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Clinical Relevance of Antithyroid Antibodies in Thyroid Disease: a retrospective study

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Inês Catarina Moreira dos Santos Correia Lume

Orientado por:

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ABSTRACT

Background: Autoimmune thyroid disease is a prevailing autoimmune disorder, however the functional and clinical roles of antithyroid antibodies have not been fully elucidated.

Objectives: To compare antithyroid antibodies and thyroid function parameters between two points in time of follow-up and to identify possible relations between antithyroid antibodies and thyroid function parameters, in Hashimoto's thyroidits.

Patients and Methods: In this retrospective observational study, we studied a database comprising 346 patients with Hashimoto's thyroiditis assisted at Hospital de Santa Maria – Centro Hospitalar Universitário Lisboa Norte, from 2009 ± 6 to 2014 ± 3. Statistical data analysis was conducted to detect differences in thyroid hormones and antibodies in serum at two different points in time (first visit and last visit of follow-up) and to infer from correlations between antithyroid antibodies and thyroid function tests.

Results: There was a significant reduction of TSH, T3, T4 and TPOAb, and a significant increase of fT4 between visits. Significant modest positive correlations between TPOAb and TgAb levels, TPOAb and TSH levels and TPOAb and fT4 levels were found at the first visit. A significant weak negative correlation between TgAb and Tg levels was reported for both visits. We identified subsets of patients who preferentially produce TPOAb or TgAb, and those who had positive Tg despite positive TgAb.

Conclusion: The follow-up change we observed in antithyroid antibodies and thyroid function shows us that Hashimoto's thyroiditis is not an immutable disease. The correlation between TPOAb and fT4 and TSH might suggest this antibody's importance in Hashimoto's thyroiditis pathogenesis. The difference in TPOAb and TgAb expression in distinct patient subsets could reflect the evolution of Hashimoto's thyroiditis, a random phenomenon or a different clinical situation altogether.

Keywords: antithyroid antibodies; autoimmune thyroid disease; Hashimoto's thyroiditis; anti-thyroid peroxidase antibody; anti-thyroglobulin antibody.

RESUMO

Introdução: A doença tiroideia autoimune é uma doença autoimune prevalente, contudo os papéis funcional e clínico dos anticorpos anti-tiroideus permanecem por esclarecer.

Objetivos: Comparar os anticorpos antitiroideus e parâmetros de função tiroideia em dois pontos do tempo distintos do seguimento e identificar possíveis correlações entre os anticorpos anti-tiroideus e parâmetros de função tiroideia, na tiroidite de Hashimoto.

Doentes e Métodos: Neste estudo retrospetivo observacional, estudámos uma base de dados de 346 doentes com tiroidite de Hashimoto seguidos no Hospital de Santa Maria – Centro Hospitalar Universitário Lisboa Norte, de 2009 ± 6 a 2014 ± 3. A análise estatística foi realizada para detetar diferenças entre as hormonas e anticorpos tiroideus séricos nas primeira e última consultas do seguimento e para inferir sobre correlações entre as hormonas e anticorpos tiroideus.

Resultados: Verificou-se uma redução significativa de TSH, T3, T4 e TPOAb, e um aumento significativo de fT4 entre consultas. Correlações modestas e positivas entre TPOAb e TgAb, TPOAb e TSH, e TPOAb e fT4 foram descritas na primeira consulta. Uma correlação modesta negativa entre TgAb e Tg foi documentada nas duas consultas. Identificámos subgrupos de doentes que produziam preferencialmente TPOAb ou TgAb, e aqueles que tinham medições positivas de Tg apesar de medições positivas de TgAb.

Conclusão: As alterações nos anticorpos anti-tiroideus e função tiroideia observadas no seguimento destes doentes mostra que a tiroidite de Hashimoto não é uma doença imutável. A correlação entre TPOAb e fT4 e TSH poderá sugerir a importância deste anticorpo na tiroidite de Hashimoto. As diferenças na expressão de TPOAb e TgAb em diferentes subgrupos de doentes poderão refletir a evolução natural da tiroidite de Hashimoto, um fenómeno aleatório ou uma situação clínica distinta.

Palavras-Chave: anticorpos anti-tiroideus; doença tiroideia autoimune; tiroidite de Hashimoto; anticorpo anti-peroxidase tiroideia; anticorpo anti-tiroglobulina.

O Trabalho Final exprime a opinião do autor e não da FML (Faculdade de Medicina de Lisboa).

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ABBREVIATIONS

fT4	Free thyroxine
fT41	Free thyroxine at the first visit
fT4L	Free thyroxine at the last visit
HAb	Heterophile antibodies
NIS	Sodium/iodide-symporter
NS	Not significant
T3	Total triiodothyronine
T31	Total triiodothyronine at the first visit
T3L	Total triiodothyronine at the last visit
T4	Total thyroxine
T41	Total thyroxine at the first visit
T4L	Total thyroxine at the last visit
TSH	Thyroid-stimulating hormone
TSH1	Thyroid-stimulating hormone at the first visit
TSHL	Thyroid-stimulating hormone at the last visit
TSH-R	Thyroid-stimulating hormone receptor
Tg	Thyroglobulin
Tg1	Thyroglobulin at the first visit
Tg	Thyroglobulin at the last visit
TgAb	Anti-thyroglobulin antibodies
TgAb1	Anti-thyroglobulin antibodies at the first visit
TgAbL	Anti-thyroglobulin antibodies at the last visit
TPO	Thyroid peroxidase
TPOAb	Anti-thyroid peroxidase antibodies
TPOAb1	Anti-thyroid peroxidase antibodies at the first visit
TPOAbL	Anti-thyroid peroxidase antibodies at the last visit
TRAb	TSH receptor antibodies

INTRODUCTION

Autoimmune thyroid diseases, including Hashimoto's thyroiditis and Graves' disease, remain the most frequent autoimmune diseases in the adult population [1], [2]. There is a female preponderance in all types of autoimmune thyroid disease [1], [2], except for IgG4-related thyroiditis [3].

Both Hashimoto's thyroiditis and Graves' disease feature diffuse lymphocytic infiltration of the thyroid gland and the presence of antibodies against thyroid autoantigens, most commonly thyroid peroxidase (TPO), thyroglobulin (Tg) and thyroid-stimulating hormone (TSH) receptor [4]. Rarely, antibodies against other thyroid-specific antigens such as triiodothyronine (T3), thyroxine (T4), sodium/iodide-symporter (NIS), pendrin, carbonic anhydrase 2 and megalin have also been identified [4]–[6].

With the exception of TSH receptor antibodies (TRAb) [7], thyroid autoantibodies, although useful for the diagnosis of autoimmune thyroid disease, are assumed to lack physiological or clinical relevance and may be determined only once, at the first visit [8]–[10].

André Gide, a french writer, stated that we call experience always repeating the same mistakes. Therefore, we decided to challenge old common assumptions by exploring data from our patients with Hashimoto's thyroiditis.

We will summarize the current clinical relevance and utility of antithyroid antibodies in thyroid disease. As a retrospective observational study, this paper will focus on examining the interrelationship of antithyroid antibodies and their influence on thyroid hormones, as well as the long-term evolution of antithyroid antibodies and thyroid function in Hashimoto's thyroiditis.

Hashimoto's Thyroiditis

Hashimoto's thyroiditis is an autoimmune condition affecting the thyroid gland, resulting from the combination of genetic susceptibility, conferred by a number of immune-related and thyroid-specific genes, with multiple environmental and existential factors [11]–[13].

Both cellular and humoral immunity play a role in Hashimoto's thyroiditis pathogenesis. As *Ajjan et al*. eloquently described, altered activity of T regulatory cells, increased function of follicular helper T cells coupled with release of DNA fragments by cell death and deranged microRNA profile contribute to a breakdown of T cell

tolerance. The subsequent initiation and perpetuation of the autoimmune process results in thyroid infiltration by T and B cells, with predominating Th1 phenotype. These lymphocytes are responsible for thyroid cell injury through: (a) CD8+ mediated cytotoxicity and apoptosis; (b) production of cytokines, which cause disruption of thyroid hormone synthesis, induce the expression of other proinflammatory molecules by the thyroid cells themselves (including MHC II molecules), promote oxidative stress, compromise thyroid follicular cell integrity and further stimulate T and B cells; and (c) antibody production [11], [14].

Antithyroid antibodies are produced by intrathyroidal B cells and, to a lesser extent, in cervical lymph nodes and the bone marrow [15], [16]. Their role in thyroid autoimmunity induced damage to the thyroid gland is unclear. Intrathyroidal complement fixation by thyroid antibodies occurs but might not lyse cells [17]. However, antithyroid antibodies may play a secondary amplifying role in the autoimmune response. By sublethal complement attack, antithyroid antibodies lead to impaired TSH responsiveness, release of pro-inflammatory molecules and oxidative cells [18], [19]. Antibody-dependent cell-mediated cytotoxicity carried out by natural killer cells and monocytes has been demonstrated *in vitro* [20]. Transplacental passage of maternal TPOAb or TgAb does not affect the fetal thyroid, which suggests that a T cell effector is required to initiate autoimmune damage to the thyroid [21].

Most forms of Hashimoto's thyroiditis ultimately evolve into hypothyroidism, although patients can be euthyroid. The progressive destruction and atrophy of the thyroid follicular unit results in the loss of production and secretion of thyroid hormones and a compensatory rise in TSH [22].

Antithyroid Peroxidase Antibodies

Anti-thyroid peroxidase antibodies (TPOAb) are polyclonal immunoglobulins directed against thyroid peroxidase, belonging to any subclass of IgG class or, rarely, IgA class [23]. Thyroid peroxidase is a key thyroid enzyme localized in the apical membrane of thyrocytes, responsible for catalyzing iodine oxidation, Tg organification and intrachain coupling of iodotyrosines to generate T4 and T3 [24].

TPOAb are found in 5–20% of the general population. The kind of TPOAb found on subjects without thyroid dysfunction seems to differ from TPOAb found patients with thyroid dysfunction, once they do not block TPO activity or interfere with actions of TPOAb found in autoimmune thyroid disease [25], [26]. The significance of TPOAb

in euthyroid individuals is uncertain [27], [28]. TPO might be an antigen for low amounts of naturally occurring autoantibodies, without pathogenic consequences [29].

Detection of TPOAb occurs in 90–95% of autoimmune thyroid disease patients, nearly 100% of Hashimoto's thyroiditis patients and 80% of Graves' disease patients [4], [30]. In contrast with TgAb, TPOAb of autoimmune thyroid disease fix complement, promote cell-mediated cytotoxicity and C3 complement-mediated cytotoxicity, contributing to thyrocyte damage, and competitively inhibit TPO's enzymatic activity [20], [26], [31]–[34].

These differences in activity of TPOAb associated with thyroid dysfunction and TPOAb not associated with thyroid dysfunction might be explained, at least in part, by different IgG subclass distribution. TPOAb from serum of patients' with thyroid dysfunction patients are predominantly IgG1, whilst TPOAb from serum of patients' without thyroid dysfunction are predominantly IgG4 [35]. Additionally, their pathogenic potential might be linked to which TPO epitope they are binding to [36].

Clinical Utility of Antithyroid Peroxidase Antibodies
1. Hashimoto's Thyroiditis and Hypothyroidism

TPOAb are a more sensitive serological marker for thyroid autoimmunity, than TgAb [25], [37]. Only positive TPOAb were found to correlate with thyroid dysfunction and hypothyroidism [25]. There is also a with a significant correlation between the degree of lymphocytic thyroiditis and TPOAb titers [38].

TPOAb measurement participates in the investigation of hypothyroidism, by establishing Hashimoto's thyroiditis as the underlying cause [10], [39].

In the *Clinical Practice Guidelines for Hypothyroidism in Adults: Cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association*, TPOAb status assessment is recommended when nodular thyroid disease or diffuse goiter are suspected to be secondary to autoimmune thyroid disease. There are no recommendations for further evaluations [10].

In the *NICE guideline for thyroid disease: assessment and management*, measuring TPOAb for adults with confirmed primary hypothyroidism is recommended. Repetition of TPOAb testing is not indicated [39].

2. Subclinical Hypothyroidism

Elevated TPOAb titers in subclinical hypothyroidism predict progression to overt hypothyroidism, at an annual progression rate of 4.3% with positive TPOAb versus 2.6% with negative TPOAb [27], [40].

TPOAb assessment is to be considered in the setting of subclinical hypothyroidism [10], [39], [41]. The *NICE guidelines* and the *2013 ETA Guideline: Management of Subclinical Hypothyroidism* do not indicate repetition of TPOAb measurement in subclinical hypothyroidism [39], [41].

Elevated TPOAb levels in patients with subclinical hypothyroidism may warrant attentive monitoring or influence the decision to institute thyroid hormone replacement [8].

Antithyroglobulin Antibodies

Anti-thyroglobulin antibodies are 330-kDa polyclonal immunoglobulins directed against thyroglobulin, belonging to the IgG class or, less often, IgA or IgM class [4], [42]–[44]. Thyroglobulin is a 660-kDa homodimeric glycoprotein stored as colloid in the follicular lumen, representing 75-80% of total thyroid protein, whose physiologic roles include supplying a template for thyroid hormone biosynthesis, storage of iodine, and modulation of thyroid follicular function [45]–[47]. Out of 40 identified epitopes in the Tg molecule, only 6 are immunogenic [48].

Abnormal production TgAb associates with higher serum Tg concentration, higher iodine content of Tg, conformational changes in Tg, and supernormal TSH levels [49].

TgAb prevalence is higher in women, with advancing age and iodine supplementation of iodine deficient areas [25], [50]–[52].

Albeit primarily found as part of the pathogenic mechanisms integrating thyroid autoimmunity [11], TgAb are also present in 10% of the general population without any evidence of thyroid disease [25], [53], [54].

Serum TgAb is a conventional marker for thyroid autoimmunity, being detected in 60% of patients with autoimmune thyroid disease, 60–80% of Hashimoto's thyroiditis patients and 50–60% of Graves' disease patients [4], [55].

The patterns of TgAb differ between autoimmune thyroid disease patients and non-autoimmune thyroid disease patients [48].

Their functional role is unclear. TgAb are not complement fixing, because Tg epitopes are too widely spaced to allow cross-linking [42]. TgAb do not cause thyroid cell destruction [42].

Clinical Utility of Antithyroglobulin Antibodies

1. Autoimmune Thyroid Disease

TgAb are less sensitive thyroid autoimmunity biomarkers, when compared with TPOAb and TRAb [56]. In the absence of TPOAb, TgAb are not associated with thyroid dysfunction [25].

Measurement of TgAb is not necessary for the diagnosis of thyroid autoimmune disease, including Hashimoto's thyroiditis [10], [39].

2. Differentiated Thyroid Cancer

TgAb holds its primary value in the context of differentiated thyroid cancer. They are found in 25–30% of patients with differentiated thyroid cancer, which compounds a two-fold higher rate than that of the general population [54], [57].

The mechanisms of TgAb production in the context of thyroid cancer may stem from an underlying autoimmune thyroiditis or from an immunologic response to thyroglobulin with increased immunogenicity, generated by tumor thyroid cells [48], [58]–[61]. In fact, altered production and secretion of Tg by tumor cells has been observed [62]–[64]. The biosynthetic process of Tg could potentially become unregulated in thyroid tumour cells, resulting in unpredictable changes in the spatial conformation and tertiary structure of Tg, leading to exposure or masking of epitopes and, thus, different Tg immunoreactivity [60], [65], [66]. Different epitope patterns of TgAb described in autoimmune thyroiditis and differentiated thyroid cells support the hypothesis of a dual mechanism [67].

