Hashimotos' Thyroiditis:

epidemiology, pathogenesis, clinic and therapy.

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Abstract

Hashimoto's thyroiditis (HT), the most frequent autoimmune thyroid disorders (AITDs), is the leading cause of hypothyroidism in the iodine-sufficient areas of the world. About 20-30% of patients suffers from HT, whose cause is thought to be a combination of genetic susceptibility and environmental factors that causes the loss of immunological tolerance, with a consequent autoimmune attack to the thyroid tissue and appearance of the disease.

The pathologic features of lymphocytic infiltration, especially of T cells, and follicular destruction are the histological hallmark of autoimmune thyroiditis (AIT), that lead to gradual atrophy and fibrosis. An important role in the immune-pathogenesis of AITDs is due to chemokines and cytokines.

In about 20% of patients, AITDs are associated with other organ specific/systemic autoimmune disorders.

Many studies have demonstrated the relationship between papillary thyroid cancer (PTC) and AITD.

The treatment of hypothyroidism, as result of AIT, consists in daily assumption of synthetic levothyroxine.

Keywords: autoimune diseases, autoimmune thyroid disorders, autoimmune thyroiditis, Hashimoto's thyroiditis, hypothyroidism, levothyroxine

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1. Introduction

Hashimoto's thyroiditis (HT) is the most frequent autoimmune thyroid disorders (AITDs). It causes a chronic inflammation of the thyroid tissue (1,2), with a condition of hypothyroidism in about 20-30% of patients (1-3).

In the beginning of 20th century Hashimoto firstly described the most significant signs of autoimmune thyroiditis (AIT), reporting patients with atrophy of follicular cells, lymphocytic infiltration, goiter and fibrosis. AIT occurs in about 0.3-1.5/1000 subjects/year, with a major frequency in women than in men (4-10 times).

The diagnosis of AIT depends on different characteristics: presence of circulating antibodies against the thyroid; a hypoechogenic and dyshomogeneous gland parenchyma at ultrasonography; elevated levels of thyroid stimulating hormone (TSH), with normal or low serum thyroid hormones (only in a fraction of patients) (1-4).

2. Epidemiology

Epidemiological studies showed that (1): A) the AIT risk is higher among women than men; B) AIT hypothyroidism is age-related; C) there is a geographic heterogeneity; D) the AIT incidence is higher in condition of iodine-sufficient with respect to the deficient one; E) the prevalence of antithyroid antibodies (ATA) differs with races, is increased with age, and is reduced with smoking.

As reported in the Whickham survey (5), the mean incidence and the prevalence of spontaneous hypothyroidism, as consequence of AIT, were respectively 3.5-5/1000 in women (mean age 57 years), and respectively 0.6-1/1000 in men (5). Other studies have collected similar data in other geographical areas (1).

The current data showed higher AIT incidence rates, with/without concurrent hypothyroidism, in the same geographical area than the older studies.

However, it is difficult to detect the underlying reasons of this phenomenon, and if this depends on current increased incidence, or other reasons, that can be related, for example, to study design or more accurate diagnostic tests.

However the increased incidence of AIT worldwide is matter of fact (6).

3. Risk factors

The cause of HT is thought to depend on a combination of genetic susceptibility and environmental risk factors, which determines the breakdown of immunological tolerance, with a resulting autoimmune attack to the thyroid itself.

3.1 Genetic susceptibility

As many epidemiological researches showed evidence for genetic susceptibility to HT, these include the following observations: 1) familiarity of AITD (it occurs in 20–30% of patients' sibling), with a sibling risk ratio of 16.9; 2) concordance rate for AITD of 29% to 55% for monozygotic twins with respect to 0-7% for dizygotic ones (7); 3) circulating ATA in about 50% of sibling of affected patients (8).

With case-control studies Simmonds et al. have found a significant association among AITD, the presence of ATA and some genes (9) [IL2RA, human leukocyte antigen (HLA), PTPN22, and CTLA4], identified by the method tag-single nucleotide polymorphism (2,8,9). Other AITD genes [FCRL3, TSH receptor (TSH-R), and HLA class I] were identified through further case-control studies, and confirmed by genome-wide association studies (GWAS).

