evaluate the risk of each NTAD for adult population, odds ratio and relative 95% confidence interval was calculated.

Statistical analyses were performed using SPSS 17.0 for Window package. $P < 0.05$ two sides was considered to be statistically significant.

Results

General characteristics of adult and pediatric study population

We compared the data from 500 adult patients (467 F, 33 M, mean age at presentation 40.2 ± 13.7 years) with those from 553 children/adolescents (449 F, 104 M, mean age at presentation 11.1 ± 2.9 years). The analytic results of both groups are presented in Table 1.

The female sex was more represented in both groups, but such a female prevalence was significantly more evident in the adults ($P < 0.0001$).

Concerning thyroid functional status, more than half of the patients were euthyroid at the first evaluation, and about forty percent were hypothyroid, without differences between the two cohorts (Table 1). However, mean serum TSH was significantly higher in children/adolescents than that in adults because the rate of overt hypothyroidism largely exceeded the rate of subclinical hypothyroidism in the former ($P < 0.0001$). Also, the wider serum TSH reference intervals for pediatric age should be considered.

In the pediatric cohort, there were 36 patients (6.5%) with transient thyrotoxicosis (Hashitoxicosis) at presentation. In such patients, serum TRAb remained undetectable during the overall follow-up period, whereas TPOAb serum levels were positive, and hyperthyroidism resolution occurred spontaneously within 3–18 months, with no relapses. No hyperthyroid individuals were found in the adult cohort (Table 1).

Table 2 Prevalence of associated non-thyroidal autoimmune diseases (NTADs) in adult and pediatric/adolescent HT patients.

Non-thyroidal autoimmune diseases	Adults	Children/adolescent	P value*
	147/500 (29.4%)[§]	104/553 (18.8%)[§]	<0.0001
Arthropathies	65 (13%)	1 (0.18%)	<0.0001
SpondyloArthritis (SpA)	40 (8%)	0	<0.0001
Psoriatic Arthritis	29 (5.8%)	0	
Other SpA	11 (2.2%)	0	
Rheumatoid arthritis (RA)	25 (5%)	1 (0.18%)	<0.0001
Connective tissue diseases	48 (9.6%)	1 (0.18%)	<0.0001
Sjogren's syndrome (SS)	28 (5.6%)	0	<0.0001
Systemic Lupus Erythematosus (SLE)	7 (1.4%)	0	0.062
Polymyositis	1 (0.2%)	0	
Systemic scleroderma (SSc)	1 (0.2%)	0	
Undifferentiated connective disease	10 (2%)	0	0.017
Vasculitis	1 (0.2%)	1 (0.18%)	0.636
Cutaneous diseases	29 (5.8%)	25 (4.5%)	0.507
Vitiligo	14 (2.8%)	15 (2.7%)	0.319
Chronic urticaria	5 (1%)	1 (0.18%)	0.408
Psoriasis	4 (0.8%)	2 (0.36%)	0.991
Erythema nodosum	3 (0.6%)	0	
Alopecia	1 (0.2%)	5 (0.9%)	0.091
Lichen	2 (0.4%)	0	
Atopic dermatitis	0	2 (0.36%)	
Type 1 diabetes mellitus	2 (0.4%)	38 (6.9%)	<0.0001
Addison's disease	5 (1%)	4 (0.7%)	0.487
Multiple sclerosis	3 (0.6%)	0	0.381
Gastro-enteric diseases	15 (3%)	40 (7.23%)	<0.0001
Celiac disease (CD)	9 (1.8%)	40 (7.23%)	<0.0001
Autoimmune hepatitis	4 (0.8%)	0	
Ulcerative colitis (UC)	2 (0.4%)	0	

*P values typed in bold are significant ($P \leq 0.05$). [§]Forty adults and 7 pediatric patients had two or more associated NTAD ($P < 0.0001$). NTAD preceded the diagnosis of HT in 70 adults (16 SpA, 14 RA, 7 SS, 4 SLE, 1 polymyositis, 5 undifferentiated connective disease, 10 vitiligo, 1 chronic urticaria, 1 psoriasis, 2 erythema nodosum, 1 multiple sclerosis, 5 CD, 1 UC) and in 75 children/adolescents (36 T1DM, 29 CD, 8 vitiligo, 2 alopecia); $P < 0.0001$ adults vs children/adolescents.