2.1 Postoperative Role of Anti-Thyroglobulin Antibodies in Differentiated Thyroid Cancer

TgAb interference renders serum Tg measurements unreliable, once it can lead to spuriously low or high results in any Tg assay [54], [68]–[73]. Of note, postoperative Tg is an instrumental biochemical marker to ascertain the effectiveness of treatment for differentiated thyroid cancer and for long-term surveillance of residual or recurrent disease, following total thyroidectomy and radioiodine remnant ablation [56], [64], [74]–[76]. Thus, TgAb and Tg must be measured concurrently in the same sample, with interpretation of the Tg level accordingly [56], [74], [77]. Moreover, it is essential that

the presence of TgAb be assessed everytime Tg is measured, once the TgAb status may change overtime [74], [78].

As stated above, the main clinical utility of TgAb is to ensure the reliability of the serum Tg in the follow-up of differentiated thyroid cancer. For patients with positive TgAb, TgAb has evolved as a surrogate marker for the follow-up of differentiated thyroid cancer [64], [79].

TgAb concentrations respond to changes in the mass of Tg-secreting thyroid tissue [80]. Hence, the TgAb trend, defined by the sequential changes in TgAb titers, proves more robust than the qualitative or single quantitative value of TgAb for predicting disease prognosis [81]–[83]. Declining TgAb levels indicate a lower risk of recurrent or persistent disease [83]–[86]. Stable TgAb levels could stem from continued Tg antigen secretion by small volume of remnant thyroid tissue or tumoral micro-foci, as well as long-lived plasma cells [87], [88]. Stable but higher concentrations of TgAb reflect a higher recurrence risk, than lower concentrations of TgAb [84]. A sustained rising trend or permanent new emergence of TgAb, on the other hand, is suggestive of persistent or recurrent disease [79], [83], [89]–[93].

Furthermore, an unsuccessful radioablation correlates with higher post-radioablation TgAb values [94], [95].

Finally, serum TgAb interference in FNA-Tg from suspicious lymph nodes of differentiated thyroid cancer patients is controversial, once it has been reported to happen in some studies [96]–[99], but not in others [100]–[102].

2.2 Preoperative Role of Anti-Thyroglobulin Antibodies in Differentiated Thyroid Cancer

High preoperative TgAb levels weakly predict thyroid cancer in thyroid nodules [42], [103]–[105]. A systematic review and meta-analysis by *Xiao et al.* established sensitivity of 16.04% and specificity of 90.67% of TgAb for the diagnosis of thyroid cancer [106]. Regardless, the ATA guidelines recommend against measurement of preoperative TgAb, considering limited evidence of its significance [56].

PATIENTS AND METHODS

Patients

In the present retrospective observational study, we examined a database comprising 346 patients diagnosed with Hashimoto's thyroiditis, who were assisted at the outpatient endocrine clinic of a public central university hospital in Lisbon (Hospital de Santa Maria – Centro Hospitalar Universitário Lisboa Norte; HSM-CHULN), from 2009 ± 6 to 2014 ± 3. All patients provided a written informed consent concerning the authorization for clinical data use in future research studies, after approval by our hospital's Ethics Committee.

The diagnosis of Hashimoto's thyroiditis was defined as TPOAb or TgAb positivity and absence of hyperthyroidism.

The following variables, retrieved anonymously from patient's clinical records and included in a specifically designed database, were analyzed: (1) sex; (2) age (in years); (3) diagnosis; (4) time since diagnosis (in years); (5) time of follow up (in years); (6) TSH, T3, T4, fT4, Tg, TPOAb, TgAb and TPOAb/TgAb ratio at the first and last visits; (7) presence of thyroid dysfunction at the first visit (defined as TSH outside the normal reference range); (8) levothyroxine therapy at the first and last visits.

Blood samples were collected in the morning (8 – 10h) at the Clinical Pathology Department, after an overnight fast. All thyroid function tests and thyroid antibodies related variables were assessed by commercially available standard chemiluminescent immunoassays, at the Clinical Pathology Department. The department complies with the standards of practice and is a registered and certified facility.

The normal reference ranges considered for these variables are presented in Table 1.

Table 1. Reference range intervals for thyroid variables

TSH	0.35 - 5.50 μU/mL
T3	60 - 180 ng/dL
T4	4.5 - 11 μg/dL
fT4	0.9 - 1.8 ng/dL
Tg	< 55 ng/mL
TPOAb	< 40 U/mL
TgAb	< 40 U/mL

Statistical Analysis

Statistical data analysis was performed using the statistics software program IBM SPSS version 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Limit of significance was set at two-tailed p-value < 0.05.

Normality was verified with the Kolmogorov-Smirnov Test. Non-parametric tests were used for non-normal distributed variables. Differences between the paired first and last visits' variables were tested with the non-parametric Wilcoxon Signed-Rank Test. Chi-square test (χ^2) was conducted to test differences between TPOAb and TgAb positivity in both visits and between visits. Non-parametric Spearman Rank Order Regression was conducted to determine the relation between antithyroid antibodies (TPOAb and TgAb) and thyroid function tests as well as other clinical variables.

Results are presented as the mean ± standard deviation or as percentage, as appropriate.

RESULTS

Study Population Profile

Demographic data were analyzed from 346 patients, 90% of which were female (n = 310). Most patients were middle aged (51 ± 17 years old at the first visit), with long standing disease (8 ± 8 years). Only 24% of patients presented with thyroid dysfunction and 49% were under levothyroxine therapy (hence, a total of 73% of patients had thyroid dysfunction), at the time of the first visit. At the last visit, after an average follow-up of 5 ± 4 years, 9% of patients presented with thyroid dysfunction and 73% were under levothyroxine therapy (hence, a total of 82% of patients had thyroid dysfunction). Biochemical and serological data of all patients are presented in Table 2 and Appendices 1 – 15.

Table 2. Thyroid Function and Serological Data of All Study Subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3

	First Visit			Last Visit		
	n	Mean ± σ	Median	n	Mean ± σ	Median
TSH	333	7.7 ± 29.3	2.9	244	3.9 ± 9.2	2.2
T3	327	131 ± 45	126	242	109 ± 32	104
T4	328	8.7 ± 2.5	8.6	239	8.6 ± 2.4	8.4
fT4	314	1.3 ± 3.3	1.1	239	1.3 ± 0.6	1.2
Tg	150	93 ± 442	18	158	50 ± 240	11
TPOAb	326	966 ± 4254	151	234	527 ± 1957	85
TgAb	325	781 ± 4088	38	230	504 ± 2412	38

TPOAb and TgAb Characteristics

The prevalence of TPOAb positivity was 66,8% (n = 231) at the first visit, and 41,3% (n = 143) at the last visit. The prevalence of TgAb positivity was 46% (n = 159) at the first visit, and 31,8% (n = 110) at the last visit.

The difference between TPOAb positivity at the first and last visits was statistically significant ($p < 0.001$). The difference between TgAb positivity at the first and last visits was statistically significant ($p = 0.001$). The difference between TPOAb positivity and TgAb positivity at the first visit was statistically significant ($p < 0.001$). The difference between TPOAb positivity and TgAb positivity at the last visit was statistically significant ($p < 0.001$).

Serum concentrations of TPOAb were higher than serum concentrations of TgAb in both visits, but this difference was only statistically significant at the first visit ($z = -5,842$, $n = 324$, $p < 0.001$).

At the first visit, 55.7% ($n = 193$) of patients presented with higher serum TPOAb concentrations than serum TgAb concentrations, 28.6% ($n = 99$) of patients presented with higher serum TgAb concentrations than serum TPOAb concentrations and only 8.1% ($n = 28$) of patients presented with equal concentrations of both antibodies (n missing $= 22$). For patients who had higher serum TPOAb concentrations, the mean time of disease was 9 ± 7 years. For patients who had higher serum TgAb concentrations, the mean time of disease was 6 ± 6 years. 76 patients presented with serum TPOAb concentrations 10 times higher than serum TgAb concentrations, while only 27 patients presented with serum TgAb concentrations 10 times higher than serum TPOAb concentrations.

At the last visit, 30.1% ($n = 104$) of patients presented with higher serum TPOAb concentrations than serum TgAb concentrations, 28.9% ($n = 100$) of patients presented with higher serum TgAb concentrations than serum TPOAb concentrations and only 5.9% ($n = 20$) of patients presented with equal concentrations of both antibodies (n missing $= 119$). For patients who had higher serum TPOAb concentrations, the mean time of disease was 9 ± 7. For patients who had higher serum TgAb concentrations, the mean time of disease was 10 ± 8. 42 patients presented with serum TPOAb concentrations 10 times higher than serum TgAb concentrations, while only 22 patients presented with serum TgAb concentrations 10 times higher than serum TPOAb concentrations.

Differences in Thyroid Antibodies and Function Between Visits

Serum concentrations of TSH, T3, T4 and TPOAb were significantly reduced between the first and last visits ($z = -2.748$, $n = 242$, $p = 0.006$; $z = -8.859$, $n = 237$, $p < 0.001$; $z = -2.091$; $n = 235$, $p = 0.037$; $z = -5.706$, $n = 230$, $p < 0.001$; respectively), as shown in Appendix 16 and illustrated in Figure 1a, 1b, 1c, 1f.

However, serum fT4 was significantly increased between the first and last visits, ($z = -3.362$, $n = 221$, $p = 0.01$), as shown in Appendix 16 and illustrated in Figure 1d.

There were no statistically significant differences between visits for serum concentrations of Tg and TgAb, even though we observed a reduction in their titers (Appendix 16 and Figure 1e, 1g).
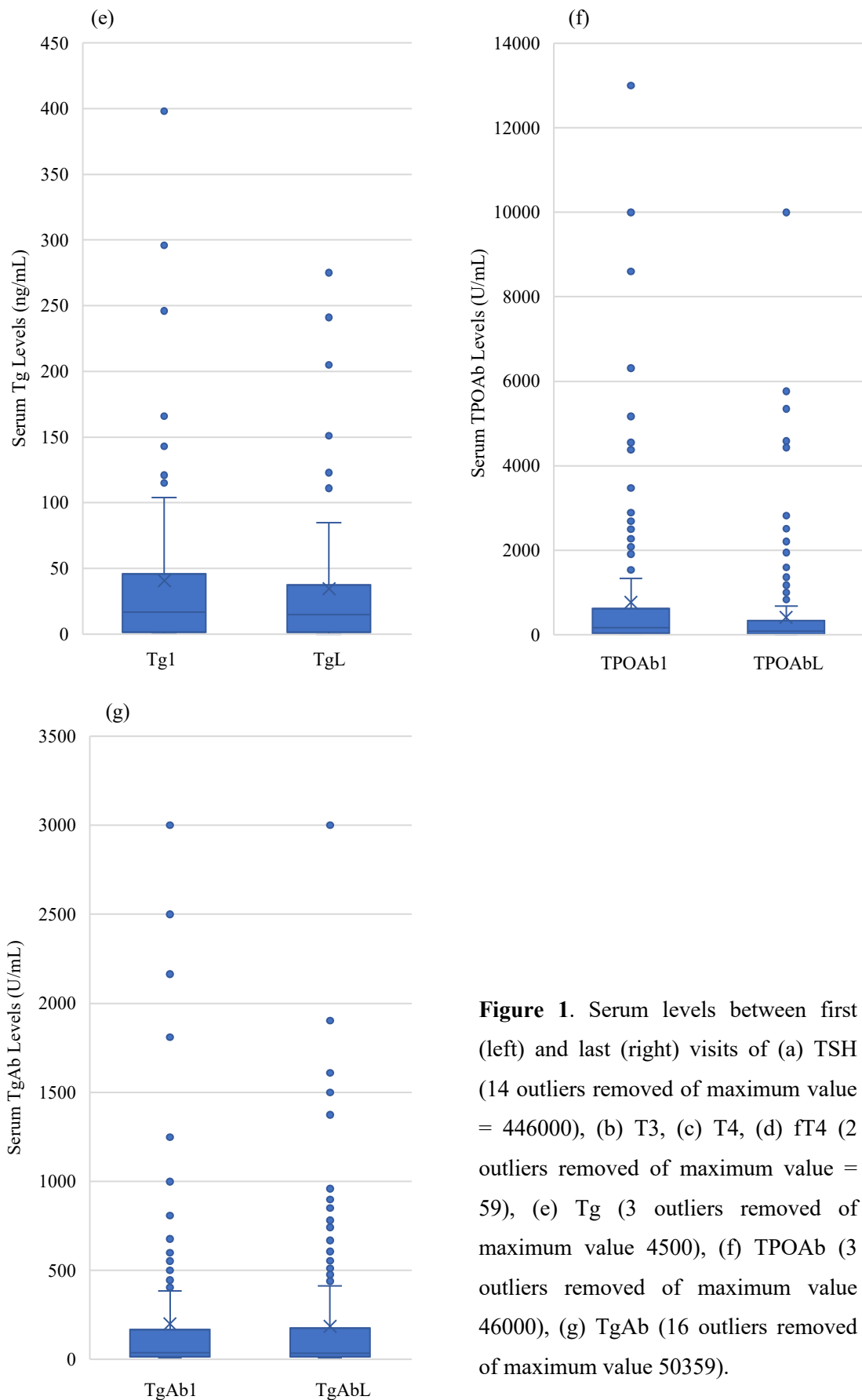
(e)

(f)

