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Is thyroid autoimmunity associated with subclinical atherosclerosis in young women with type 1 diabetes mellitus?

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Abstract

Introduction: It has been hypothesized that autoimmunity may contribute to cardiovascular complications and may be an important trigger for processes leading to atherosclerosis, especially in type 1 diabetes mellitus (T1DM). This pilot study aimed to answer the question of whether markers of thyroid autoimmunity are associated with increased carotid intima-media thickness (cIMT) in young, asymptomatic T1DM women.

Material and methods: The study population consisted of 102 women, including 72 with T1DM and 30 healthy controls. All patients had thyroid hormones within the normal range. According to the antiperoxidase antibodies (aTPO) titre, the T1DM women were divided into an aTPO-positive (T1DM aTPO+) (n = 41) and an aTPO-negative (T1DM aTPO–) (n = 31) group. In all patients, aTPO, thyroglobulin antibody (aTG) titres, thyroid-stimulating hormone (TSH), free thyroxine (FT3), free triiodothyronine (FT4), lipid parameters, glycated haemoglobin, thyroid ultrasonography, and cIMT assessment were evaluated. The association of cIMT with different risk factors related to thyroid autoimmunity was determined.

Results: Carotid intima-media thickness was significantly greater in T1DM aTPO+ females (0.66 ± 0.10 mm) than in T1DM aTPO– (0.59 ± 0.11 mm) and healthy controls (0.58 ± 0.10 mm) (p = 0.007, p = 0.001, respectively). In all women cIMT was significantly, positively correlated with aTPO (p = 0.005, r = 0.273), Hashimoto's thyroiditis (HT) duration (p = 0.00015, r = 0.367), levothyroxine dose per week (p = 0.006, r = 0.269), and ultrasound features of HT (p = 0.004, r = 0.281) and inversely with fT3 concentration (p = 0.014, r = –0.243) and FT3/FT4 ratio (p = 0.042, r = –0.201). A logistic regression analysis showed that HT duration (OR: 1.102, 95% CI: 1.008–1.206, p = 0.032) and a positive history family of HT (OR: 3.909, 95%CI: 1.014–15.071, p = 0.045) were risk factors for increased cIMT. However, multivariate regression analysis showed that the studied parameters related to thyroid autoimmunity are not independent risk factors for increased cIMT.

Conclusions: We expanded the data on cIMT in young women with T1DM and showed that thyroid autoimmunity, and in particular the duration of exposure to anti-thyroid antibodies, despite adequate levothyroxine substitution, is associated with subclinical atherosclerosis in young women with T1DM. However, thyroid-related parameters are not independent risk factors for increased cIMT in euthyroid women. **(Endokrynol Pol 2022; 73 (2): 301–308)**

Key words: *type 1 diabetes; thyroid autoimmunity; anti-thyroid antibodies; atherosclerosis*

Introduction

Type 1 diabetes mellitus (T1DM) has an autoimmune aetiology and is associated with many complications, of which cardiovascular (CV) diseases caused by accelerated atherosclerosis remain the most challenging in clinical practice [1]. Coexistence of autoimmune disorders, of which Hashimoto's thyroiditis (HT) is the most common [2], is believed to amplify the elevated CV risk. However, evidence that thyroid autoimmunity is a predictor of atherosclerosis is controversial.

An increasing number of reports indicate the involvement of immunological factors in the development of diabetic complications, of which the most significant association is suspected in CV complication in T1DM [3]. It seems that an imbalance in the immune system may be a key trigger of processes underlying atherosclerosis, which include inflammation [4], endothelial dysfunction [5], and accumulation of prothrombotic factors [6]. Interestingly, autoimmune factors in CV complications appear to be particularly relevant in women. It was found that the antioxidant and anti-inflammatory effects of oestrogen observed in healthy women are due to the regulatory Th2, macrophage, and T regulatory cell immune response and are probably disrupted in the presence of autoimmune

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disease [7]. This may explain the increased CV risk in premenopausal women with T1DM compared to those without diabetes as well as the sex differences in CV disease development [8]. In fact, a recent meta-analysis has reported that women with diabetes have a 58% and 13% greater risk of coronary heart disease and all-cause mortality, respectively, compared to men with the same disease [9]. The latest guidelines of the European Society of Cardiology suggest that CV complications increase most in young women with early onset T1DM, highlighting the need for further research on new CV risk factors specific to this group [10].