In summary, at HT presentation, both euthyroidism and hypothyroidism were equally frequent in the two cohorts, but the proportions of subclinical and overt hypothyroidism were significantly different, and thyrotoxicosis was definitely more frequent in the pediatric group.

Association with NTADs in adults and children

The whole spectrum of autoimmune disorders that were found in association with HT in our study population, and their frequencies, are reported in Table 2. As shown, the prevalence of patients with associated NTADs was significantly higher in adults than that in the pediatric cohort: 29.4 vs 18.8% ($P < 0.0001$). Moreover, the number of adult patients who suffered from two or more associated NTAD was significantly higher than that of children/adolescents (40 vs 7; $P < 0.0001$).

The epidemiological distribution of NTADs was strongly different in the two cohorts. In fact, in the adult patients the most frequent associated diseases were arthropathies, mostly psoriatic arthritis and rheumatoid arthritis (RA), and connective tissue diseases, mostly Sjögren syndrome (SS). In the pediatric/adolescent population, these disorders were absent or rarely found, and the most prevalent autoimmune comorbidities were represented by CD and T1DM (Table 2). Skin diseases were diagnosed in both cohorts with a similar prevalence,

vitiligo being the most common. Also, Addison's disease occurred in the two cohorts with a similar prevalence (Table 2).

Such a different clustering of autoimmune disorders in relation with age was even more evident when we stratified all HT patients by age (median and interquartile range) in relation to the type of associated comorbidity (Table 3). When comparing median age at diagnosis between cases with different autoimmune diseases, those with coexisting arthropathies were significantly older compared with patients with no coexisting autoimmune diseases ($P < 0.00001$), as well as compared to those suffering from T1DM, vitiligo, alopecia or CD ($P < 0.0001$). Similarly, subjects affected by connective diseases were older compared to cases without any associated autoimmune disease ($P < 0.001$) and to those with coexisting T1DM or CD ($P < 0.001$) (Table 3). Also, HT patients with comorbidities, as a whole, were older than those without any other autoimmune disease ($P < 0.0001$).

In addition to the differences in aggregation based on age, there was also a different pattern in regard to the presenting autoimmune disease. HT preceded the diagnosis of NTAD in 77 (52%) adults and 29 (28%) children/adolescents ($\chi^2 = 14.98$; $P < 0.0001$). Within the pediatric/adolescent group, T1DM and CD were usually diagnosed before HT ($P < 0.0001$), whereas HT usually preceded the diagnosis of connective tissue diseases in adults ($P = 0.0041$). In our adult cohort, arthropathies were

Table 3 Median age at HT presentation and interquartile range (years) in all patients (adults plus children/adolescents), in relationship with the most prevalent comorbidities.*

Coexisting non-thyroidal autoimmune diseases (NTADs)	Patients n	Median age at presentation	Interquartile range
Psoriatic arthritis	29	46	37–57
Rheumatoid arthritis (RA)	26	50.5	40.7–63
Sjogren syndrome (SS)	28	49	42.2–60.5
Systemic Lupus Erythematosus (LES)	7	32	20–34
Undifferentiated connective tissue disease	10	41.5	35–50
Vitiligo	29	12.9	9.2–38
Chronic urticaria	6	33	18.4–46
Coeliac disease (CD)	49	14.6	11.4–16.8
Type 1 diabetes mellitus (T1DM)	40	11.9	9–14
Psoriasis	6	15.5	9.4–21
Alopecia	6	12	10.2–14.8
None	802	14.6	10.8–36