(g)
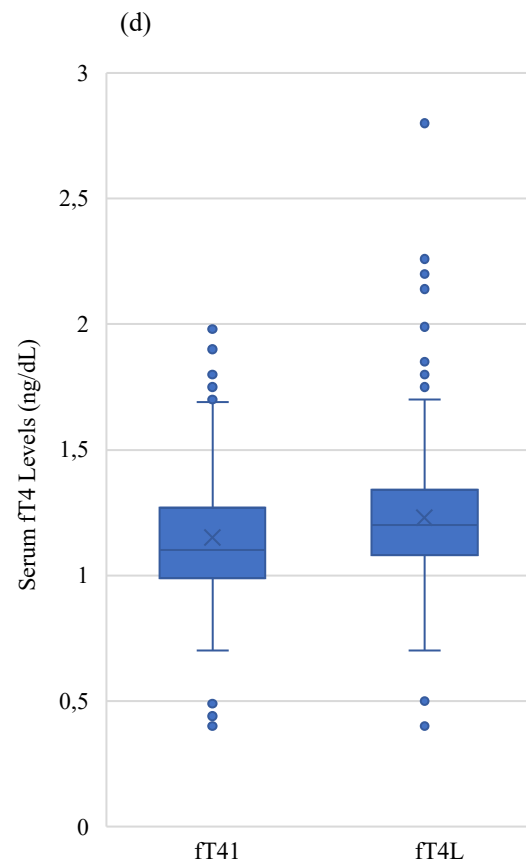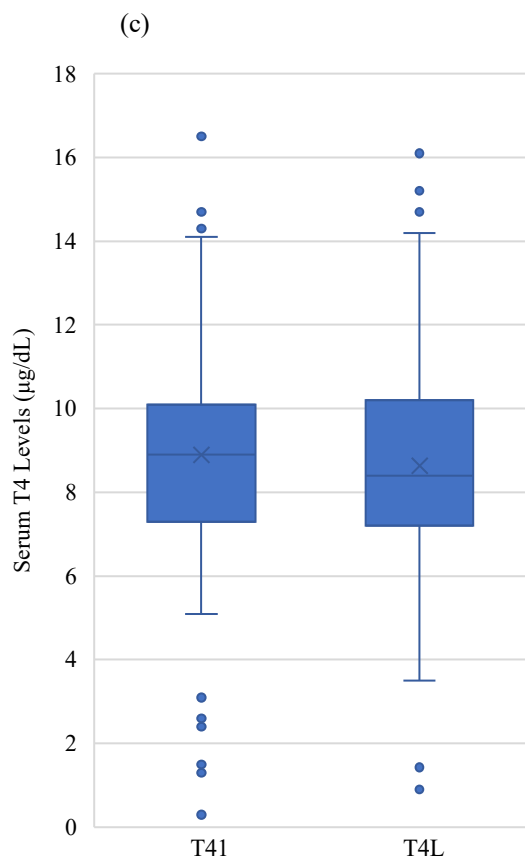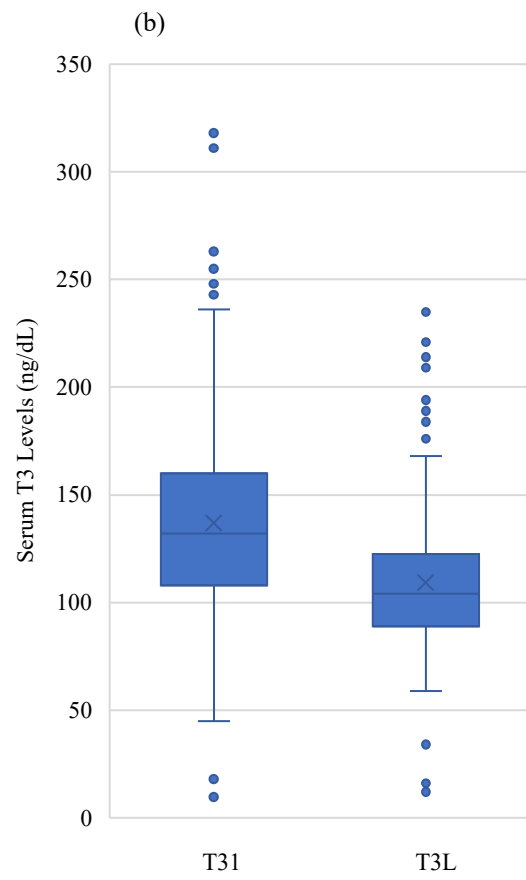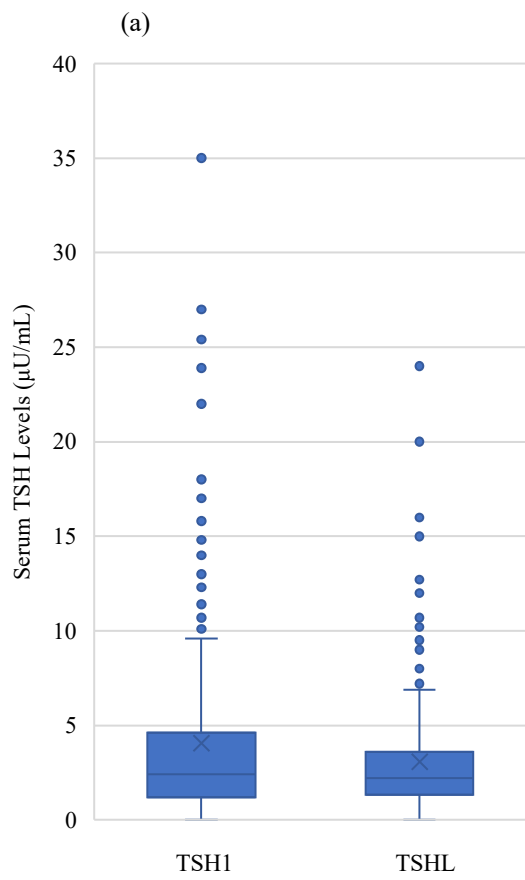
Figure 1. Serum levels between first (left) and last (right) visits of (a) TSH (14 outliers removed of maximum value = 446000), (b) T3, (c) T4, (d) fT4 (2 outliers removed of maximum value = 59), (e) Tg (3 outliers removed of maximum value 4500), (f) TPOAb (3 outliers removed of maximum value 46000), (g) TgAb (16 outliers removed of maximum value 50359).

Influence of Thyroid Antibodies on Other Thyroid Parameters in the Absence of Therapy

1. First Visit

At the first visit of non-treated patients, there was a significant yet weak positive correlation between the serum TPOAb levels and serum TgAb levels ($r = 0.275$, $n = 170$, $p < 0.001$), as shown in Appendix 17. Increases in serum TPOAb levels correlated with increases in serum TgAb levels.

There was a significant weak positive correlation between the serum TPOAb levels and serum TSH levels ($r = 0.212$, $n = 169$, $p = 0.006$), as shown in Appendix 17. Increases in serum TPOAb levels correlated with increases in serum TSH levels.

There was a significant weak negative correlation between the serum TPOAb levels and serum fT4 levels ($r = -0.194$, $n = 158$, $p = 0.014$), as shown in Appendix 17. Increases in serum TPOAb levels correlated with decreases in serum fT4 levels.

Serum TPOAb levels did not significantly correlate with serum T3, T4 or Tg, as shown in Appendix 17.

There was a significant weak negative correlation between the serum TgAb levels and serum Tg levels ($r = -0.319$, $n = 80$, $p = 0.004$), as shown in Appendix 18. Increases in serum TgAb levels correlated with decreases in serum Tg levels.

Serum TgAb levels did not significantly correlate with serum TSH, T3, T4 or fT4, as shown in Appendix 18.

2. Last Visit

At the last visit, serum TPOAb levels did not significantly correlate with serum TgAb, TSH, T3, T4, fT4 or Tg, as shown in Appendix 19.

There was a significant weak negative correlation between the serum TgAb levels and serum Tg levels ($r = -0.370$, $n = 46$, $p = 0.011$), as shown in Appendix 20. Increases in serum TgAb levels correlated with decreases in serum Tg levels.

Serum TgAb levels did not significantly correlate with serum TSH, T3, T4 or fT4, as shown in Appendix 20.

Study Population Subset with Positive Tg and Positive TgAb

At the first visit, there were 13 patients who presented with positive Tg and positive TgAb. They were all women. Their descriptive data is shown on Table 3.

At the last visit, there were 10 patients who presented with positive Tg and positive TgAb. They were all women. Their descriptive data is shown on Table 4.

Table 3. Demographic, biochemical and serological data of patients with positive Tg and positive TgAb, at the first visit

	n	Mean ± σ
Age	13	51 ± 16
Time since diagnosis	3	11 ± 12
TSH1	13	14,5 ± 40,8
T31	13	121,9 ± 60,8
T41	13	7,6 ± 3,2
FT41	12	1 ± 0,2
Tg1	13	102,8 ± 49,4
TPOAb1	12	992 ± 2842,5
TgAb1	13	955,8 ± 2720,5
TSHL	5	2,2 ± 1,3
T3L	5	113,8 ± 17
T4L	5	9,7 ± 1,97
FT4L	4	1,1 ± 0,1
TgL	5	74,8 ± 49,2
TPOAbL	5	106,6 ± 123,4
TgAbL	5	320,4 ± 469,2

Table 4. Demographic, biochemical and serological data of patients with positive Tg and positive TgAb, at the last visit

	n	Mean ± σ
Age	8	56 ± 21
Time since diagnosis	2	7,5 ± 4,9
TSH1	10	3,5 ± 2,63
T31	10	130,5 ± 54,7
T41	10	8,4 ± 2,3
FT41	10	1 ± 0,2
Tg1	5	197,8 ± 142,95
TPOAb1	10	802,6 ± 902,4
TgAb1	10	244,4 ± 554,249
TSHL	10	3,4 ± 2,6
T3L	10	105,6 ± 27,1
T4L	9	8,1 ± 2,99
FT4L	10	1,2 ± 0,3
TgL	10	138,6 ± 51,7
TPOAbL	10	1714,2 ± 3210,6
TgAbL	10	254,2 ± 155,1

DISCUSSION

Although autoimmune thyroid disease is a prevailing autoimmune disorder and the most common cause of primary hypothyroidism in iodine sufficient populations, the functional and clinical role of antithyroid antibodies has not been fully elucidated [1], [107].

While TPOAb is thought to be the most accurate serological biomarker for thyroid autoimmunity, with demonstrated pathogenic consequences, TgAb is less useful and its role is uncertain in autoimmune thyroid disease [25].

The determination of possible relations between TPOAb and TgAb with thyroid hormones and their temporal evolution would be helpful in enlightening their pathologic role autoimmune thyroid disease.

Study Population Profile

In the present study, patients were mainly female and middle-aged, which is in agreement with demographic distribution usually described for autoimmune thyroid disease [1]. Older age and female sex are crucial risk factors for the development of Hashimoto's thyroiditis [11]. In addition, the frequency of TPOAb and TgAb positivity increases with age in women [25], [27]. The female preponderance is likely due to sex steroid effects on the immune response, but a skewed X chromosome inactivation and fetal microchimerism have been proposed as alternative explanations [11].

Long standing disease in Hashimoto's thyroiditis, as was the case for our study population, is also a common finding as the initial course of disease is most often silent, and formal diagnosis takes place years after disease onset [108]. 73% of patients in our study population had thyroid dysfunction at presentation, but most were already under levothyroxine therapy. Nevertheless, an euthyroid presentation and subclinical hypothyroidism in Hashimoto's thyroiditis are not uncommon [10], [22], demonstrating the indolent nature of the underlying autoimmune process and the scope of the compensatory action of TSH [109].

Relationship between TPOAb and TgAb

Autoantibody prevalence in Hashimoto's thyroiditis has been reported as nearly 100% for TPOAb and 60–80% for TgAb [5, 6]. In our study, all patients were positive for one or the other antibody, as antibody positivity was one of the selection criteria for the study population. Furthermore, TPOAb was more frequently measurable than TgAb

(66,8% versus 46% at the first visit; 41,3% versus 31,8% at the last visit; respectively), with a statistically significant difference, which is consistent with antithyroid antibody status described in previous studies [30], [37], [110]–[114].

In comparison to TgAb, TPOAb displays higher serum concentrations in autoimmune thyroid disease [9]. Overall, serum concentrations of TPOAb were higher than TgAb, in our study sample, but this difference was only statistically different for the first visit. However, if one considers only the qualitative value of the antibodies (positive versus negative), the proportion of TPOAb positivity was significantly higher than the proportion of TgAb positivity in both visits. Additionally, the wide range in quantitative values of TPOAb and TgAb we found was also documented in different studies [111], [115], [116].

Nonetheless, almost one third of patients in both visits had higher serum TgAb concentrations than TPOAb. Thus, there were patients who preferentially produced TPOAb, while others mainly developed TgAb. *Carlé et al.* found no differences in patient characteristics (sex, age, iodine status and goiter) between these two patient groups, in their study population, and proposed that a random phenomenon might determine which antibody is predominantly generated [111].

We reported a significant positive but only modest correlation between the two antibodies (TPOAb and TgAb) in our study population of Hashimoto's thyroiditis patients, a similar finding to other population-based studies. However, this correlation was only found at the first visit. *Carlé et al.* analyzed autoantibody status in 186 patients with primary hypothyroidism and observed a significant positive weak correlation between log TPOAb and log TgAb ($r^2 = 0.11$, $p < 0.001$, n = 145) [111]. Likewise, a review by *Caturegli et al.* analyzed 6200 serum samples from the 2007–2008 NHANES survey and 4977 serum samples tested by the Johns Hopkins Immunology Laboratory and noted, again, a significant positive weak correlation between TPOAb and TgAb in both groups ($r^2 = 0.21$; $r^2 = 0.46$; $p < 0.0001$; respectively) [22]. *Feldt-Rasmussen et al.* defined a significant positive weak correlation between the quantitative concentrations of TPOAb and TgAb (Spearmann's rho = 0.65, $p < 0.001$), for both thyroid diseases and non-thyroid autoimmune diseases.

It is worth questioning if the weak nature of the correlation between TPOAb and TgAb and the occurrence of predominant production of one antibody or the other are related.

Caturegli et al. have argued that the weak correlation between the two autoantibodies suggests they are expressed at different stages of disease, with TgAb preceding the appearance of TPOAb [22]. An immune escalation mechanism has been proposed, where TgAb would be present at disease onset as an initial response from the innate immune system and TPOAb would represent an ensuing adaptive immune response [22], [117]. In fact, this was demonstrated in mouse models of spontaneous autoimmune thyroiditis [118]. In human subjects, diagnosis of Hashimoto's thyroiditis usually takes places years after disease onset, hence TPOAb are expected to be more prevalent and at higher serum titers [22].

Could this mechanism simultaneously explain the weak antibody correlation and the reason why certain patients mainly developed TgAb instead of TPOAb? Following the immune escalation model, patients who mainly expressed TgAb would be at earlier stages of disease. Interestingly, *Carlé et. al* also found that serum TSH tended to be lower in patients predominantly positive for TgAb, even though this was not statistically significant [111]. Serum TSH is expected to increase as thyroid hormone production decreases, which would be the outcome of progressive autoimmune thyroiditis [109]. By this line of thinking, higher TSH levels, like higher TPOAb levels, would be expected at later stages of disease.

One question remains to be answered: why was the correlation between TPOAb and TgAb not found at the last visit? If the last visit represents a later stage of disease, according to the immune escalation hypothesis, the discrepancy between TPOAb and TgAb production and corresponding serum levels would be even more skewed towards TPOAb at this stage. Thus, a correlation would be less likely at later stages of disease.

27 and 22 patients had serum TgAb concentrations 10 times higher than serum TPOAb concentrations at the first and last visits, respectively. It is questionable if such a considerable disparity fits the immune escalation model or if this subset of patients represents a different clinical situation, such as a specific immune response triggered by differentiated thyroid cancer [48], [58]–[61], [119]. In fact, TgAb has been shown to respond to changes in the mass of Tg-secreting thyroid tissue [80]. Simultaneously, the majority of differentiated thyroid cancer show an increased preoperative serum Tg [120].

Differences in Thyroid Antibodies and Function Between Visits

In the present study, TSH, T3, T4 and TPOAb titers were reduced and fT4 titers were increased between visits. TgAb and Tg levels were decreased between visits, but such difference was not statistically significant. Despite this, the number of TgAb positive cases was significantly reduced between visits. The same applied to the number of TPOAb positive cases. We equate two possible reasons to explain these findings.

First, one cannot overlook the possible influence of levothyroxine, as the percentage of patients under levothyroxine therapy in our study increased from 49% to 73% between visits. A recent systematic review and meta-analysis reviewing 25 randomized clinical trials concerning the clinical efficacy of levothyroxine in the treatment of overt and subclinical hypothyroidism reported, when comparing the levothyroxine group and the placebo group: a significant decrease in TSH and increase in fT4 in both overt or subclinical hypothyroidism; and no significant difference in T3 for overt hypothyroidism, but a significant decrease in T3 for subclinical hypothyroidism [121]. Thyroid hormone replacement achieves normal TSH titers, by correcting the overstimulation of the hypothalamic-pituitary-thyroid axis [109], [122]. Reduced TSH levels could contribute to reduced T3, T4 and Tg levels, by the decrease of stimulation of the thyroid gland. Previous research reports decrease of TPOAb levels following levothyroxine therapy [112], [123]–[127]. *Chiovato et al.* argued that such a decrease in TPOAb titers could not be due to spontaneous fluctuations of antibody, as they did not find a similar significant decrease in euthyroid patients with Hashimoto's thyroiditis [112]. TPO gene expression and activity as well as targeting of TPO to the thyrocyte membrane is a TSH enhanced phenomenon [24], [128]–[130]. As argued by other authors, the suppression of serum TSH levels by levothyroxine therapy could cause a reduction in expression of the TPO autoantigen, halting the ensuing autoimmune response and decreasing TPOAb levels [112], [126], [127]. In fact, *Guclu et al.* also showed a reduction in inflammatory markers (serum IL-12 and IFN-γ) involved in the pathogenesis of Hashimoto's thyroiditis, and we hypothesize it could mirror an overall decrease in the autoimmune response [126]. The reduced stimulation of the thyroid gland by TSH could also account for a decrease of TgAb. This protein has been shown to be dependent on TSH [45], [49].