Furthermore GWAS and Immunochip identified other AITD risk genes: a) FOXE1, which is involved in thyroid morphogenesis and in the binding response elements into thyroglobulin (Tg) and thyroid peroxidase (TPO) promoters; b) GDCG4p14 and RNASET2, expressed on CD4+ and CD8+ cells (9); c) BACH2, expressed during the maturation of B-cells.

Noteworthy, seven out of eleven known susceptibility genes take part in the role of T cells; these findings underline the importance of T cells in the AITD immune-pathogenesis (2).

In a family with an early onset of HT, apparently autosomal dominant, it has also been described a splice site variant in the gene of thyroglobulin, TG c.1076-1G>C; this is the first reported monogenic form of AITD. Further studies are still needed to understand how this variant gene can lead to autoimmunity (10).

3.2 AITD Susceptibility Genes: Thyroid Antigens

Several antibodies and specific T cells directed against thyroid antigens have been described in chronic autoimmune thyroiditis, and the major antigens are Tg, TPO and TSH-R. However, there may be a reaction between sera obtained from patients with AITD and a lot of other antigens, both specific and non-specific.

The product of Tg gene, a homodimeric glycosylated iodo-protein of about 660 kDa, represents the main protein inside the thyroid gland (approximately 80%). The synthesis of thyroid hormones depends on the Tg, which in turn is synthetized by thyrocytes. After the synthesis, in some cases Tg enters into the circulation through a passage which in part depends on the thyroid volume. Entering into the vascular system, Tg is exposed to the immune cells.

Tg antigenicity depends on its iodination level, as shown in animal models of thyroiditis. Since Tg could be the

initial trigger, the best model of human AIT (11) consists in mice immunized with Tg.

Linkage studies demonstrate the importance of Tg gene, located on chromosome 8, in AITD; in particular it has been shown a significant linkage with the locus located on chromosome 8q (12). It has been demonstrated a significant association between aminoacid variants of Tg and AITD, and a significant statistical relationship between a single nucleotide polymorphism (SNP) present at the level of exon 33 of the Tg gene and DRb1-Arg74 (a variant of HLA-DR which contains an arginine at the position 74), both contributing to a high AITD risk (13). However, it is not yet established a direct association between the Tg SNPs and the pathogenic Tg peptides.

There's a growing body of evidence regarding the epigenetic modifications and its potential role in the autoimmunity process. One recent topic of research has been the DNA methylation, more precisely the methylation of the ICAM-1 gene promoter in patients with AIDT, with results pointing to hypomethylation of the DNA in these patients (14).

Another issue regarding genetics and the activation of the immune system is held in a Chinese study conducted to evaluate the activation of dendritic cells (DC) by exosomes. The exosomes of patients with HT were taken mainly by the CD4+ monocytes and CD11c+ DCs and behaved differently than the exosomes of the control group: they could present antigens to DCs and bind toll like receptor 2/3 causing the activation of the DC via NFKb pathway, hence, leading to a balance loss in the CD4+ T lymphocyte differentiation, possibly, and potentially, contributing to the HT onset (15).

Anti-thyroglobulin antibodies (AbTg) usually recognize native Tg, as well as a limited range of epitopes present on Tg molecule. The main epitope attacked by the AbTg, collected from patients with AIT or Graves' Disease (GD), is the region II, that is the immune-dominant domain located in the protein core.

Thyroid hormones (T4, and T3) synthesis depends also on TPO, a globular 107 kDa glycosylated haemoprotein responsable for the Tg iodination and the coupling of the residues of iodothyronines. Anti-thyroid peroxidase antibodies (AbTPO) recognizes its conformational epitopes, in particular at the level of an immune-dominant region, which consists of two overlapping regions, the region A and the region B; the latter represents the main target of human AbTPO, probably acting as the starting epitope of the AITD immune-pathogenesis (16).

TSH-R, located at the level of the baso-lateral membrane of thyroid follicular cells, is the major regulator of metabolism, cell growth, and secretion of thyroid hormones. It consists of the extracellular subunit (A), of about 55 kDa, and the transmembrane subunit (B), of about 40 kDa, joined together by disulfide bonds. A and B subunits could be divided; in this case the cell surface can lose the A subunit, with a consequent repercussions on

the immune system (17). TSH receptor autoantibodies (TRAb) bind TSH-R in specific binding sites.