The following statistically significant comparisons were recorded: Psoriasis arthritis vs vitiligo, CD, T1DM, psoriasis and alopecia ($P < 0.0001$); RA vs vitiligo, CD and T1DM ($P < 0.0001$), and alopecia ($P = 0.0004$); SS vs LES ($P = 0.0004$), vitiligo, CD and T1DM ($P < 0.0001$), psoriasis ($P = 0.002$), alopecia ($P = 0.0001$); LES vs T1DM ($P = 0.0001$); undifferentiated connective tissue disease vs CD and T1DM ($P < 0.0001$). Data for patients with no other autoimmune comorbidities were also displayed. Comparing patients affected by each of the aforementioned rheumatic disorders with patients without NTADs, the difference in median age was significant ($P < 0.0001$). It was highly significant also the comparison between the median age of patients with NTADs as a whole and that of patients without any comorbidity ($P < 0.0001$). *We performed pairwise comparisons between median ages of patients affected by different NTADs using Mann–Whitney test. Only NTADs affecting six or more patients were included, to allow calculation of interquartile range. According to Bonferroni's correction, the 'adjusted' significance level is $0.050/78 = 0.0006$.

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Table 4 Demographic and clinical parameters of adults and children/adolescents with HT and associated non-thyroidal autoimmune diseases (NTADs).

Parameters	Adults	Children/adolescent	P value
Patients with NTAD	147/500 (29.4%)	104/553 (18.8%)	<0.0001
Age (year)			
Mean $\pm$ S.D. (range)	43.40 $\pm$ 14.13 (18–80)	11.13 $\pm$ 3.4 (2.8–18)	–
Sex			
Female	142 (96.6%)	77 (74%)	<0.0001
Male	5 (3.4%)	27 (26%)	
F:M ratio	28:01:00	2.8:1	–
Functional status (n (%))			
Euthyroidism	86 (58.5%)	69 (66.4%)	0.256
Hypothyroidism	61 (41.5%)	28 (26.9%)	0.025
Subclinical	43 (29.25%)	15 (14.4%)	0.188
Overt	18 (12.25%)	13 (12.5%)	
Thyrotoxicosis	0	7 (6.7%)	–
TSH (μ IU/L)*	5.54 $\pm$ 11.79, 2.38	8.56 $\pm$ 24.85, 2.92	0.2
FT4 (pmol/L)*	13.10 $\pm$ 4.76, 13.72	15.24 $\pm$ 4.98, 14.75	<0.0001
Tg-Ab (IU/L)*	325.41 $\pm$ 973.09, 113	1103.20 $\pm$ 3830.98, 175	0.019
TPO-Ab (IU/L)*	340.66 $\pm$ 489.69, 187.75	1359.80 $\pm$ 4255.29, 386	0.004

*Data are expressed as mean $\pm$ S.D., median in italics. Normal values are specified under 'Materials and methods' section. P values typed in bold are significant ($P \leq 0.05$).

diagnosed before or after HT at the same extent ($P=0.403$), as well as CD ($P=0.8531$). Skin diseases were diagnosed before or after HT at the same extent both in children ($P=0.2363$) and adults ($P=0.9484$), without differences between age groups ($P=0.3899$).

Comparing the two subgroups of adult and pediatric/adolescent patients with associated NTADs (Table 4), we found that the female sex was more represented in the two subgroups of patients with associated NTADs, but once again, such a female prevalence was more evident in the adult cohort, indicating that autoimmunity tends to involve mainly women with increasing age. Concerning thyroid functional status, hypothyroidism was found in a higher percentage of adults compared to children/adolescents with comorbidities (41.5% vs 26.9%; $P=0.025$).

Comparing in each cohort the HT patients with and without NTADs, some differences emerged. In adults, subjects with associated NTADs were significantly older than those without comorbidities (43.40 $\pm$ 14.13 vs 38.9 $\pm$ 13.35 year; $P<0.0001$), with a F:M ratio 28:1 in the former and 12:1 in the latter. Thus, adults with comorbidities were more frequently females and older than those without NTADs. In the pediatric/adolescent cohort, there were no differences in age at presentation between subjects with or without NTADs, but the F:M ratio was significantly lower in the former (2.8:1 vs 4.9:1; $P=0.046$); thus, males were more represented among children/adolescents with comorbidities. Moreover, subdividing these patients according to their pubertal

status, we did not find any significant difference in the prevalence of NTADs between pubertal (69/363 or 19%) and pre-pubertal (35/190 or 18%) subjects ($P=0.866$).