Second, we must consider the natural history of Hashimoto's thyroiditis. The destructive process in chronic lymphocytic thyroiditis entails atrophy of the follicular epithelium paired with variable degrees of fibrosis of the thyroid gland [131]. Follicular

atrophy and colloid loss could lead to the gradual loss of thyroid hormonogenesis, accounting for the reduction in T3, T4 and Tg. In a similar fashion to what was demonstrated in thyroid cancer, the reduction of thyroid antibodies could be explained by the atrophy and fibrosis of the thyroid gland. In differentiated thyroid cancer, complete removal of thyroid tissue by total thyroidectomy and radioablation therapy, with its antigenic elements and intrathyroidal lymphocytes, is expected to yield full disappearance of all major anti-thyroid antibodies in the serum, with a median disappearance time of 3 years for TgAb and of 6.3 years for TPOAb [80]. *Chiovato et al.* reported a statistically significant correlation between the disappearance of thyroid tissue and that of anti-thyroid antibodies [80]. In this scenario, the elimination of inciting thyroid antigens removes the stimulus that drives antibody production and antigen presentation to the immune system, whilst the elimination of intrathyroidal lymphocytes equates the eradication of the main source of anti-thyroid antibody production. We hypothesize that the atrophy of the thyroid gland could result in the partial depletion of thyroid autoantigens and intrathyroidal B cells, thereby partially removing the stimulus and main sources for production of antithyroid antibodies. To the best of our knowledge no studies correlate the degree of thyroid atrophy with expression of thyroid autoantigens and respective antibodies.

Influence of Thyroid Antibodies on Other Thyroid Parameters

A significant yet weak positive correlation between the serum TPOAb levels and serum TSH levels was found at the first visit, but not at the last visit. This correlation between TSH levels and TPOAb titers was found in hypothyroid patients and in euthyroid individuals in other studies [28], [113], [115], [132]–[134]. The relationship between TPO and TSH highlighted above could also explain such an association. Conversely, *Roos et al.* suggested that this correlation, and the fact that TSH and TPOAb were independent predictors of future development of hypothyroidism, could mean that the maintenance of euthyroidism in the presence of TPOAb required a compensatory increase in TSH levels [28].

TPOAb titers negatively, but weakly, correlated with fT4 levels only at the first visit. TPOAb have pathogenic potential in Hashimoto's thyroiditis – they fix complement, promote cell-mediated cytotoxicity and C3 complement-mediated cytotoxicity, and competitively inhibit TPO's enzymatic activity [20], [26], [31]–[34] –, but its translation *in vivo* and the importance it takes in the overall autoimmune process

is dubious. TPOAb were found to correlate with thyroid dysfunction [25]. The negative correlation between TPOAb and fT4 can clarify the positive correlation between TPOAb and TSH. If TPOAb decrease fT4, it would result in a compensatory increase of TSH.

TPOAb were not found to correlate with T3 or T4. A study encompassing 311 participants (261 diagnosed with autoimmune thyroid diseases) found a significant correlation between serum T3 and TPOAb as well as TgAb [115]. Another study of 2425 subjects under suspicion of thyroid disease showed a correlation between serum T4 and TPOAb [134]. This inconsistent relationship of TPOAb with T3 and T4 could be caused by the binding of these hormones to thyroxine-binding globulin and other transport plasma proteins.

We found no correlation between TgAb and TSH, even though abnormal TSH levels were associated with abnormal Tg levels and increased production of TgAb [45], [49], [113].

TgAb titers did not correlate with T3, T4 or fT4. TgAb are not complement fixing and do not cause thyroid cell destruction [42], hence it could have an even smaller probability of influencing thyroid hormone levels.

Predictably, there was a significant negative but weak between serum TgAb and Tg levels, at both visits. Because TgAb are directed against Tg, this correlation is expected. TgAb interference nullifies serum Tg measurements, once it can lead to false results in any Tg assay [68], [69]. Theoretically, the presence of TgAb leads to the negativization of Tg measurements, which could explain the weak correlation. Moreover, the TgAb interference does not seem to be completely concentration dependent, as low concentrations of TgAb might interfere with Tg measurements and high concentrations of TgAb might not [54], [70], [135], further explaining the significant but weak correlation. In a practical scenario, this means it is difficult to predict the degree of Tg under estimation or over estimation based on the TgAb concentration [120].

Study Population Subset with Positive Tg and Positive TgAb

A small number of patients presented with positive Tg and TgAb. The expected TgAb interference in Tg quantitation methods results in spuriously low Tg results, which makes this population subset unexpected. A few hypotheses could substantiate this clinical scenario. First, TgAb interference can cause falsely high Tg results,

especially in radioimmunoassays (RIA) [72], [73]. In addition, as stated above, the TgAb concentration does not show a linear correlation with TgAb interference [54], [70], [135]. Second, the presence of heterophile antibody (HAb), whose screening is not routinely done, may result in falsely high serum Tg immunometric assays (IMA) measurements [71]. Third, if these subsets represent patients with differentiated thyroid cancer, the true value of Tg can be very elevated, largely surpassing the capacity of TgAb interference. Again, it has been shown that the majority of differentiated thyroid cancer show an increased preoperative serum Tg [120]. Fourth, the heterogeneity of both Tg and TgAb offer an additional explanation [48], [58]–[61]. In the setting of thyroid cancer, TgAb could be directed against normal Tg, as part of the Hashimoto's thyroiditis response, and the assays could be measuring tumoral Tg. The opposite also applies: TgAb could be directed against a tumoral Tg with increased immunogenicity, as part of a cancer specific immune response, and the assays could be measuring normal Tg.

Study Limitations

This study has potential limitations, which weaken our findings. First, this is a retrospective and single center study with a relatively small sample of patients. Therefore, our study population characteristics might not match those of the general Hashimoto's thyroiditis population. In addition, our study's database was based in patient's clinical records, and both might be subject to a registry bias. Considering the indolent nature of Hashimoto's thyroiditis, the follow-up period might not have been enough to reflect the magnitude of change in thyroid function and antibodies. Another limitation would be that therapeutic adherence to levothyroxine was not evaluated. Unfortunately, we did not have the support of the Clinical Pathology Department to further investigate a few of our hypothesis to explain our findings.

Conclusion

Our knowledge on Hashimoto's thyroiditis remains ever expanding. The clarification of relationship and temporal evolution of antithyroid antibodies and thyroid function could help elucidate Hashimoto's thyroiditis pathogenesis and, eventually, help define the management of these patients. The follow-up change we observed in antithyroid antibodies and thyroid function shows us that Hashimoto's thyroiditis is not an immutable disease. The correlation between TPOAb and fT4 and TSH might suggest

this antibody's importance has been disregarded. The difference in TPOAb and TgAb expression in distinct patient subsets could reflect the evolution of Hashimoto's thyroiditis, a random phenomenon or a different clinical situation altogether.

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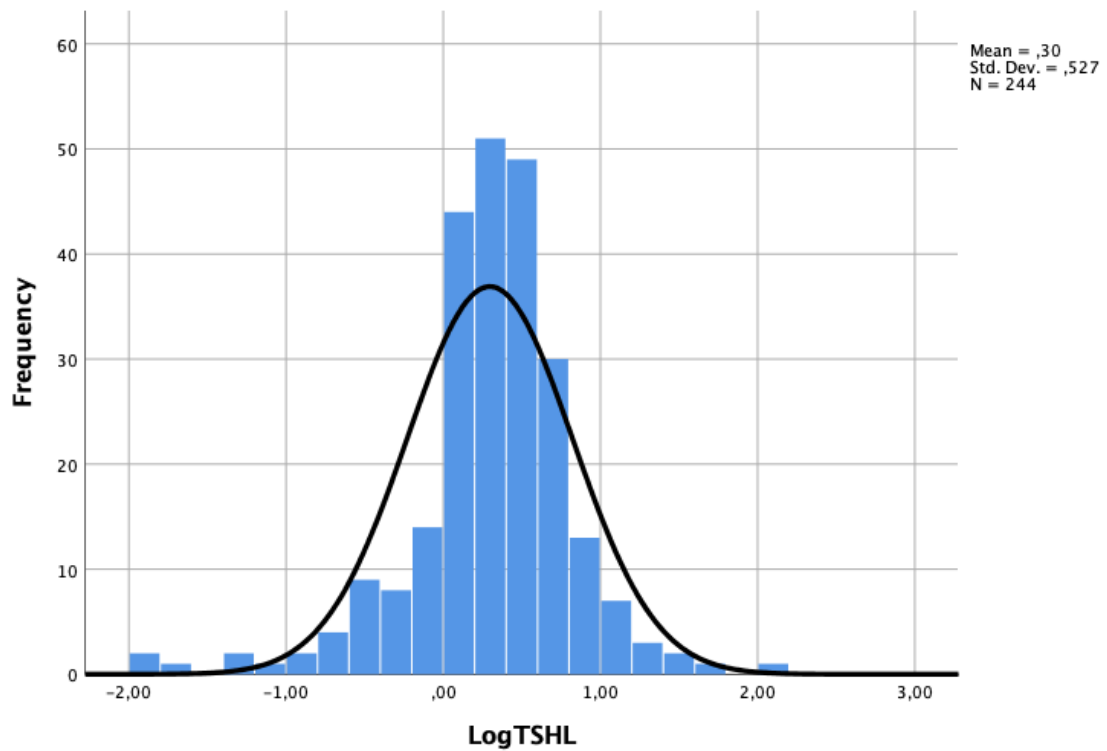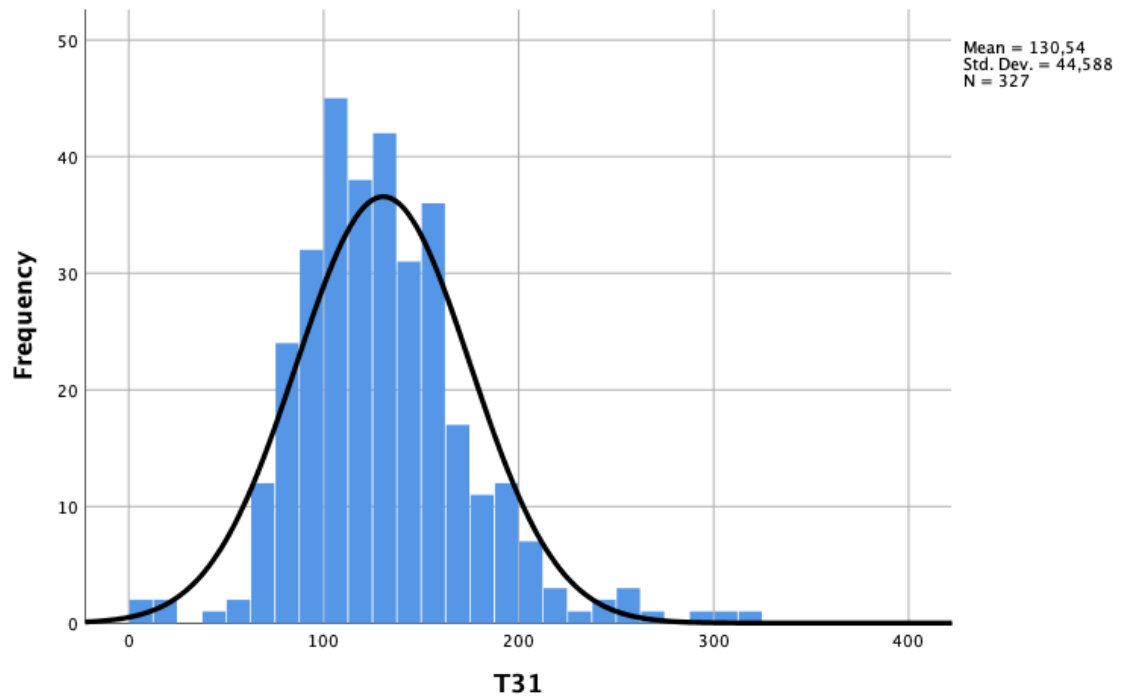
APPENDICES