It is estimated that up to 25% of people with T1DM have elevated thyroid antibodies, and about half of them will develop clinical autoimmune thyroid disease [11]. There is increasing evidence that coexistence of diabetes and thyroid disease leads to endothelial damage, which has a significant impact on the development of macro- and microangiopathic complications [12]. Despite the proven impact of already subclinical hypothyroidism on the increased CV risk in young patients with T1DM [13], the data on the influence of the thyroid autoimmunity on the CV system in euthyroid patients are still inconclusive [14, 15]. Thus, the aim of this study was to answer the question of whether thyroid markers of immunological response are associated with elevated carotid intima-media thickness (cIMT) in young women with T1DM, providing new insight into CV risk factors in this group of patients.

Material and methods

Study population

A total of 72 female with T1DM were prospectively recruited from the patients of the Diabetes Outpatient Clinic of the Central Clinical Hospital of the Ministry of Internal Affairs in Warsaw between May 2018 and October 2020. Data on duration of diabetes, daily insulin dose, levothyroxine dose per week, presence of microangiopathy complications, and family history of HT were collected from the medical interview and past medical records. The inclusion criteria were diagnosis of T1DM, age 18–40 years, female sex, glycated haemoglobin (HbA_{1c}) < 10%, body mass index (BMI) < 30 kg/m^2, and current euthyreosis status in laboratory tests. The exclusion criteria were as follows: smoking behaviour, pregnancy, hypertension, history of any other CV disease, infectious disease, kidney disease, and taking any medication other than insulin or levothyroxine. The diagnosis of HT was established based on standard criteria: positive aTPO or thyroglobulin antibody (aTG) and a typical ultrasound image. Based on laboratory tests, patients with T1DM were divided into antiperoxidase antibody (aTPO)-positive (T1DM aTPO+) and aTPO-negative (T1DM aTPO–) subgroups.

The control group consisted of healthy medication-free, age-matched women. Inclusion criteria were as follows: age 18–40 years, female sex, BMI < 30 kg/m^2, and no history of diabetes, CV disease, hypothyroidism, or any other disease. Patients with positive antithyroid antibodies and with HT features on ultrasound examination were excluded from the control group.

The study protocol was approved by the Ethics and Surveillance Committee for Research in Human and Animal Sciences at the Central Clinical Hospital of the Ministry of Internal Affairs in Warsaw and was conducted according to the Declaration of Helsinki. All participants had signed an informed consent form before they were included within the study.

Anthropometric, laboratory, and ultrasound data

Interview and physical examination were performed for all study participants. The body mass index (BMI) was determined as body weight [kg]/height [metres] squared. For laboratory tests, thyroid-stimulating hormone (TSH) (normal values 0.27–4.2 μIU/mL), free thyroxine (FT4) (normal values 0.93–1.7 ng/dL), free triiodothyronine (FT3) (normal values 2–4.4 pg/mL, aTG (positive values were > 115 IU/mL), aTPO (positive values were > 34 IU/mL), HbA_{1c}, and lipid concentrations were performed in the morning after an overnight fast in the hospital laboratory using standard methods. The fT3:fT4 ratio was derived by dividing plasma concentrations of fT3 by those of fT4.

All patients underwent thyroid ultrasonography and bilateral B-mode ultrasound to evaluate cIMT according to the guidelines presented by the Polish Ultrasonography Society [16, 17]. Carotid arteries were examined using a 10 MHz linear transducer probe using a high-definition echograph (Canon Aplio a) and were performed by the same experienced sonographer. cIMT was measured three times on each side (right and left), and the mean value was calculated for all six measurements. The volume of the thyroid gland considered in the study was the sum of the volumes of the right and left lobe. The analysis included the assessment of the features of HT — reduced echogenicity of the parenchyma and hyperechoic echoes from the stromal connective tissue.

Statistical analysis

Analysis was performed using Statistica 13. Continuous variables are presented as the mean ± 1 SD, while categorical variables are presented as numbers (percentage). The Lilliefors and Shapiro-Wilk tests were used to verify the normal distribution of data. Student's t-test for normally distributed data, the Mann-Whitney U test and Kruskal-Wallis ANOVA for variables non-normally distributed, and the chi-square test for categorical variables were used to compare the differences between the groups. Correlations and their significance were calculated using the non-parametric Spearman test. cIMT was assumed to be a binary variable, and the threshold value of IMT for pathology was adopted at level 0.75 mm (IMT ≥ 0.75 mm was calculated as pathology). Logistic regression analyses and multivariate logistic regression analysis were performed to evaluate effects of various factors on cIMT. The statistical significance level was set at $p < 0.05$.