3.3 Iodine Intake

HT is the most common cause of hypothyroidism in iodine-sufficient areas of the world. An excessive iodine intake is linked with a higher AIT prevalence, while in iodine deficient areas a lower prevalence is shown. For example, in China, AIT is found in 0.3% of patients of mildly iodine deficient areas, while in 1.3% of those with excessive iodine intake (4).

3.4 Stress

The induction of immune suppression by non-antigen-specific mechanisms can associate emotional or psychologic stress to AIT, probably because of the effects of cortisol on immune cells, followed by immune hyperactivity which leads to the thyroid autoimmunity (4).

3.5 Pregnancy and Sex Steroids

Sex steroids have a pathogenetic role in AIT considering the higher incidence of this disease among the female gender. However, as the older women may experience HT more than younger women, probably the presence/absence of estrogen is of limited importance (4).

Some thyroid self-antigens are located on the X-chromosome. For this reason, it has been hypothesized that the random X chromosome inactivation (XCI), also known as lyonization, may influence the incidence of AITD in female and male sex. XCI has been suggested to be skewed in many such female patients with AITD. For example, skewed XCI has been shown in 34% of female twins with AITD, and only 11% of controls (18).

CD4+CD25+ regulatory T cells increase during pregnancy, leading to reduced function of both T and B cells. The rebound from this immune-suppression probably contributes to the development of postpartum HT. The immune-suppression which occurs during the pregnancy is associated with a shift to Th2 immune-preponderance and a change in cytokine profiles (4).

During pregnancy several mechanisms modulate the immune system. These factors together are the maternal-fetal interface as a local site of immune privilege (19).

Also the progesterone, after its release by the placenta, has an important role as a modulator of the immune system.

About 20% of postpartum thyroiditis patients develop the classical HT in later years (4,20).

3.6 Fetal Microchimerism

Inside the maternal thyroid glands of patients with AITD, fetal cells have been identified. Such cells may react against the thyroid gland and play a significant role in the development of HT. But, to date, this is only an hypothesis (4).

3.7 Radiation Exposure

High rate of ATA have been described in children exposed to nuclear disasters, like Chernobyl's accident, with a consequent increased risk of problems like thyroid cancer and thyroid dysfunctions (21).

Volzke et al. conducted a population study on 4299 subjects, of which 160 were professionally exposed to ionizing radiation; with this study it has been shown that the exposition to ionizing radiation longer than 5 years made that subjects particularly at high risk of AIT. For these reasons, a relationship between AITD and background radiation has been hypothesized, however additional studies are needed (22).

3.8 Selenium

The thyroid gland is the largest reservoir of selenium of the whole body.

Selenoproteins like glutathione peroxidases (GPxs) and thioredoxin reductases, present at the level of thyrocytes, are able to control the redox state and protect the cells from the oxidative damage; in particular GPx-3 inhibits the oxidative capacity of H2O2. As low selenium levels are associated to immune dysfuction (23), a reduced selenium assumption is considered a risk factor for AITD development.

The selenium supplementation has an important impact on the serum levels of AbTPO, as reported by six randomized clinical trials (23,24). In 3/6 studies, selenium decreased AbTPO with respect to placebo. On the other hand, selenium intake seems to be important in the prevention of thyroid dysfunction, postpartum surge of AbTPO, and worsening of mild Graves' ophthalmopathy (GO) (24).

A randomized trial evaluates the impact of selenium supplementation on the TSH levels and interferon (IFN)-γ inducible chemokines [chemokine (C-X-C motif) ligand (CXCL)9, CXCL10, CXCL11] in patients with subclinical hypothyroidism due to HT. The patients were administered 83 mcg of selenomethionine daily, for 4 months. Approximately, half of the patients of the study became euthyroid (responders) and the other half remained hypothyroid (non responders); and there was no change in the levels of chemokines, AbTPO, nor iodine in neither groups. After 6 months of selenium withdraw, 83% of the responders remained euthyroid against only 14% in the group of non responders (25).

As reported by Van Zuuren et al. in Cochrane Database of Systematic Review, data at present do not allow confident decision making about the use of selenium supplementation for HT (24,26).

3.9 Vitamin D

The vitamin D plays a role in AIT, as investigated in the past years (27).