Mean = 51
Std. Dev. = 16,925
N = 325
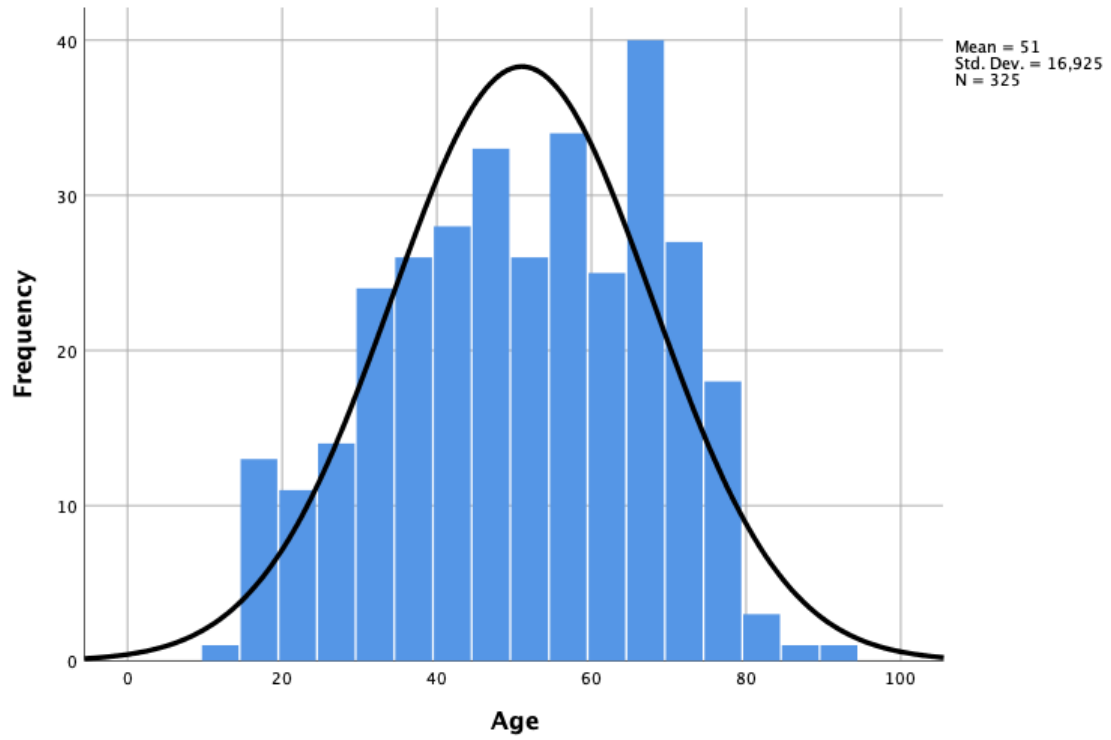
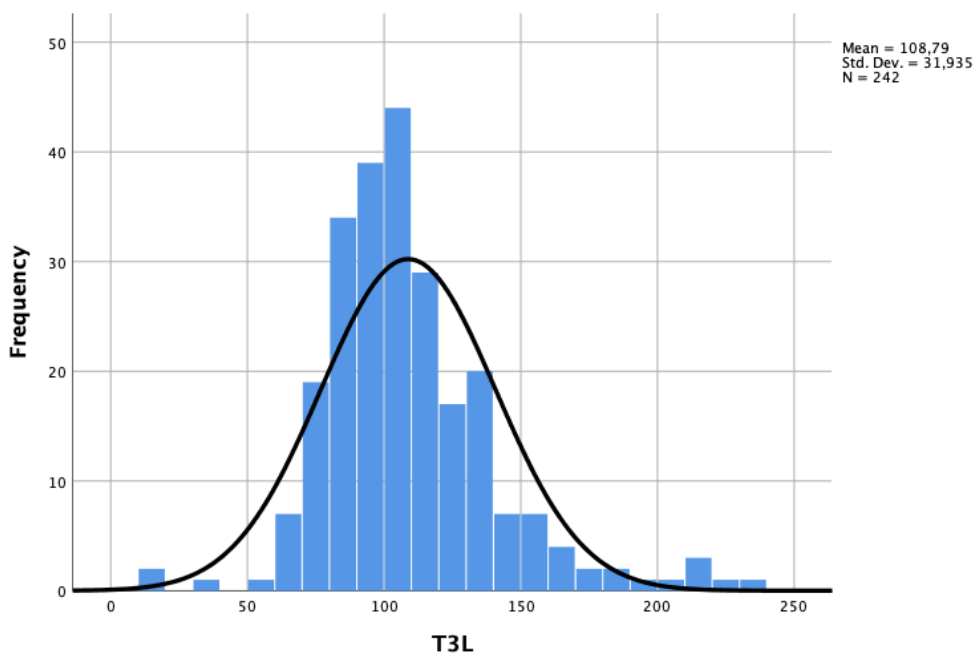**Appendix 1**. Age distribution of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
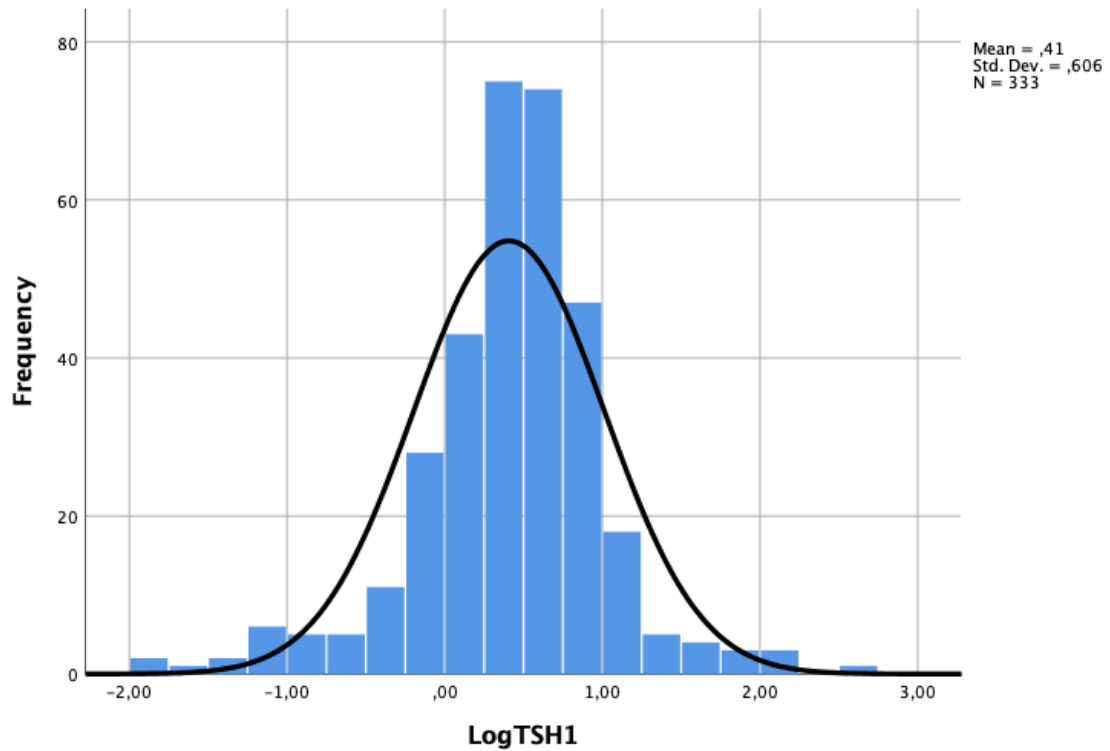
Appendix 2. Log transformed serum TSH distribution from the first visit of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
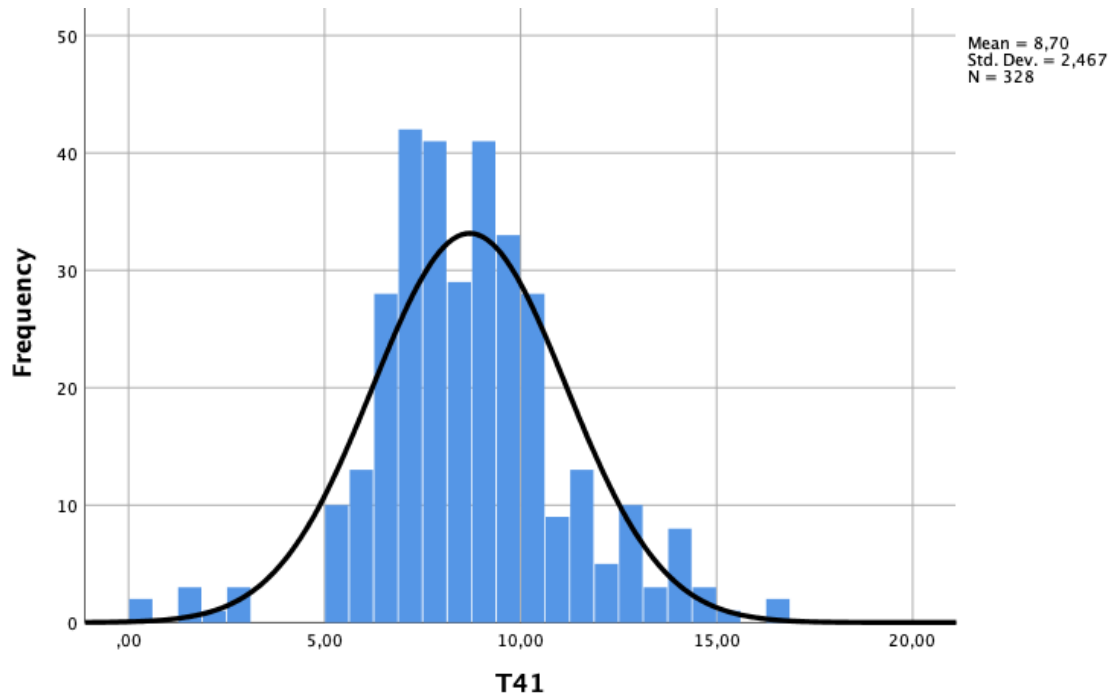

Appendix 3. Log transformed serum TSH distribution from the last visit of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
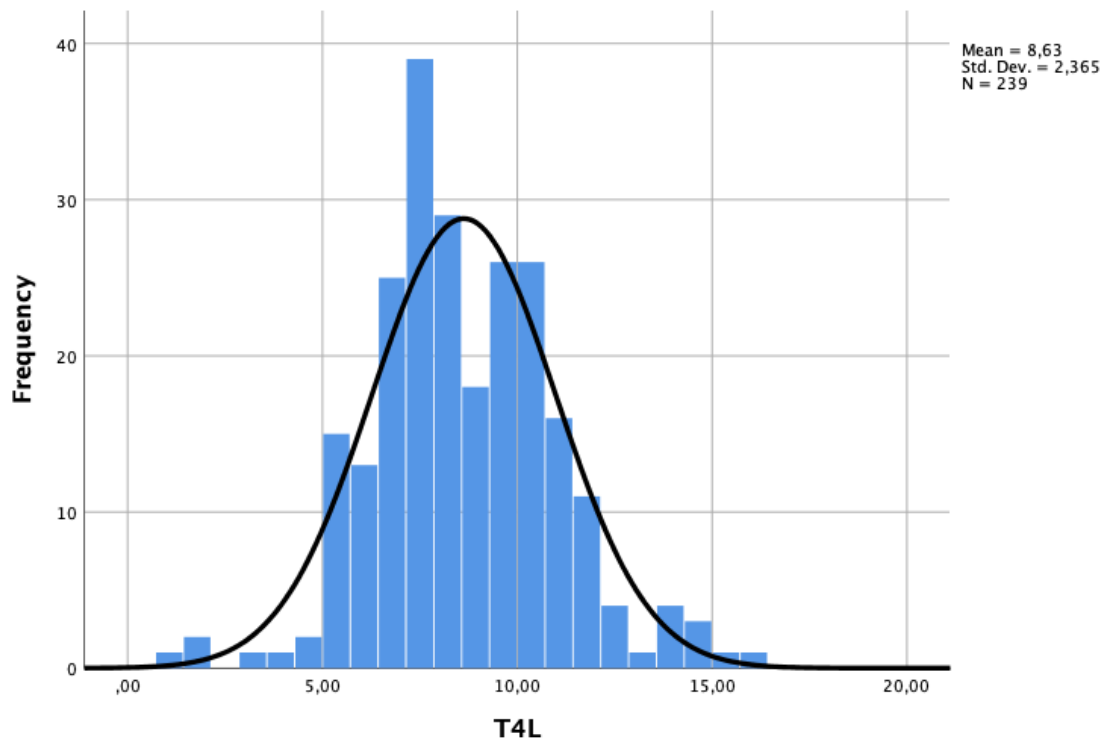
Appendix 4. Serum T3 distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
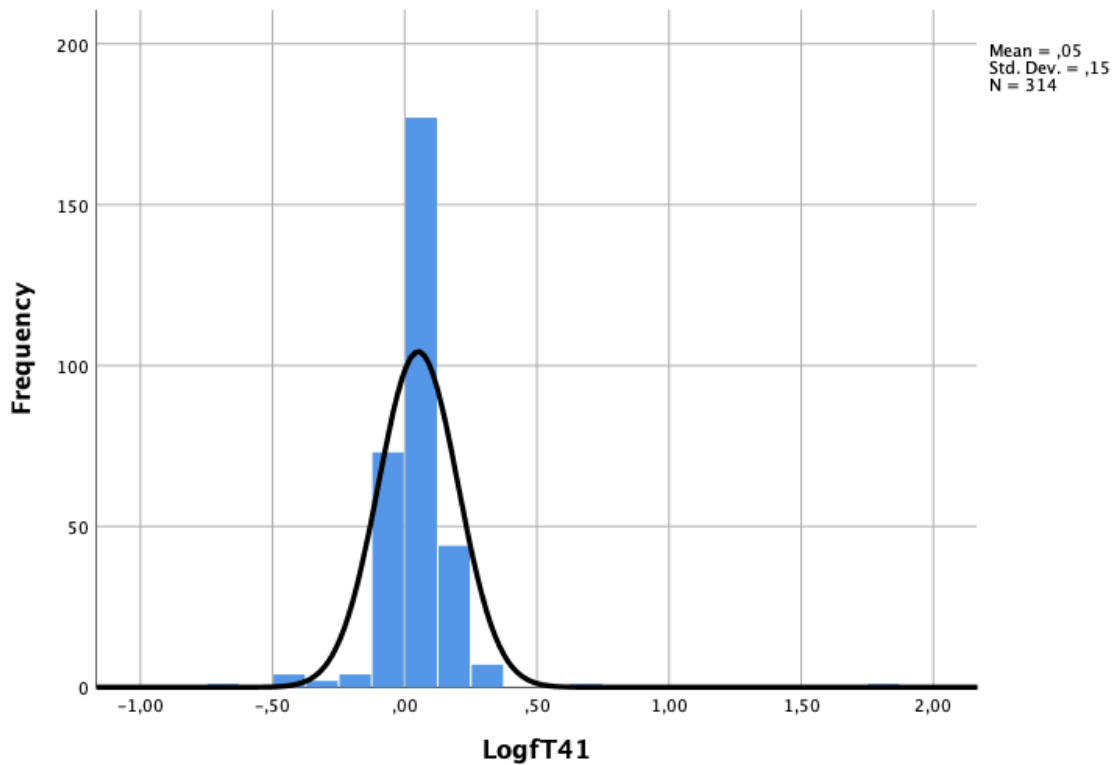

Appendix 5. Serum T3 distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Mean = 8,70
Std. Dev. = 2,467
N = 328

Appendix 6. Serum T4 distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Mean = 8,63
Std. Dev. = 2,365
N = 239

Appendix 7. Serum T4 distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
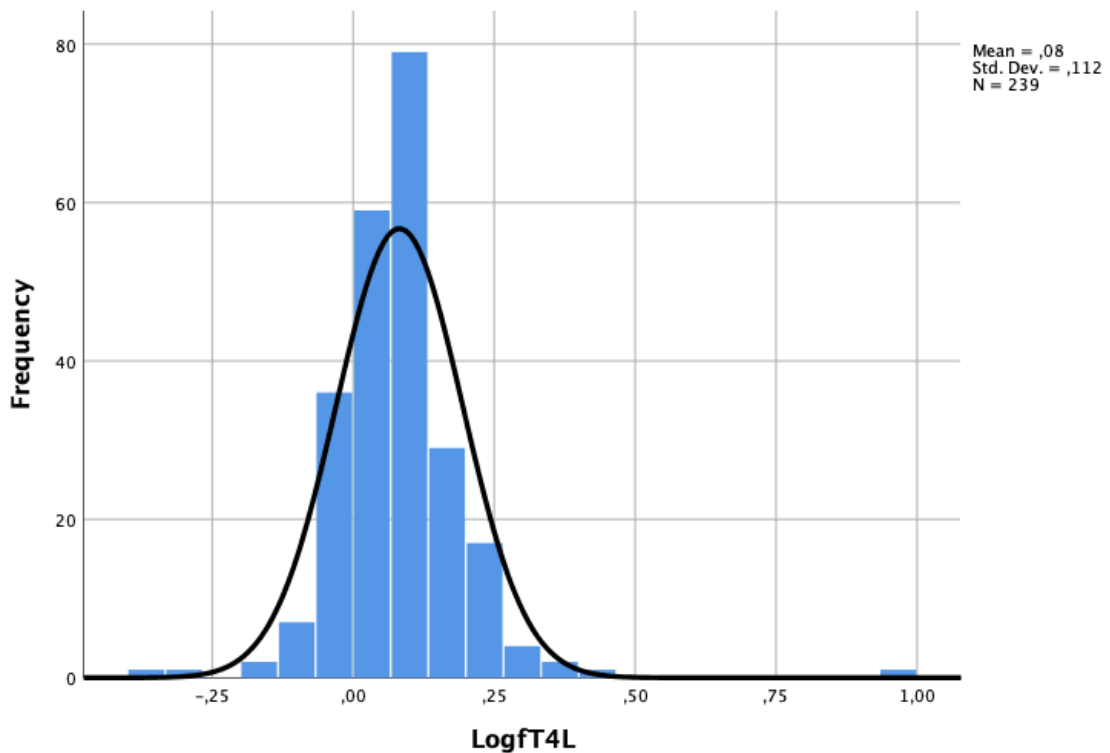
Appendix 8. Log transformed serum fT4 distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Appendix 9. Log transformed serum fT4 distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Appendix 10. Log transformed serum Tg distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
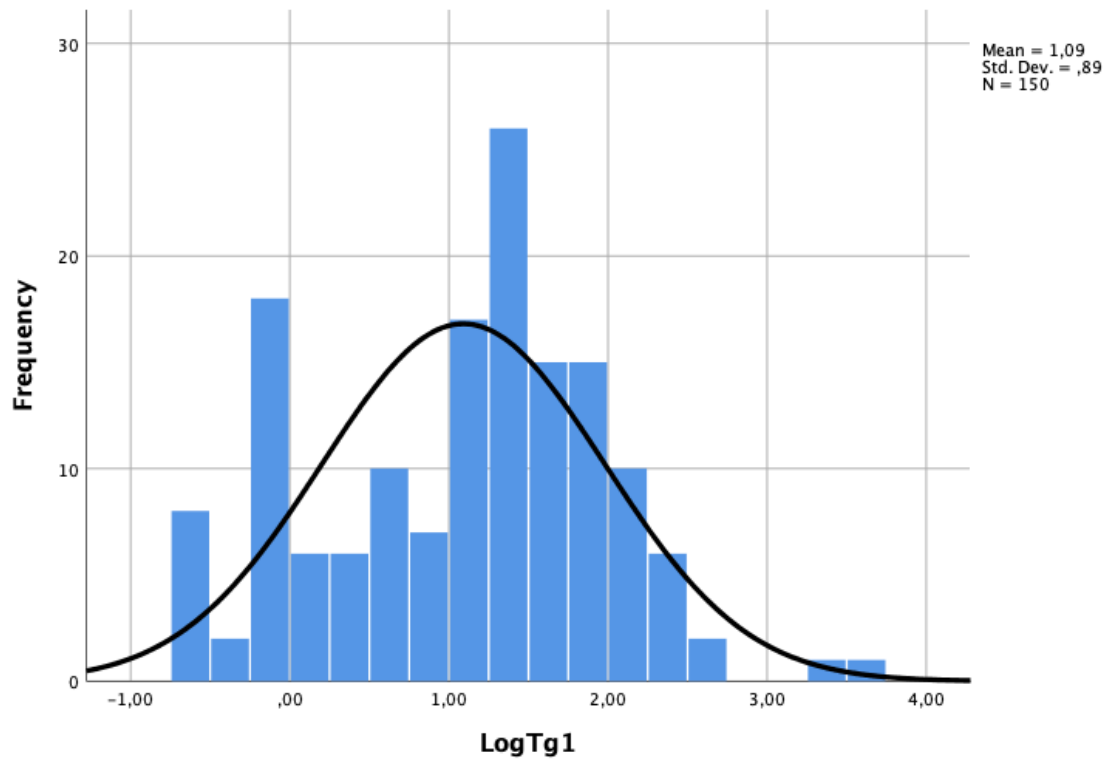