Results

The general characteristics of analysed groups are presented in Table 1. The study population comprised 102 females, including 30 controls with mean age 26.57 ± 3.68 years and 72 participants with T1DM with mean age 26.26 ± 4.86 years and with mean diabetes duration of 12.10 ± 6.31 years and HbA1c at the time of the analysis 8.01 ± 1.51%. T1DM patients were divided into two subgroups depending on the titre of aTPO: T1DM aTPO+ (n = 41) and T1DM aTPO– (n = 31). Among studied patients, in the T1DM aTPO+ group, 26 (63,41%) were treated chronically with levothyroxine. Among studied patients in the T1DM aTPO– group, 7 (22.58%) were treated chronically with levothyroxine but without typical elevated antibodies. All study participants had thyroid hormones within normal

Table 1. *Anthropometric, clinical, biochemical, and ultrasound characteristics of the studied groups*

	T1DM (n = 72)	T1DM aTPO+ (n = 41)	T1DM aTPO– (n = 31)	Controls (n = 30)	p (T1DM vs. Controls)	p (T1DM aTPO+ *vs.* T1DM aTPO–)
Age [years]	26.26 ± 4.86	27.02 ± 4.89	25.26 ± 4.70	26.57 ± 3.68	0.561	0.130
BMI [kg/m^2]	22.66 ± 2.87	22.89 ± 2.98	22.36 ± 2.74	22.78 ± 3.28	0.921	0.556
Diabetes ± duration (years)	12.10 ± 6.31	12.68 ± 7.33	11.32 ± 4.63	0	< 0.001***	0.549
Daily insulin dose [units]	39.26 ± 17.89	38.79 ± 19.40	39.87 ± 15.98	0	< 0.001***	0.587
Presence of diabetes microangiopathy complications (%)	9.0	9.7	12.9	0	< 0.001***	0.679
HbA_{1c} (%)	8.01 ± 1.51	8.14 ± 1.30	7.85 ± 1.77	5.18 ± 0.26	< 0.001***	0.248
Total cholesterol [mg/dL]	177.29 ± 32.52	177.90 ± 32.47	178.48 ± 33.10	172.87 ± 28.80	0.690	0.821
LDL-C [mg/dL]	90.87 ± 29.51	90.29 ± 29.78	91.65 ± 29.61	84.6 ± 28.20	0.476	0.830
HDL-C [mg/dL]	70.11 ± 18.51	71.51 ± 18.61	68.26 ± 18.51	72.70 ± 20.94	0.586	0.297
Triglyceride [mg/dL]	86.28 ± 42.68	80.51 ± 40.90	93.90 ± 44.44	79.23 ± 28.57	0.801	0.117
HT duration [years]	3.83 ± 5.61	6.76 ± 5.99	0	0	< 0.001***	< 0.001***
L-thyroxine dose per week [μg]	245.94 ± 308.11	355.07 ± 326.86	101.61 ± 210.84	0	< 0.001***	< 0.001***
TSH [μIU/mL]	2.16 ± 1.72	2.33 ± 2.10	1.93 ± 1.03	2.33 ± 1.03	0.096	0.659
ft4 [ng/dL]	1.24 ± 0.22	1.27 ± 0.24	1.21 ± 0.18	1.25 ± 0.17	0.779	0.120
ft3 [pg/mL]	2.90 ± 0.45	2.91 ± 0.42	2.89 ± 0.49	3.25 ± 0.54	< 0.001***	0.435
fT3/fT4 ratio	0.237 ± 0.04	0.23 ± 0.05	0.24 ± 0.04	0.26 ± 0.05	0.022*	0.470
aTPO [IU/mL]	118.34 ± 180.44	198.77 ± 205.79	11.97 ± 7.09	8.81 ± 5.79	< 0.001***	< 0.001***
aTG [IU/mL]	197.92 ± 485.94	316.41 ± 615.98	41.21 ± 91.54	68.41 ± 125.96	< 0.001***	< 0.001***
Thyroid volume [mL]	11.68 ± ± 3.37	11.86 ± 3.68	11.43 ± 2.95	11.70 ± 3.99	0.586	0.651
Ultrasound features of HT (%)	61.11	100	9.6	0	< 0.001***	< 0.001***
Positive family history of HT (%)	55.56	60.97	48.39	53.33	0.839	0.294
cIMT mean [mm]	0.63 ± 0.11	0.66 ± 0.10	0.59 ± 0.11	0.58 ± 0.10	0.028*	0.007**