Different polymorphisms in vitamin D receptor have an important role in AIT risk, like BsmI and TaqI polymorphisms (27).

As showed by a meta-analysis, serum vitamin D levels were lower in AIT subjects with respect to controls (27).

As different trials have shown different results about vitamin D supplementation in AIT, a Chinese study questioned the role of vitamin D in AIT. However, a recent systematic review and meta-analysis showed that after 6 months of vitamin D supplementation AbTPO and AbTg levels significantly decrease, supporting the idea of its effectiveness in reducing ATA concentrations. Nevertheless, other long term-studies are needed to confirm these results (27).

3.10 Viruses

The impact of viruses on AITD pathogenesis has been investigated with contradictory findings.

However, different studies confirmed the association between hepatitis C virus (HCV) and AITD (28,29). Thyroid diseases are common among chronic hepatitis C (CHC) patients, especially females with elevated AbTPO levels are at increased risk of developing hypothyroidism. Several studies demonstrated a high prevalence of AIT in patients with HCV and mixed cryoglobulinemia (MC+HCV). In particular, a case-control study showed circulating AbTg and AbTPO levels, and hypothyroidism, were more frequently observed in MC+HCV subjects, in comparison with negative subjects for HCV. Moreover, in CHC and MC+HCV patients, also in presence of AIT, it has been shown an elevated prevalence of papillary thyroid cancer (PTC). Furthermore, it has been shown the presence of HCV in the thyroid tissue of CHC subjects (30), and it has been shown that HCV is able to enter into thyrocytes stimulating the production of inflammatory cytokines (31).

3.11 Drugs

The treatment with IFN-alpha may induces the onset of AIT, and women with preexisting high AbTPO levels seem to be at risk of hypothyroidism. It can exert toxicity on the thyroid cells or provocate a destructive immune responses resulting in autoimmune hypothyroidism (32).

Alemtuzumab therapy (anti-CD52 MAB), or therapy with active anti-retroviral drugs, cause a depletion of cells like lymphocytes; however, when CD4+ T-cells increase (recovery phase), the appearance of autoreactive clones may occur. Therefore, both alemtuzumab therapy and active anti-retroviral therapy could induce the immune reconstitution syndrome, expressed as Graves' hyperthyroidism (GH) and less frequently as hypothyroidism (24).

4. Immune-pathogenesis

The lymphocytic infiltration (expecially of T cells) in the thyroid gland represents the principal feature of AITD; in this way, the thyroid gland can be gradually replaced. This can lead to fibrosis and atrophy of thyrocytes (2). An increase of the CD4/CD8 ratio is caused by a decrease of the CD8+ T cells circulating levels, is found in patients with postpartum thyroiditis, AIT, and also in other AITD such as GD. Moreover, also T lymphocytes at active state, expressing HLA-DR, are higher. The infiltration of CD8+ and/or CD4+ cells can be often found in the activated state in thyroid tissues but in case of AIT CD4+ can be predominant (2).

The regulatory T cells (Tregs) are known for their important role in maintaining an immune balance, and different researches have been aiming to elucidate better this pathway. Studies have shown a decrease of Tregs in patients with HT (33).

The PD-1/PDL1 pathway is an important pathway for peripheral immune tolerance, and has already been demonstrated to be activated in HT patients, but probably not to the extend to inhibit disease progression, pointing this as one possible treatment approach. All that taken together leads one to believe that the deficiency of Tregs frequency, as well as an aberrant expression of Helios and PD-1 may play an important part in the autoimmune damage caused to the thyroid in HT (33,34).

In thyroid tissue (in presence of AIT) there are B cells, arranged in lymphoid follicles, in the end with germinal centers. Meanwhile intra-thyroid B cells are responsible of the production of antibodies, and *in vivo* studies reported that the thyroid gland can be the principal source of ATA, like B cells of juxta-thyroid lymph node or the others in bone marrow.

5. ATA

5.1 AbTPO

AbTPO can be used to diagnose thyroiditis and thyroid dysfunction (35). Circulating AbTPO are present in about 80-90% of patients with AIT, and in about 50-60% of patients with GD. The determination of circulating AbTPO has a sensitivity of 90% for the diagnosis of AITD.

As demostranted by a prospective study, AbTPO positivity can be used to predict the switch from subclinical hypothyroidism to overt hypothyroidism. In addition, in female patients with AbTPO there is an increased risk of postpartum thyroiditis.