Appendix 11. Log transformed serum Tg distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
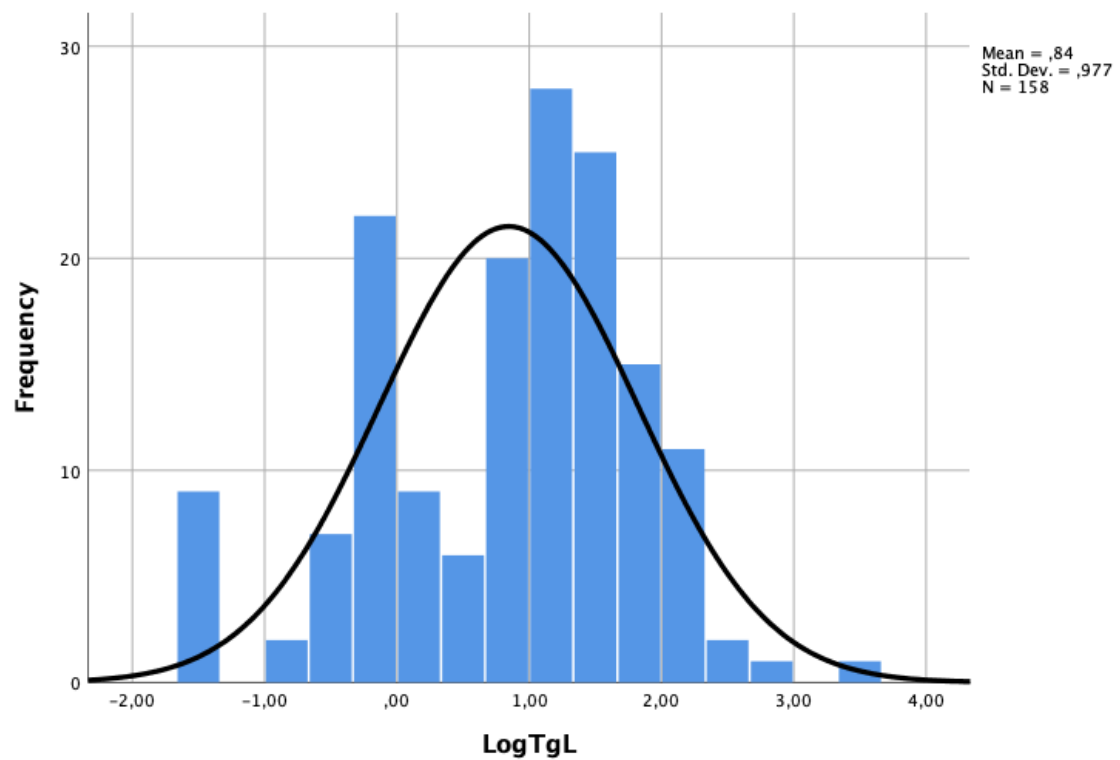
Appendix 12. Log transformed serum TPOAb distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Appendix 13. Log transformed serum TPOAb distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Mean = 1,84
Std. Dev. = ,756
N = 325

Appendix 14. Log transformed serum TgAb distribution, from the first visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.

Mean = 1,83
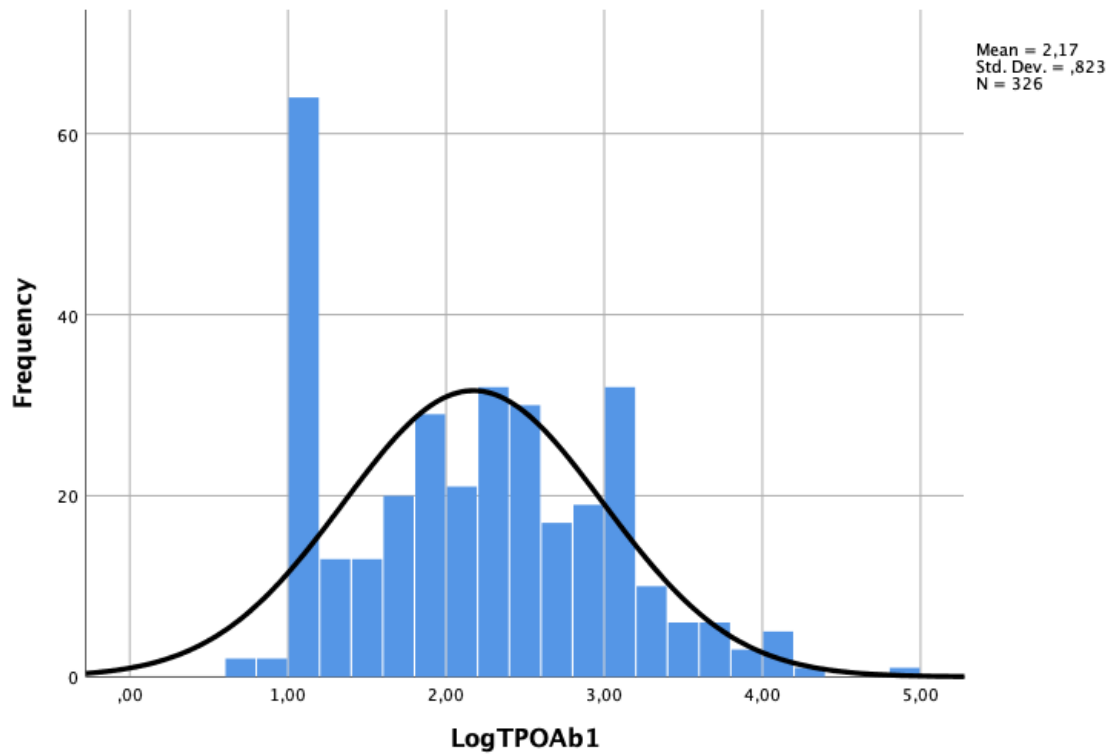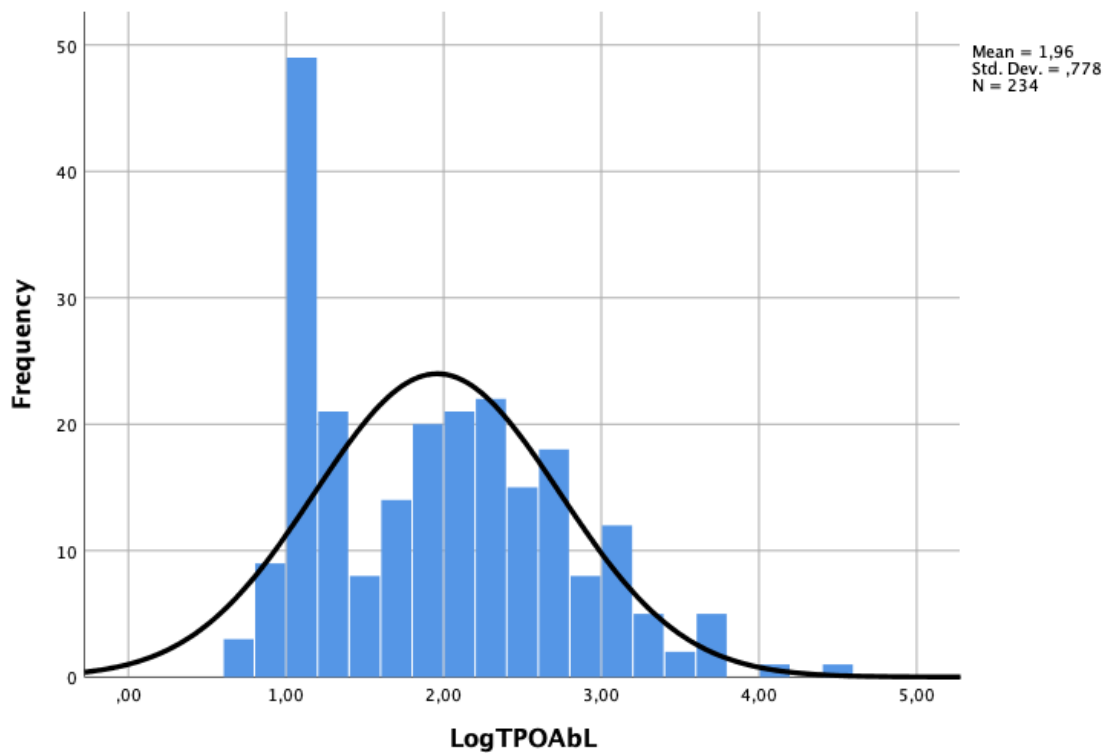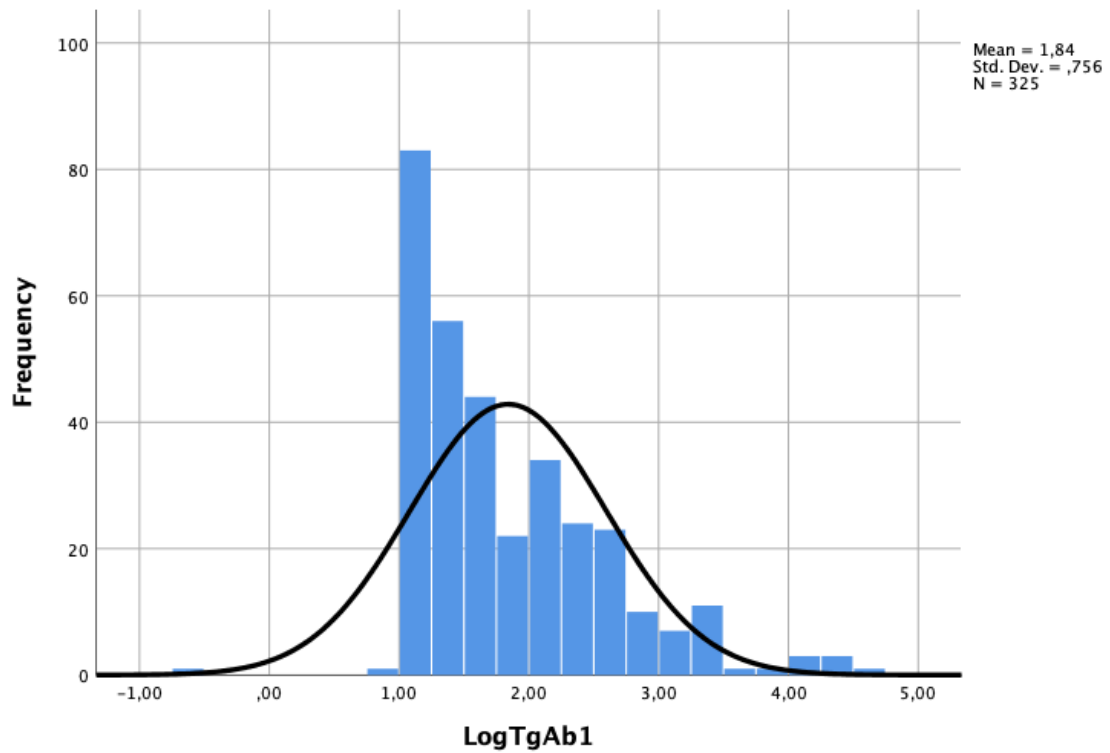Std. Dev. = ,729
N = 230

Appendix 15. Log transformed serum TgAb distribution, from the last visit, of all study subjects from HSM-CHULN, followed from 2009 ± 6 to 2014 ± 3.
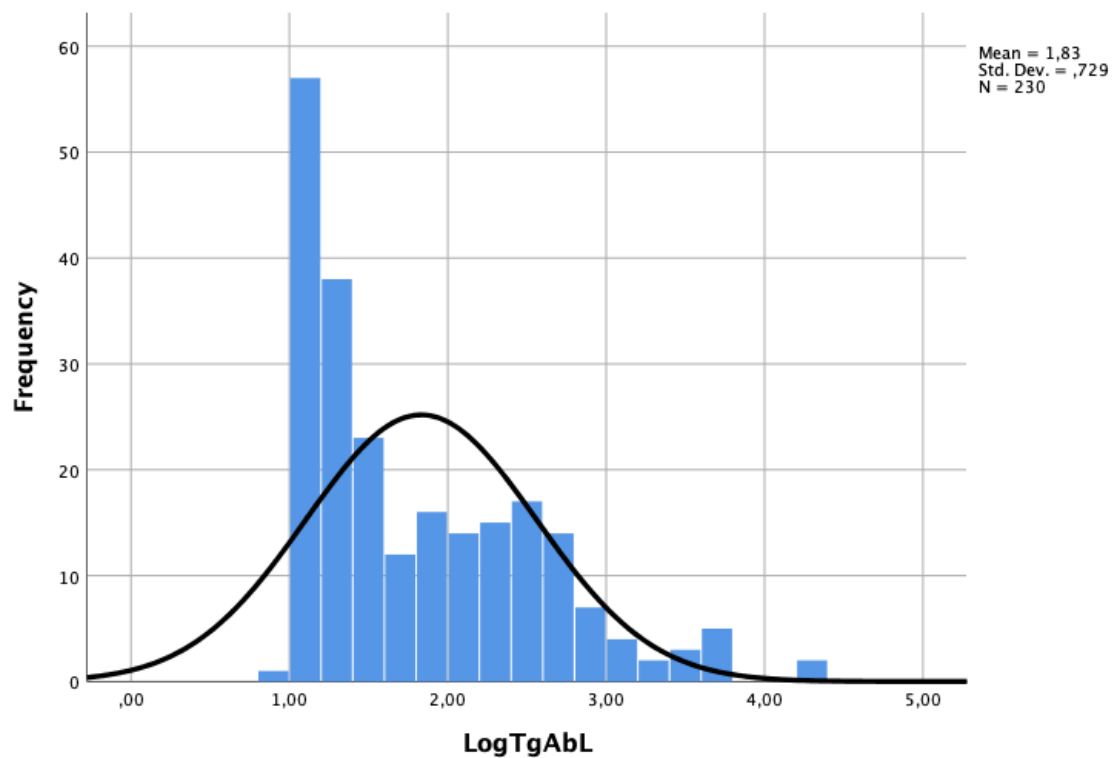
Appendix 16. Results of the Wilcoxon Signed-Rank Test to detect differences between first and last visits in all variables