As shown in Table 5, the relative risk, expressed as odds ratio (OR) of developing an additional autoimmune disorder was greater in adults than in children/adolescents, and in adulthood it was even higher than the OR of having more than two NTADs. Among the various NTADs, the relative risk was the greatest for RA (OR 21.107), suggesting a strong disease association (Table 5).

Also, the observed prevalence rates of NTADs were compared to those reported in the Italian population, when data were available either from the current

Table 5 Relative risk (odds ratio) of the single non-thyroidal autoimmune disease, comparing prevalence in adult vs pediatric/adolescent cohort (for prevalence values >0).*

Coexisting non-thyroidal autoimmune disease (NTAD)	Odds ratio	95% Confidence interval
Any NTAD	1.798	1.349–2.396
Two or more NTAD	5.18	2.217–12.105
Rheumatoid arthritis (RA)	21.107	2.811–158.465
Vasculitis	0.705	0.044–11.409
Vitiligo	0.625	0.287–1.357
Chronic urticaria	3.627	0.417–31.511
Type 1 diabetes mellitus (T1DM)	0.024	0.006–0.102
Coeliac disease (CD)	0.104	0.048–0.228
Psoriasis	1.427	0.256–7.937
Alopecia	0.136	0.016–1.179

*OR was calculated for all autoimmune comorbidities, which were represented in both groups.

literature (31, 32, 33, 34) or from our Health Department (www.salute.gov.it). Most of the NTADs were reported more frequently in our HT subjects than in the general population: psoriatic arthritis, 5.8% vs 1% in the general population; RA, 5% vs 1%; SS, 5.6% vs 3%; LES, 1.4% vs 0.07%; T1DM in children/adolescents, 6.8% vs 1.5%; CD 1.8% vs 0.7% in adults, 7.2% vs 1.25% in children/adolescents; vitiligo, 2.8% in adults and 2.7% in children/adolescents vs 2.0% in the general population. Although limited by the paucity of contemporary population prevalence data, our data support the presence of a true, non-casual disease association and an increased risk of other autoimmune disease in HT patients, mainly for connective tissue diseases in adult age, for T1DM in pediatric age and for CD and vitiligo in both age groups.

Discussion

In the present study, we have evaluated the association between HT and other autoimmune diseases in more than 1000 well-characterized, newly diagnosed HT patients at any age of life to delineate the clinical patterns of diseases aggregation in relation with age. To the best of our knowledge, this is the largest cohort so far reported in the literature, which includes even pediatric/adolescent patients along with adults with HT.

It is currently known that increasing age and female gender are associated with increasing risk of developing autoimmune disorders (7, 10), but scanty data are available on their possible role in conditioning the aggregation of autoimmune diseases. The present study was aimed to ascertain whether the age at HT presentation might *per se* influence the aggregation of NTADs in patients with the same autoimmune disorder (namely HT) by conditioning a different clustering of diseases in pediatric/adolescent and adult age.

A previous multicenter study by Boelaert and coworkers (13) have estimated the prevalence and the relative risk of other autoimmune diseases in a well-characterized cohort of adult Caucasian subjects (mean age 43 years) suffering from HT ($n=495$) or Graves' disease ($n=2791$). The estimated prevalence of another NTAD was 14.3% in HT and 9.67% in Graves' disease cases, and the relative risks of all other coexisting autoimmune disorders were significantly increased in both. In this cohort, even if women definitely outnumbered men (2744 F and 542 M), there were no significant differences in the prevalence of associated NTADs between men and women. In the same manner, no differences in age at diagnosis were

reported between HT cases with or without associated NTADs, whereas Graves's disease cases with coexisting RA and pernicious anemia were older than cases without any associated NTADs. However, comparison between the whole group of patients with different NTADs and those without any associated NTAD was not provided in the study (13). Thus, the study from Boelaert and coworkers provided strong evidence for true diseases association, but no additional information about the possible influence of age on such an association as it was beyond the aim of the study.