Many studies have suggested that AbTPO have an important role in the immune-pathogenesis of hypothyroidism in AIT. In fact, AbTPO are able to induce two different types of cytotoxicity, the antibody-dependent cellular cytotoxicity by natural killer (NK) cells, and the complement-dependent cytotoxicity, contributing to the thyrocyte death, and thyroid atrophy (35).

5.2 AbTg

AbTg are present in circulation in 60-80% of patients with AIT, and 40-60% of patients with GD. The determination of circulating AbTg has a sensitivity of 30-50% for the diagnosis of AIT. As demostranted by a prospective study, AbTg, unlike AbTPO, cannot be used to predict the switch from subclinical hypothyroidism to overt hypothyroidism. Furthermore, IgG and IgM AbTg at low affinity were also detected in patients without AITD, and IgG1 or IgG4 AbTg cannot stimulate the complement and are not pathogenetic for the appearance of hypothyroidism in AIT (35).

5.3 TRAb

TRAbs are a feature of GD, and have a specific pathogenic role. 90-95% of GD patients are usually positive for TRAb; however, 5% of GD patients are positive for AbTPO, but negative for TRAb. TRAb binds TSH-R in specific binding sites, but the way is not still known. TRAb with thyroid-stimulating activity (agonist) are responsible for the hyperthyroidism of GD. In rare cases, TRAb act as antagonists and prevent the TSH-R binding and stimulating activities of TSH and can cause hypothyroidism (36). TRAb might have also a neutral activity, and in this case the binding the TSH-R has no metabolic effects on thyroid cells.

6. AITD, chemokines, cytokines

Several studies have shown the important role of cytokines and chemokines in the immune-pathogenesis of AIT and GD. An amplification feedback loop, in which IFN-γ and tumor necrosis factor (TNF)-α, produced by Th1 lymphocytes recruited within the thyroid tissue, induce thyrocytes to release CXCL10, responsible of initiating and perpetuating of the autoimmune process (37,38). After release, CXCL10 binds its receptor, chemokine (C-X-C motif) receptor 3 (CXCR3) on Th1 lymphocytes, attracting them into the target tissue and enhancing and

perpetuating the inflammatory process. This process has a pathogenic role in different autoimmune disorders, both organ-specific [GD, GO, type 1 diabetes (T1D)], and systemic [systemic lupus erythematosus (SLE), psoriasis, systemic sclerosis (SSc), sarcoidosis, MC, Sjögren's syndrome (SS) (39-41).

IFN-γ and TNF-α also stimulate CD4+, CD8+ and NK cells, but also target cells (such as thyrocytes) to synthesize and release CXCL10, and other IFN-γ dependent chemokines (37,38). From the target tissue, for example the thyroid, IFN-γ dependent chemokines enter the circulation. In fact, in AIT patients with high grade of lympho-monocytic infiltration (with an hypoechoic pattern at the ultrasound) and in hypothyroid ones, high circulating levels of IFN-γ dependent chemokines were found (37,38). Therefore, IFN-γ dependent chemokines could be considered a marker of a thyroid inflammation with more aggressive characteristics, which causes hypothyroidism and thyroid destruction (37,38). Future studies are needed to investigate new treatments that block IFN-γ dependent chemokines in patients with AIT.

7. Thyroid autoimmunity & other autoimmune diseases

There is an association between AITD and other organ specific/systemic autoimmune disorders; in fact it's not unusual to find patients with more than one immune-mediated endocrinopathy. As a result, polyglandular autoimmune syndromes (PAS), characterized by the failure of different endocrine glands, occur.

A recent study analyzed 15,000 adults with endocrine disorders and, among these, 360 patients are suffering from PAS. The high percentage of these patients shows Addison's disease, hypogonadism, GD, AIT, vitiligo (Vit), alopecia, pernicious anemia and T1D. In most cases the first manifestation of PAS was the T1D-AITD association (48%) (42).