Variables	z	p-value	n	n detailed
TSH	-2.748	0.006	242	Negative: 137
				Positive: 103
				Ties: 2
T3	-8.859	<0.001	237	Negative: 184
				Positive: 53
				Ties: 0
T4	-2.091	0.037	235	Negative: 135
				Positive: 96
				Ties: 4
fT4	-3.362	0.01	221	Negative: 88
				Positive: 124
				Ties: 9
Tg	-0.744	NS (0.457)	71	Negative: 36
				Positive: 33
				Ties: 2
TPOAb	-5.706	<0.001	230	Negative: 155
				Positive: 59
				Ties: 16
TgAb	-1.126	NS (0.260)	224	Negative: 108
				Positive: 89
				Ties: 27

Appendix 17. Results of the Spearman Rank Order Regression to detect relations between TPOAb and TgAb, TSH, T3, T4, fT4 and Tg at the first visit of non-treated patients

Variables	r	n	p-value
TgAb1	0.265	170	< 0.001
TSH1	0.212	169	0.006
T31	-0.010	167	NS (0.897)
T41	-0.003	169	NS (0.973)
fT41	-0.194	158	0.014
Tg1	-0.060	80	NS (0.595)

Appendix 18. Results of the Spearman Rank Order Regression to detect relations between TgAb and TPOAb, TSH, T3, T4, fT4 and Tg at the first visit of non-treated patients

Variables	r	n	p-value
TSH1	-0.012	169	NS (0.881)
T31	-0.327	167	NS (0.327)
T41	-0.038	169	NS (0.627)
fT41	-0.098	158	NS (0.221)
Tg1	-0,319	80	0.004

Appendix 19. Results of the Spearman Rank Order Regression to detect relations between TPOAb and TgAb, TSH, T3, T4, fT4 and Tg at the last visit of non-treated patients

Variables	r	n	p-value
TgAbL	0.188	67	NS (0.127)
TSHL	0.139	70	NS (0.251)
T3L	0.060	68	NS (0.625)
T4L	-0.119	67	NS (0.339)
fT4L	-0.112	71	NS (0.354)
TgL	0.178	49	NS (0.220)

Appendix 20. Results of the Spearman Rank Order Regression to detect relations between TgAb and TPOAb, TSH, T3, T4, fT4 and Tg at the last visit of non-treated patients

Variables	r	n	p-value
TSHL	-0.026	67	NS (0.832)
T3L	0.069	66	NS (0.582)
T4L	0.060	65	NS (0.635)
fT4L	0.113	68	NS (0.359)
TgL	-0.370	46	0.011

RESUMO ALARGADO EM PORTUGÊS

Introdução

As doenças autoimunes da tiróide, incluindo a tiroidite de Hashimoto e doença de Graves, permanecem as doenças autoimunes mais frequentes na população adulta. Existe uma preponderância do sexo feminino em todos os tipos de doenças autoimunes da tiróide, exceto na tiroidite relacionada com IgG4.

Tanto a tiroidite de Hashimoto quanto a doença de Graves exibem uma infiltração da glândula tiroideia e a presença de anticorpos contra autoantigénios tiroideus, mais frequentemente contra a peroxidase tiroideia, tiroglobulina e recetor da hormona estimuladora da tiróide.

Com a exceção dos anticorpos contra o recetor da TSH, os autoanticorpos tiroideus, embora úteis para o diagnóstico de doença autoimune da tiróide, assumem-se desprovidos de relevância fisiológica ou clínica e poderão ser determinados apenas uma vez, na primeira consulta.

André Gide, escritor francês, disse que chamamos experiência à repetição dos mesmos erros. Como tal, decidimos desafiar as suposições tradicionais ao explorar dados dos nossos doentes com tiroidite de Hashimoto.

Começaremos por resumir a relevância e utilidade clínicas atuais dos anticorpos antitiroideus neste Trabalho Final de Mestrado. Como estudo retrospetivo observacional, iremos examinar a inter-relação dos anticorpos antitiroideus e a influência dos anticorpos antitiroideus sobre outros parâmetros da função tiroideia bem como a evolução dos anticorpos antitiroideus e função tiroideia ao longo do tempo, na tiroidite de Hashimoto.

Os anticorpos anti-peroxidase são imunoglobulinas policlonais firigidas contra a peroxidase tiroideia, uma enzima tiroideia chave, localizada na membrana apical dos tirócitos e responsável por catalisar a oxidação do iodo, organificação da Tg e emparelhamento intra-cadeia dos resíduos de tirosina iodinados, de forma a gerar T4 e T3.

Os TPOAb são encontrados em 5-20% da população geral. Os TPOAb encontrados nos indivíduos sem disfunção tiroideia parecem diferir dos TPOAb encontrados na doença autoimune da tiróide, na medida em que não bloqueiam a atividade da TPO ou interferem com as ações dos TPOAb associados à doença autoimune da tiróide. O seu significado é incerto nesta população sem doença tiroideia.

A deteção de TPOAb ocorre em 90-95% dos doentes com doença autoimune da tiróide, quase 100% dos doentes com tiroidite de Hashimoto e 80% dos doentes com doença de Graves. Estes TPOAb associados à doença autoimune da tiróide fixam complemento, promovem a citotoxicidade celular e citotoxicidade mediada por C3, contribuindo para danificar os tirócitos, bem como inibem competitivamente a ativiade enzimática da TPO.

Os TPOAb constituem o marcador serológico mais sensível para a presença de autoimunidade tiroideia, especialmente em comparação com TgAb. Apenas a presença de TPOAb positivos se correlacionou com a presença de disfunção tiroideia. Adicionalmente, verifica-se uma correlação significativa entre o grau de tiroidite linfocítica e os níveis de TPOAB.

A medição de TPOAb participa na investigação do hipotiroidismo, contribuindo para definir a respetiva etiologia como tiroidite de Hashimoto. Por outro lado, a sua determinação também se encontra recomendada para a avaliação do hipotiroidismo subclínico, já que níveis elevados de TPOAb predizem a progressão para hipotiroidismo manifesto (taxa anual de progressão de 4.3% se TPOAb positivo versus 2.6% para TPOAb negativo). Nos doentes com hipotiroidismo subclínico, níveis elevados de TPOAb poderão justificar uma monitorização cautelosa ou influenciar a decisão sobre o início do tratamento. A repetição da medição dos TPOAb não está recomendada em guidelines internacionais, quer no hipotiroidismo manifesto quer no hipotiroidismo subclínico.

Os anticorpos anti-tiroglobulina são imunoglobulinas policlonais dirigidas contra a tiroglobulina, uma glicoproteína armazenada no colóide tiroideu, responsável por fornecer o molde para a síntese de hormonas tiroideias, armazenamento de iodo e modulação da função folicular tiroideia.

Apesar de reconhecidos como parte integrante dos mecanismos de autoimunidade tiroideia, os TgAb encontram-se presentes em 10% da população geral sem evidência de doença tiroideia.

A deteção de TgAb ocorre em ocorre em 60% dos doentes com doença autoimune da tiróide, quase 60-80% dos doentes com tiroidite de Hashimoto e 50-60% dos doentes com doença de Graves. Como tal, é um marcador menos sensível que os TPOAb para a autoimunidade tiroideia. Na ausência de TPOAb, os TgAb não se associam a disfunção tiroideia. Na doença autoimune da tiróide, o seu papel funcional é incerto, uma vez que não fixam complemento nem causam destruição das células tiroideias.

A medição dos TgAb não é necessária para o diagnóstico de doença autoimune da tiróide e não incorpora a abordagem do hipotiroidismo.

Os TgAb cumprem o seu principal papel na neoplasia diferenciada da tiróide, onde registam uma prevalência de 25-30% (uma taxa duas vezes superior à população geral).

No contexto pós-tratamento, a determinação dos TgAb pretende garantir a fiabilidade da Tg como marcador de doença neoplásica persistente ou recorrente, após eliminação completa de tecido tiroideu através de tiroidectomia total e terapêutica radioablativa. Isto porque a presença de TgAb inviabiliza as medições de Tg por mecanismos de interferência, em qualquer dos métodos de quantificação utilizados. A constatação de que as concentrações de TgAb respondem a alterações na massa de tecido tiroideu secretor de Tg resultou na evolução do papel dos TgAb na neoplasia diferenciada da tiróide. A tendência dos níveis de TgAb provou ser um marcador de substituição robusto da Tg para detetar doença persistente ou recorrente. Uma tendência descendente de TgAb indica baixo risco de persistência ou recorrência de doença. Uma tendência ascendente ou aparecimento de novo de TgAb é sugestivo de doença persistente ou recorrente.

No contexto pré-operatório, níveis elevados de TgAb predizem fracamente o diagnóstico de cancro da tiróide em nódulos tiroideus, mas a sua medição para tais efeitos diagnósticos não é recomendada em normas de orientação clínica internacionais.

Doentes e Métodos

1. Doentes

No presente estudo retrospetivo observacional, examinámos uma base de dados abrangendo 346 doentes diagnosticados com tiroidite de Hashimoto, seguidos na consulta externa de Endocrinologia de um hospital público central em Lisboa (Hospital de Santa Maria — Centro Hospitalar Universitário Lisboa Norte; HSM-CHULN), de 2009 ± 6 até 2014 ± 3. Todos os doentes assinaram um consentimento informado para autorização da utilização dos seus dados clínicos em investigações futuras, após aprovação pela Comissão de Ética do referido hospital.

O diagnóstico de tiroidite de Hashimoto foi definido como TPOAb ou TgAb positivos e ausência de hipertiroidismo.

As seguintes variáveis, recolhidas anonimamente e incluídas na base de dados, foram analisadas: (1) sexo; (2) idade (em anos); (3) diagnóstico; (4) tempo decorrido

desde diagnóstico (em anos); (5) tempo de seguimento (em anos); (6) TSH, T3, T4, fT4, Tg, TPOAb, TgAb e rácio TPOAb/TgAb nas primeira e última consultas; (7) presença de disfunção tiroideia na primeira consulta (definida por TSH fora do intervalo de referência normal); (8) terapêutica com levotiroxina na primeira e última consultas.

As amostras sanguíneas dos doentes foram colhidas no período da manhã (8 – 10h) no Departamento de Patologia Clínica, após um jejum noturno. Todas as variáveis relacionadas com a função tiroideia e anticorpos antitiroideus foram avaliadas por métodos padrão de imunoquimioluminescência, utilizando ensaios comerciais disponíveis no Departamento de Patologia Clínica do HSM-CHULN. Este departamento está em conformidade com os padrões de prática clínica e é uma instalação registada e certificada. Os intervalos de referência normais considerados encontram-se apresentados na Tabela 1.

2. Análise Estatística

A análise estatística dos dados foi realizada utilizando o programa de software estatístico IBM SPSS versão 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). O limite de significância estatística foi estabelecido para valor-p bicaudal < 0.05.

A normalidade dos dados foi verificada com o teste Kolmogorov-Smirnov. Foram utilizados testes não paramétricos para as variáveis com distribuição não normal. As diferenças entre variáveis emparelhadas das primeira e última consultas foram detetadas com o teste não paramétrico Wilcoxon Signed-Rank. O teste Qui-Quadrado (χ^2) foi realizado para testar as diferenças no número positividade de TPOAb e TgAb em ambas visitas e entre visitas. O teste não paramétrico Spearman Rank Order Regression foi realizado para determinar a relação entre os anticorpos antitiroideus (TPOAb e TgAb) e os testes de função tiroideia bem como outras variáveis clínicas.

Os resultados são apresentados como média ± desvio-padrão ou percentagem, como apropriado.

Resultados

1. Perfil da População em Estudo

Os dados demográficos de 346 doentes foram analisados, 90% dos quais eram do sexo feminino (n = 310). A maioria dos doentes era de meia idade (51 ± 17 anos na primeira consulta), com doença de longa duração (8 ± 8 anos). Apenas 24% dos doentes apresentavam-se com disfunção tiroideia e 49% estavam sob tratamento com

levotiroxina (pelo que, um total de 73% de doentes apresentava disfunção tiroideia), na primeira consulta. Na última consulta, após um tempo de seguimento médio de 5 ± 4 anos, 9% dos doentes apresentavam-se com disfunção tiroideia e 73% dos doentes encontravam-se medicados com levotiroxina (pelo que, um total de 82% de doentes apresentava disfunção tiroideia).

Os dados bioquímicos e serológicos de todos os doentes estão representados no Tabela 2 e Anexos 1 – 15.

2. Características de TPOAb e TgAb

A prevalência de TPOAb positivo foi 66,8% (n = 231) na primeira consulta, e 41,3% (n = 143) na última consulta. A prevalência de TgAb positivo foi 46% (n = 159) na primeira consulta, e 31,8% (n = 110) na última consulta.

A diferença entre a positividade de TPOAb entre a primeira e última consultas foi estatisticamente significativa ($p < 0.001$). A diferença entre a positividade de TgAb entre a primeira e última consultas foi estatisticamente significativa ($p = 0.001$). A diferença entre a positividade de TPOAb e a positividade de TgAb na primeira consulta foi estatisticamente significativa ($p < 0.001$). A diferença entre a positividade de TPOAb e a positividade de TgAb na última consulta foi estatisticamente significativa ($p < 0.001$).

As concentrações séricas de TPOAb foram superiores às concentrações séricas de TgAb em ambas consultas, mas esta diferença foi estatisticamente significativa apenas na primeira consulta ($z = -5,842$, n = 324, $p < 0.001$).

Na primeira consulta, 55.7% (n = 193) dos doentes apresentavam concentrações séricas de TPOAb superiores às concentrações séricas de TgAb, 28.6% (n = 99) dos doentes apresentavam concentrações séricas de TgAb superiores às concentrações séricas de TPOAb e apenas 8.1% (n = 28) apresentavam concentrações iguais de ambos anticorpos (n em falta = 22). Para os doentes com concentrações séricas de TPOAb superiores, o tempo médio de doença foi 9 ± 7 anos. Para os doentes com concentrações séricas de TgAb superiores, o tempo médio de doença foi 6 ± 6 anos. 76 doentes apresentavam concentrações séricas de TPOAb 10 vezes superiores às concentrações séricas de TgAb, enquanto apenas 27 doentes apresentavam concentrações séricas de TgAb 10 vezes superiores às concentrações séricas de TPOAb.

Na última consulta, 30.1% (n = 104) dos doentes apresentavam concentrações séricas de TPOAb superiores às concentrações séricas de TgAb, 28.9% (n = 100) dos doentes apresentavam concentrações séricas de TgAb superiores às concentrações

séricas de TPOAb e apenas 5.9% (n = 20) apresentavam concentrações iguais de ambos anticorpos (n em falta = 119). Para os doentes com concentrações séricas de TPOAb superiores, o tempo médio de doença foi 9 ± 7 anos. Para os doentes com concentrações séricas de TgAb superiores, o tempo médio de doença foi 10 ± 8 anos. 42 doentes apresentavam concentrações séricas de TPOAb 10 vezes superiores às concentrações séricas de TgAb, enquanto apenas 22 doentes apresentavam concentrações séricas de TgAb 10 vezes superiores às concentrações séricas de TPOAb.