*p < 0.05, **p < 0.01, ***p < 0.001; *Data presented as mean ± SD or N (%); BMI — body mass index; HbA_{1c} — glycated haemoglobin; LDL-C — low-density lipoprotein cholesterol; HDL-C — high-density lipoprotein cholesterol; HT — Hashimoto's thyroiditis; TSH — thyroid-stimulating hormone; fT4 — free thyroxine; fT3 — free triiodothyronine; aTPO-anti — thyroid peroxidase antibody; aTG — anti-thyroglobulin antibody; cIMT — carotid intima-media thickness

range. There were no differences in age, BMI, lipids parameters, TSH, fT4 levels, thyroid volume, and positive family history of HD between patients with and without T1DM (p > 0.05). FT3 and the fT3/fT4 ratio were significantly lower in the T1DM group than in controls (p < 0.001, p = 0.022, respectively). The studied subgroups of T1DM also did not differ significantly in age, BMI, lipid parameters, TSH, fT4, thyroid volume, and positive family history of HD as well as in fT3, mean diabetes duration, daily insulin dose, presence of diabetes microangiopathy complications, and HbA_{1c}. As intended, the groups differed significantly in anti-thyroid antibody titres (aTPO and aTG) and the presence of features of HT in ultrasonography (Tab. 1).

To compare the cIMT values between groups, the mean of all six measurements from both sides were calculated. We found that mean cIMT was significantly higher in T1DM patients than in controls (p = 0.028) and significantly higher in aTPO-positive T1DM patients than aTPO-negative T1DM patients (p = 0.007). Conversely, there was no significant difference between cIMT in the T1DM aTPO– group and controls (p = 0.704). Figure 1 shows the differences in cIMT between groups: the T1DM aTPO+ *vs.* T1DM aTPO– *vs.* controls.

We evaluated the association of cIMT with thyroid parameters including clinician date — HT duration, levothyroxine dose per week reflecting the severity of HT,

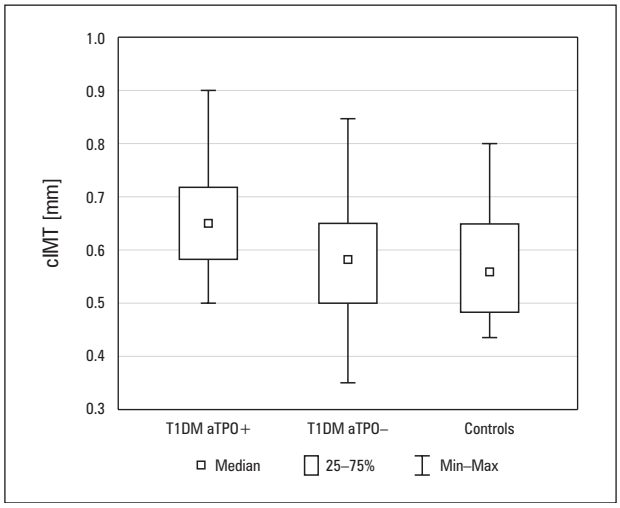

Figure 1. *Carotid intima-media thickness (cIMT) in women with type 1 diabetes mellitus (T1DM) and positive antithyroid peroxidase antibody titre (aTPO+) vs. women with T1DM and negative aTPO titre (aTPO–) (p = 0.007) vs. healthy controls (p = 0.001)*

family history of HT; laboratory date — TSH, FT3, FT4, aTPO, aTG, and ultrasound data — thyroid volume, ultrasound features of HT. In Table 2, we present results of the correlation analysis between cIMT and studied variable factors in all women (n = 102), in women with T1DM (n = 72), and in controls (n = 30).