In the present study on a large cohort, we not only just collected the clinical records but also screened all the participants for common autoimmune diseases. Furthermore, unlike previous studies, our HT population consisted of both adults (550 cases, comparable by number, sex and age to the study by Boelaert and coworkers) and pediatric/adolescent (553 cases, aged <18 years) patients. This has an important significance for the diseases prevalence estimates as some of the NTADs investigated tend to occur early in life (i.e. T1DM and CD), whereas others tend to occur later (i.e. RA).

In our study, the prevalence of associated NTADs in the whole cohort of HT subjects was 23.8%, and it was significantly higher in adults. Also the number of patients suffering from two or more associated NTADs was significantly higher in adults than in children/adolescents. Moreover, subjects with associated NTADs were significantly older than those without comorbidities. The relative risk of developing NTADs increased with age as adults had a nearly twofold risk compared to children/adolescents. Thus, we report an increasing prevalence of coexisting NTADs with age, as well as an increased OR for one or more other autoimmune disorders.

Female subjects were more frequently affected by autoimmune comorbidities with increasing age. However, such a female preponderance was not evident in children/adolescents. Under the age of eighteen, the female: male ratio was significantly lower in HT patients with NTADs compared to those without comorbidities, suggesting that sex-related factors are less important in conditioning autoimmune diseases aggregation at younger age. Accordingly, we did not find any difference in NTADs prevalence when subdividing these subjects according to their pubertal status.

Although most disease associations described in the present study have already been reported individually in the literature, the present data provide novel information about their epidemiological distribution and clustering, and first highlight that there are significant differences

in the prevalence and peculiar aggregations of NTADs in different ages. In fact, the NTADs distribution was strongly different in the two cohorts. In adults, the most frequently associated diseases were arthropathies (psoriatic arthritis and RA) and connective tissue diseases (mostly SS), and the OR was the greatest for RA (more than 20-folds). On the contrary, in the pediatric/adolescent cohort, these disorders were absent or rarely found, and the most prevalent ones were T1DM and CD. Skin diseases were represented with similar prevalence in both cohorts, vitiligo being the most common. Accordingly, when comparing median age at diagnosis between cases with different NTADs, patients with coexisting arthropathies and/or connective diseases were significantly older compared with those reporting T1DM, vitiligo, alopecia or CD.

Very recently, similar findings have been reported in another organ-specific autoimmune disease that often clusters with HT, i.e. CD (20). In a retrospective study including more than 400 CD patients, the occurrence of another autoimmune disease was reported in 25.2% of the patients (the most prevalent being T1DM, microscopic colitis and autoimmune thyroid diseases), and the age of CD diagnosis was significantly higher in patients with a coexisting autoimmune disorder compared to those with CD alone ($P = .002$). Older age at the time of CD diagnosis, and not the time of follow-up or the disease duration, was an independent predictor of having a concomitant autoimmune disorder. The authors hypothesized that CD diagnosis later in life, thus possibly prolonged inflammation, may favor the occurrence of other autoimmune disorders (20).

It is conceivable that, beside the genetic background, autoimmune disorders may share common exogenous factors potentially involved in the development and progression of the disease, such as vitamin D deficiency, increased oxidative stress, improved hygiene and intestinal microbiota dysbiosis, whose role in autoimmunity is intensely debated at present (6, 7, 35, 36, 37). Specially, there is growing evidence on the role of microbiome in various age-related disorders, including rheumatoid arthritis and spondyloarthritis (37). This hypothesis may account for the high rate of association between HT and rheumatic diseases with increasing age in our cohort.

In conclusion, the present study confirms a high prevalence of NTADs in HT patients, and for the first time, delineates different patterns of aggregation of autoimmune disorders in relation to age in these patients. Age at presentation of HT may influence autoimmune diseases clustering, favoring the association of some

specific NTADs, such as T1DM and CD in children/adolescents and rheumatic diseases in adulthood. As a consequence, different types of NTADs should be investigated/suspected in relation with patient's age. Moreover, the association between HT and NTADs increases with age, and adult HT subjects have both increased prevalence and risk of concomitant NTADs in comparison with children/adolescents. Common age-related autoimmune mechanisms may contribute to the pathogenesis of coexisting autoimmune diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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