A recent important study first demonstrated a significant association of HT and systemic autoimmune/organ specific disorders, with respect of healthy controls, or patients with multinodular goiter, age and gender matched, and with a similar iodine intake (43). The results of the study demonstrated a significant increase of the prevalence of autoimmune disorders in AT patients (with respect to both controls), for the following diseases: chronic autoimmune gastritis (CAG), Vit, rheumatoid arthritis (RA), polymialgia rheumatica, celiac disease, diabetes, SS, multiple sclerosis, SLE, sarcoidosis, alopecia, psoriathic arthritis, SSc, and HCV-related cryoglobulinemia. Similar findings have observed in patients with GD (44).

The reasons of the association of AITD with other autoimmune disorders reside both in a common genetic susceptibility, such as in environmental factors. A similar genetic susceptibility is present for example in patients with AIT, and T1D. About the association of rheumatologic diseases (for example SS, Ssc, SLE, RA), with

AITD (43,44) genetic studies have been conducted.

As reported by a study on thirty-five families with frequent cases of SLE and AITD, the principal locus of susceptibility for these two disease, the 5q14.3–q15 locus, could be the potential cause of genetic susceptibility (45). In addition, HLA class II antigens were investigated in eighty-five patients with scleroderma, with the result that HLA-DR15 allele was more prevalent in patients with elevated AbTPO levels, with respect to patients without AbTPO, leading to hypothesize the association between the HLA-DR15 allele, AbTPO production and thyroid autoimmunity.

Environmental factors are also important in the association of AITD with autoimmune diseases (43,44,46).

As suggested by several recent studies, different forms of AITD (AT, and GD), as well as different rheumatic disorders (SLE, RA, SSc, MC), show elevated tissue/serum levels of CXCL10. This leads to hypothesize that in these disorders there is a common immune-pathogenic via, in which Th1 orientated immune response prevails, both during the initial phase and the active phase.

8. AITD & thyroid cancer

To date, there are no doubts about the association between AIT and PTC (25). Fiore et al. analyzed the attendance of PTC, elevated levels of TSH and ATA in 13,738 patients with AITD (3914 in treatment with L-thyroxine (L-T4) and 9824 without treatment) (47). The prevalence of PTC was greater in nodular-AIT patients than in those with nodular goiter. There was also an increase of TSH levels in patients with PTC. In patients under LT-4 therapy both TSH value and PTC incidence were lower (47). Similar findings were observed in other studies (48).

Thyroid autoimmunity and high TSH levels are considered independent risk factors for TC in different papers (2). A high prevalence of PTC has been also found in CHC and MC patient, particularly in presence of AIT (30). Among patients with both PTC and AITD, 5-10% of these may develop an aggressive disease and require systemic therapy (30).

Regarding the outcomes and clinical progression, a recent meta-analysis that included 71 published articles totalizing 44034 patients, of whom 11132 had HT, showed a negative association between PTC with comorbid HT and aggressive behavior of cancer (49).

9. Diagnosis

The circulating concentration of autoantibodies (expecially AbTPO and AbTg) and TSH, and clinical and biochemical signs, such as the classical features on thyroid ultrasound are the criteria used for the diagnosis

today. Fine needle aspiration cytology and RAI absorption are less used (3).

9.1 Clinical features

The thyroid gland is firm and enlarged in the goitrous form, while it is not palpable in the atrophic form of AIT.

The atrophic form of AIT has less frequency in younger female patients. Moreover, the classic/goitrous form of AIT is less frequent in men than in women; it develops frequently around fifty years old. Patients with AIT and goiter may have different local or systemic clinical features. Dysphonia, dysphagia and dyspnea represent the local features, due to the enlargement of the thyroid that narrows the adjacent cervical structures.

About 25-30% of patients have thyroid dysfunctions, ranging from subclinical hypothyroidism, with thyroid hormones at the range levels and high levels of TSH, to overt hypothyroidism (3).

Systemic clinical pictures occur as a result of primary hypothyroidism. The symptoms and signs of hypothyroidism are many, changeable and not specific, and, due to the wide and deep action of thyroid hormones on organs and tissues (3). Different systems, like cardiovascular, pulmonary, hematopoietic, gastrointestinal, urinary, reproductive, neuro-psychiatric, skeletal, skin and appendages, are influenced by hypothyroidism, including AIT hypothyroidism (3). However, the symptoms of hypothyroidism are not simple to identify due to an overlap with the aging manifestations (3).