3. Diferença entre a Função e Anticorpos Tiroideus da Primeira e Última Consultas

Os níveis de TSH, T3, T4 e TPOAb sofreram um redução significativa entre as primeira e última consultas, (z = -2.748, n = 242, p = 0.006; z = -8.859, n = 237, p < 0.001; z = -2.091; n = 235, p = 0.037; z = -5.706, n = 230, p < 0.001; respetivamente), como representado no Anexo 16 e ilustrado na Figure 1a, 1b, 1c, 1f.

Todavia, os níveis de fT4 aumentaram significativamente entre as primeira e última consultas, (z = - 3.362, n = 221, p = 0.01), como representado no Anexo 16 e ilustrado na Figura 1d.

Não se observou uma diferença estatisticamente significativa entre consultas para a Tg e TgAb, apesar de os seus níveis terem diminuído (Anexo 16 e Figura 1e, 1g).

4. Influência dos Anticorpos Antitiroideus sobre as Outras Variáveis Tiroideias na Ausência de Terapêutica

4.1 Primeira Consulta

Na primeira consulta dos doentes não tratados, relata-se uma correlação positiva significativa, mas modesta, entre os níveis séricos de TPOAb e TgAb (r = 0.275, n = 170, p < 0.001), como ilustrado no Anexo 17. O aumento dos níveis séricos de TPOAb correlacionou-se com aumento dos níveis séricos de TgAb.

Verificou-se uma correlação positiva significativa, mas modesta, entre os níveis séricos de TPOAb e os níveis séricos de TSH (r = 0.212, n = 169, p = 0.006), como ilustrado no Anexo 17. O aumento dos níveis séricos de TPOAb correlacionou-se com aumento dos níveis séricos de TSH.

Observou-se uma correlação negativa significativa, mas modesta, entre os níveis séricos de TPOAb e os níveis séricos de fT4 (r = -0.194, n = 158, p = 0.014), como ilustrado no Anexo 17. O aumento dos níveis séricos de TPOAb correlacionou-se com redução dos níveis séricos de fT4.

O TPOAb sérico não se correlacionou com os níveis séricos de T3, T4 ou Tg.

Observou-se uma correlação negativa significativa, mas modesta, entre os níveis séricos de TgAb e os níveis séricos de Tg (r = -0.319, n = 80, p = 0.004), como ilustrado no Anexo 18. O aumento dos níveis séricos de TgAb correlacionou-se com redução dos níveis séricos de Tg.

O TgAb sérico não se correlacionou com os níveis séricos de TSH, T3, T4 ou fT4.

4.2 Última Consulta

Na última consulta dos doentes não tratados, o TPOAb sérico não se correlacionou com os níveis séricos de TgAb, TSH, T3, T4, fT4 ou Tg.

Observou-se uma correlação negativa significativa, mas modesta, entre os níveis séricos de TgAb e os níveis séricos de Tg (r = -0.370, n = 46, p = 0.011), como ilustrado no Anexo 20. O aumento dos níveis séricos de TgAb correlacionou-se com redução dos níveis séricos de Tg.

O TgAb sérico não se correlacionou com os níveis séricos de TSH, T3, T4 ou fT4.

5. Subgrupo da População em Estudo com Tg Positiva e TgAb Positivos

Na primeira consulta, 13 doentes apresentavam Tg positiva e TgAb positivos. Todos os doentes eram do sexo feminino. Os dados descritivos deste subgrupo populacional estão demonstrados na Tabela 3.

Na última consulta, 10 doentes apresentavam Tg positiva e TgAb positivos. Todos os doentes eram do sexo feminino. Os dados descritivos deste subgrupo populacional estão demonstrados na Tabela 4.

Discussão

Apesar de a doença tiroideia autoimune constituir uma doença autoimune prevalecente e a causa mais comum de hipotiroidismo primário em populações sem carência de iodo, os papéis funcional e clínico dos anticorpos anti-tiroideus permanecem por esclarecer.

Enquanto que o TPOAb é considerado o marcador serológico mais preciso para autoimunidade tiroideia, com consequências patogénicas demonstradas, o TgAb é menos útil e possui um papel mais incerto.

A determinação de possíveis relações entre TPOAb e TgAb e as hormonas tiroideias bem como as respetivas evoluções temporais seria útil para esclarecer o papel patogénico dos anticorpos na doença tiroideia autoimune.

1. Perfil da População em Estudo

No presente estudo, os doentes eram principalmente do sexo feminino e meia idade, o que é consistente com a distribuição demográfica habitualmente descrita para doença tiroideia autoimune. A idade avançada e o sexo feminino são fatores de risco cruciais para o desenvolvimento de tiroidite de Hashimoto. Adicionalmente, a frequência de positividade TPOAb e TgAb aumenta com a idade nas mulheres. A preponderância feminina deve-se provavelmente aos efeitos das hormonas sexuais na resposta imune, mas uma inativação enviesada do cromossoma X e microquimerismo fetal foram propostos como mecanismos alternativos.

Uma longa duração de doença na tiroidite de Hashimoto, como na nossa população em estudo, é um achado comum, já que o curso inicial da doença é frequentemente silencioso e o diagnóstico formal é realizado anos após o início da doença. 73% dos doentes tinham disfunção tiroideia à apresentação, mas a maioria estava sob terapêutica com levotiroxina. Não obstante, uma apresentação eutiróide ou hipotiroidismo subclínico na tiroidite de Hashimoto não são incomuns, demonstrando a natureza indolente do processo autoimune subjacente e a capacidade de compensação da TSH.

2. Relação entre TPOAb e TgAb

A prevalência de autoanticorpos na tiroidite de Hashimoto é quase 100% para TPOAb e 60–80% para TgAb. Neste estudo, todos os doentes eram positivos para um ou o outro anticorpo, uma vez que a positividade de anticorpos for um dos critérios de seleção da população em estudo. Os TPOAb foram mais frequentemente mesuráveis do que os TgAb (66,8% versus 46% na primeira consulta; 41,3% versus 31,8% na última consulta; respetivamente), com diferença estatisticamente significativa, o que é consistente com o estado de anticorpos anti-tiroideus observado em estudos anteriores.

Em comparação com os TgAb, os TPOAb demonstram maiores concentrações séricas na doença tiroideia autoimune. Globalmente, as concentrações séricas de TPOAb foram mais elevadas que as concentrações séricas de TgAb, na nossa população em estudo, mas esta diferença foi apenas estatisticamente significativa na primeira consulta. Contudo, quando se considera apenas o valor qualitativo dos anticorpos, a proporção de casos positivos para TPOAb foi significativamente mais elevada que a proporção de casos positivos para TgAb em ambas consultas. Adicionalmente, a grande variabilidade de valores quantitativos obtidos para TPOAb e TgAb foi também documentada em estudos diferentes.

Todavia, quase um terço dos doentes em ambas visitas apresentavam concentrações séricas de TgAb mais elevadas que as concentrações séricas de TPOAb. Como tal, alguns doentes produziam preferencialmente TPOAb, enquanto que outros principalmente desenvolviam TgAb. *Carlé et al*. não encontrou diferenças referentes às características dos doentes (sexo, idade, estado de iodo, bócio) entre estes dois subgrupos, na população do seu estudo, tendo proposto que um fenómeno aleatório seria responsável por qual anticorpo é predominantemente gerado.

Descrevemos uma correlação significativa positiva, mas apenas modesta, entre os dois autoanticorpos (TPOAb and TgAb), na nossa população em estudo de tiroidite de Hashimoto, um achado semelhante a outros estudos.

É legítimo questionar se a natureza fraca da correlação entre TPOAb e TgAb e a ocorrência de produção predominante de um anticorpo ou outro estão relacionadas.

Caturegli et al. argumentaram que esta correlação fraca entre os anticorpos sugeria que seriam expressos em fases diferentes da doença, com os TgAb precedendo o aparecimento de TPOAb. Um mecanismo de escalação imune for proposto, onde os TgAb estariam presentes na instalação da doença, na forma de uma resposta inicial do sistema imune inato, e os TPOAb representariam a conseguinte resposta imunitária adaptativa. De facto, isto foi demonstrado em modelos de ratos. Nos doentes humanos, o diagnóstico de tiroidite de Hashimoto é realizado anos após a instalação da doença, pelo que se espera uma maior prevalência e títulos de TPOAb.

Seria possível que este mecanismo explicasse, simultaneamente, a correlação fraca entre os anticorpos e o motivo pelo qual alguns doentes desenvolvem principalmente TgAb em vez de TPOAb? Adequando-se ao modelo de escalação imune, doentes que expressassem principalmente TgAb estariam em fases precoces da doença. Curiosamente, *Carlé et al.* observou que os valores TSH sérico tendiam a ser mais reduzidos nos doentes predominantemente positivos para TgAb, apesar de não ser uma diferença estatisticamente significativa. Os níveis séricos de TSH aumentam com a progressão da doença, sendo expectáveis níveis mais elevados de TSH, tal como níveis mais elevados de TPOAb, nas fases mais avançadas de doença.

Uma questão permanece por esclarecer: por que razão esta correlação entre TPOAb e TgAb não foi encontrada na última consulta? Se a última consulta representar uma fase mais tardia da doença, de acordo com a hipótese de escalação imune, a discrepância entre a produção e níveis séricos de TPOAb e TgAb seria ainda mais

desviada para os TPOAb nesta fase. Consequentemente, uma correlação seria menos provável nas fases mais avançadas da doença.

27 e 22 doentes apresentaram concentrações séricas de TgAb 10 vezes mais elevadas que as concentrações séricas de TgAb nas primeira e última consultas, respetivamente. É questionável se esta disparidade considerável poderia enquadrar-se no modelo de escalação autoimune ou se este subgrupo de doentes representa uma situação clínica diferente, como uma resposta imune específica despoletada pela neoplasia diferenciada da tiróide.

3. Diferenças entre os Anticorpos Anti-Tiroideus e Função Tiroideia entre Consultas

No presente estudo, os níveis séricos de TSH, T3, T4 e TPOAb diminuíram e os níveis séricos de fT4 aumentaram entre consultas. Os níveis séricos de TgAb e Tg diminuírem entre consultas, mas tal diferença não foi estatisticamente significativa. Não obstante, o número de casos positivos de TgAb diminui significativamente entre consultas. O mesmo se aplica aos casos positivos de TPOAb. Equacionamos duas razões possíveis para explicar estes achados.

Em primeiro lugar, não podemos ignorar a potencial influência da levotiroxina, na medida em que a percentagem de doentes sob terapêutica com levotiroxina neste estudo aumentou de 49% para 73% entre consultas. Uma revisão sistemática e meta análise recente estudou 25 estudos clínicos randomizados, sobre a eficácia clínica da levotiroxina no tratamento de hipotiroidismo manifesto e subclínico, tendo descrito, ao comparar o grupo sob levotiroxina e o grupo placebo: uma redução significativa de TSH e aumento significativo de fT4 tanto no hipotiroidismo manifesto como no subclínico; e ausência de diferença de T3 no hipotiroidismo manifesto, mas uma redução significativa de T3 no hipotiroidismo subclínico. A terapêutica de substituição hormonal tiroideia alcança níveis normais de TSH ao corrigir a sobre-estimulação do eixo hipotálamo-hipófise-tiróide. Os níveis reduzidos de TSH poderiam contribuir para a redução de T3, T4 e Tg, ao diminuir a estimulação da glândula tiroideia. Estudos anteriores descreveram diminuição dos níveis de TPOAb após terapêutica com levotiroxina. *Chiovato et al.* argumentou que tal diminuição não se poderia dever a flutuações espontâneas do anticorpo, uma vez que não observou essa mesma diminuição nos doentes eutirodeus com tiroidite de Hashimoto. A expressão genética e atividade da TPO bem como a localização da TPO na membrana dos tirócitos é um fenómeno estimulado pela TSH. Tal como discutido por outros autores, a supressão da TSH pela

levotiroxina poderia causar uma redução da expressão de TPO, diminuindo a resposta autoimune e os níveis de TPOAb.

Em segundo lugar, devemos considerar a história natural da tiroidite de Hashimoto. O processo destrutivo da tiroidite linfocítica crónica engloba a atrofia do epitélio folicular a par de graus variáveis de fibrose da glândula tiroideia. A atrofia folicular e perda de colóide poderá levar à perda gradual da hormonogénese tiroideia, sendo responsável pela redução de T3, T4 e Tg. À semelhança do que ocorre na neoplasia diferenciada da tiróide, a redução dos autoanticorpos tiroideus poderia ser explicada pela atrofia e fibrose da glândula tiroideia. Na neoplasia diferenciada da tiróide, a eliminação completa do tecido tiroideu, por tiroidectomia total e terapêutica de radioablação, determina o desaparecimento de anticorpos anti-tiroideus da circulação, na ausência de doença persistente ou recorrente, com um tempo de desaparecimento médio de 3 anos para os TgAb e 6.3 anos para os TPOAb. Colocamos a hipótese de que a atrofia da glândula tiroideia poderá resultar na depleção parcial de autoantigénios tiroideus e linfócitos B intra-tiroideus, removendo parcialmente o estímulo e principais fontes de produção de anticorpos anti-tiroideus.

4. Influência dos Anticorpos Anti-Tiroideus sob Outras Variáveis

Encontrámos uma correlação significativa positiva, mas modesta, entre os níveis séricos de TPOAb e TSH na primeira consulta, mas não na última consulta. Esta correlação foi encontrada em doentes hipotiroideus e eutiroideus, noutros estudos. A relação entre a TPO e a TSH realçada acima pode explicar tal associação.

Os níveis de TPOAb correlacionam-se negativamente com os níveis de fT4 na primeira consulta. O TPOAb tem potencial patogénico na tiroidite de Hashimoto, mas a sua tradução *in vivo* e a importância que assume no processo autoimune global é dúbio. A correlação negativa entre TPOAb e fT4 poderia clarificar a correlação positiva entre TPOAb e TSH. Se os TPOAb diminuem a fT4, tal resultaria num aumento compensatório de TSH.

Os TPOAb não se correlacionam com T3 e T4, o que está em desacordo com outros estudos na literatura. A relação inconsistente entre TPOAb e T3 e T4 poderá ser causada pela ligação das hormonas tiroideias às proteínas plasmáticas de transporte.

Não houve correlação entre TgAb e TSH, apesar de uma produção anormal de TgAb associar-se a níveis de TSH anormais.

Os níveis séricos de TgAb não se correlacionaram com T3, T4 ou fT4. Os TgAb não fixam complemento nem causam destruição das células tiroideias.

Previsivelmente, verificámos uma correlação significativa negativa, mas fraca, entre os níveis séricos de TgAb e Tg, em ambas consultas. Uma vez que os TgAb são direcionados contra a Tg, esta correlação é expectável. A correlação é fraca porque a interferência dos TgAb anula as medições de Tg e não é concentração-dependente.

5. Conclusão

As alterações nos anticorpos anti-tiroideus e função tiroideia observadas no seguimento destes doentes mostra que a tiroidite de Hashimoto não é uma doença imutável. A correlação entre TPOAb e fT4 e TSH poderá sugerir a importância deste anticorpo na tiroidite de Hashimoto. As diferenças na expressão de TPOAb e TgAb em diferentes subgrupos de doentes poderão refletir a evolução natural da tiroidite de Hashimoto, um fenómeno aleatório ou uma situação clínica distinta.

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