10. Treatment

The main therapy of AIT is medical. The treatment of AIT hypothyroidism serves to counteract the symptoms, but does not cure the disease. Synthetic LT-4 is the therapy that patient must take daily for all life, given at doses of 1.5-1.7 μg per kg (3). Different formulations of L-T4 are now on the market (tablets, liquid solutions, or soft gel capsules) **Table 1**. While L-T4 in tablet is usually prescribed for the treatment of hypothyroidism, recent studies suggest that the liquid formulations, or the soft gel capsules, can be used to cure hypothyroidism in patients with malabsorption (such as patients with atrophic gastritis, lactose intolerance, bariatric surgery, gastric paresis, and others), or in patients that need to be treated with drugs that interfere with L-T4 absorption (PPI, sucralphate, calcium, etc). The different formulations consent to personalize the therapy in relation to personal characteristics of each patient, to obtain a stable and durable condition of biochemical euthyroidism (50-57).

With increasing age the incidence of hypothyroidism increases and also the reference range for TSH.

Many studies suggest that mild increases of TSH, in older people, are not related to important aspects, such as altered quality of life, cognition, mortality or events related to cardiovascular system. Signs and/or quality of life

don't improve in older subjects if treated for mild subclinical hypothyroidism.

Older people have less need for thyroid hormones, which is why it should be possible to accept a higher TSH threshold in these people before proceeding to treatment for mild subclinical hypothyroidism. Therefore for older people it would be necessary to carry out randomized studies to understand the relationship between risk and benefit in the treatment of subclinical hypothyroidism in these patients (58,59).

Rarely thyroidectomy is recommended in patients with AIT. The reasons for a thyroidectomy are manifold: severe signs or symptoms of local compression, or nodular disease with a "suspicious" cytology for malignancy, or sometime cosmetic reasons for a goiter.

HT patients can have persistent symptoms (chronic fatigue, weakness, nervousness, irritability, frequent mood swing, impaired sexual activity) and other symptoms related to a low quality of life even though being euthyroid with L-T4 medical treatment. Many studies showed that women with high levels of circulating AbTPO are more prone to have these problems; the pathogenetic mechanisms underlying these manifestations are not yet known and need further studies (60), pointing to the fact that there are more therapeutic targets that need to be explored (T4 plus T3 combination therapy, altered thyroid hormones action, as well as the autoimmune process *per se*).

A very recent study from Norway enrolled 150 HT patients who had persistent symptoms even though being euthyroid, randomized to thyroidectomy, versus medical treatment. During follow-up, only the surgical group demonstrated improvement: mean general health score increased from 38 to 64 points; fatigue score decreased from 23 to 14 points; chronic fatigue frequency decreased from 82% to 35%. Futhermore interestingly, median serum AbTPO titers decreased from 2232 to 152 IU/mL (61). This reinforces the questions of the role of AbTPO in the residual symptomatology of the AIT, and further studies are still required.

11. Future Directions of Research

To date, much progress has been made in knowledge and understanding of AIT. Neverthless the mechanisms that induce the breaking of the tolerance of the immune system, with consequent autoimmune response towards the thyroid gland and the beginning of the disease, are still unclear. In subjects with a genetic predisposition, also enviromental factors can contribute to the beginning of the immune system's anomaly, but the molecular mechanisms that regulate this interaction are not yet clear. It would be appropriate, as for other autoimmune diseases, to study the main passage that causes the autoimmune attack to discover new molecules that lead to an arrest of the autoimmune process. Recent clinical studies, carried out on patients with autoimmune disorders, tested and studied some molecules that interfere with CXCL10 (the principal Th1 chemokine of the AIT

pathogenesis) or CXCR3 (its receptor), but other studies are needed for AIT.

Recently, two new oral L-T4 formulations (liquid and soft gel capsule) have been introduced in the pharmacological treatment of AIT hypothyroidism.

Only in a few studies conducted up to now it has been shown that the gel formulation can improve the absorption of L-T4 in patients with disorders like gastritis or malabsorption, in relation to the assumption of coffee, while the liquid one could be better for patients undergoing bariatric surgery or with an increase in gastric pH, a condition that can occur when patients use proton-pump inhibitors or suffer from atrophic gastritis (62).

In hypothyroid patients with drug interference, malabsorption, or gastric disorders, the liquid formulation allows a better control of the TSH levels than tablet formulation; furthermore, it increases the absorption of the LT-4 in comparison to the formulation in tablet, where malabsorption is due to interferences with coffee or food for breakfast (53). In the new era of precision medicine, the different formulations consent to personalize the therapy in relation to personal characteristics of each patient, to obtain a stable and durable condition of biochemical euthyroidism.

Moreover, the use of L-T4 as liquid solution has been studied also in pregnancy, newborns and infants, suggesting a better bioequivalence than L-T4 in tablet (53,63,64).

In some studies on patients with AIT and subclinical hypothyroidism, treated with myo-inositol in combination with selenium, an improvement of thyroid function has been shown (65).

Summary

HT, the most frequent AITD, is the leading cause of hypothyroidism in the iodine-sufficient areas of the world. It is more common in wowen than in men (about 4-10 time), and it has an age-related frequency. The cause of HT is a combination of genetic susceptibility and environmental factors that causes the loss of immunological tolerance. The diagnosis of AIT depends on: 1) circulating anti-thyroid antibodies; 2) hypoechogenic and dyshomogeneous gland parenchyma at ultrasonography; 3) elevated TSH levels with normal or low serum thyroid hormones (only in about 25-30% of patients).

Lymphocytic infiltration, especially of T cells, and follicular destruction are the histological AIT hallmark, that lead to gradual atrophy and fibrosis. An important role in the immune-pathogenesis of AITDs is due to chemokines and cytokines, involved in an amplification feedback loop, responsible of the initiation and perpetuation of the autoimmune process.

In about 20% of patients, AITDs are associated with other systemic/organ specifics autoimmune disorders.

Sometimes the coexistence of different immune-mediated endocrinopathy can be found in patients, with the result of a PAS characterized by the failure of different endocrine glands and non-endocrine organ.

Many studies have demonstrated the relationship between papillary PTC and AITD. Since 10–30% of patients with PTC and AITD shows an aggressive form of the disease, this association seems to be clinically important.

The treatment of hypothyroidism, as result of AIT, consists in daily assumption of synthetic L-T4 (1.5–1.7 μg per kg/day).

Conflict of interest

The authors have nothing to declare.

Practice Points:

- Hashimoto's thyroiditis (HT), is the most frequent autoimmune thyroid disorders, and the leading cause of hypothyroidism in the iodine-sufficient areas of the world. About 20-30% of patients suffers from it.
- The cause of HT depends on a combination of genetic susceptibility and environmental risk factors, which determines the breakdown of immunological tolerance, with a resulting autoimmune attack to the thyroid itself.
- Several antibodies and specific T cells directed against thyroid antigens have been described in chronic autoimmune thyroiditis, and the major antigens are Tg, TPO and TSH-R.
- AITDs are associated with other systemic/organ specifics autoimmune disorders, and with PTC.
- The circulating concentration of autoantibodies (expecially AbTPO and AbTg) and TSH, and clinical and biochemical signs, such as the classical features on thyroid ultrasound are the criteria used for the diagnosis today.
- The treatment of AIT hypothyroidism consists in daily assumption of synthetic L-T4.

Research agenda:

- The mechanisms that induce the breaking of the tolerance of the immune system in AIT are still unclear.
- The cause of HT depend on a combination of genetic susceptibility and environmental risk factors; further studies are needed to understand how the different variant genes could lead to autoimmunity.
- Recently, two new oral L-T4 formulations (liquid and soft gel capsule) have been introduced in the

pharmacological treatment of AIT hypothyroidism; these different formulations consent to personalize the therapy in relation to personal characteristics of each patient, to obtain a stable and durable condition of biochemical euthyroidism.

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Table 1: New formulations of L-T4: liquid solution and soft gel capsule.

Formulations of L-T4	Indication of therapy
Liquid solution	• patients undergoing to total thyroidectomy for thyroid cancer (without malabsorption) (50); • patients with lactose intollerance (52); • patients with malabsorption due to interferences with coffee or food for breakfast (53); • patients undergoing bariatric surgery (55); • patients with an increase in gastric pH, a condition that can occur when patients use proton-pump inhibitors or suffer from atrophic gastritis (57); • pregnancy, newborns and infants (63,64).
Soft gel capsule	• patients with gastritis (53); • patients with malabsorption related to coffee (62).