In the whole population, cIMT was positively correlated with age, HT duration, levothyroxine dose per week, aTPO, and ultrasound features of HT and negatively correlated with fT3 levels and free triiodothyronine/free thyroxine (fT3/fT4) ratio. In the T1DM group (n = 72), we also found a significantly positive correlation between cIMT and age, duration of HT, and levothyroxine dose per week, but without significant correlation with other parameters. There was also no significant correlation between all cIMT measurements and the studied parameters in the control group.

To examine risk factors of cIMT among thyroid parameters, univariate logistic regression analyses were performed (Tab. 3).

Our results showed that the increased cIMT was significantly associated with HT duration with an odds ratio of 1.102 (p = 0.032, 95% CI: 1.008–1.206) and positive family history of HT with an odds ratio of 3.909 (p = 0.045, 95% CI: 1.014–15.071). Interestingly, we did not show statistical significance for age as a major risk factor for atherosclerosis, which is probably due to the narrow age range in the study group (18–40 years), the young age of the study population (88% of the studied women were under 31 years of age), and the relatively small sample size. Surprisingly, logistic regression analysis showed that higher aTG was a beneficial factor against cIMT (p = 0.006) with an odds ratio of 0.995. However, in other tests we did not confirm these results. The Mann-Whitney U test indicated that the group of patients with thicker cIMT had a higher median aTg and a lower mean aTg than the group with thinner cIMT. The discrepancy is because incidentally several patients in our study had very high aTG titre and thin cIMT thickness, causing a statistical artefact.

Table 2. ***Analysis of the intima-media thickness correlation with selected variables in all women (n = 102), in women with diabetes type 1 mellitus (T1DM) (n = 72), and in controls (n = 30)***

Variable *vs.* cIMT	All patients					
	All women (n = 102)		T1DM (n = 72)		Controls (n = 30)	
	r	p	r	p	r	p
Age	0.266	0,007**	0.268	0.023*	0.220	0.241
HT duration	0.367	0.00015***	0.358	0.002**	–	–
L-thyroxine dose per week	0.269	0.006**	0.242	0.040*	–	–
TSH [uIU/L]	–0.123	0.217	–0.236	0.044	0.304	0.102
fT4	0.057	0.572	0.066	0.583	0.036	0.849
fT3	–0.243	0.014*	–0.133	0.266	–0.291	0.119
fT3/fT4	–0.201	0.042*	–0.098	0.411	–0.279	0.136
aTPO	0.273	0.005**	0.226	0.055	–0.043	0.820
aTG	0.179	0.072	0.103	0.390	–0.048	0.800
Thyroid volume	–0.086	0.391	–0.096	0.424	–0.201	0.288
Ultrasound features of HT	0.281	0.004**	0.225	0.057	–	–
Positive family history of HT	–0.006	0.946	0.014	0.906	–0.050	0.792

*p< 0.05. **p < 0.01, ***p < 0.001. HT — Hashimoto's thyroiditis; TSH — thyroid-stimulating hormone; fT4 — free thyroxine; fT3 — free triiodothyronine; aTPO — anti-thyroid peroxidase antibody; aTG — anti-thyroglobulin antibody; cIMT — carotid intima-media thickness

Table 3. *Univariate logistic regression analyses of thyroid risk factors for intima-media thickness*

Dependent variable	Predictors	Odds ratio	95% CI	p value
cIMT	Age	1.083	0.961–1.219	0.183
	HT duration	1.102	1.008–1.206	0.032
	L-thyroxine dose per week	1.001	0.999–1.002	0.315
	TSH	0.778	0.457–1.323	0.348
	fT4	1.908	0.134–27.402	0.629
	fT3	0.315	0.081–1.221	0.091
	fT3/fT4	0.000	0.000–45.553	0.160
	aTPO	1.000	0.997–1.004	0.730
	aTG	0.995	0.992–0.999	0.006
	Thyroid volume	0.860	0.712–1.038	0.112
	Ultrasound features of HT	3.119	0.967–10.058	0.054
	Positive family history of HT (%)	3.909	1.014–15.071	0.045

HT — Hashimoto's thyroiditis; TSH — thyroid-stimulating hormone; fT4 — free thyroxine; fT3 — free triiodothyronine; aTPO — anti-thyroid peroxidase antibody; aTG — anti-thyroglobulin antibody; cIMT — carotid intima-media thickness; CI — confidence interval

Therefore, we cannot consider the obtained result from the logistic regression analysis regarding aTG as reliable. On the other hand, for reduced echogenicity of the thyroid gland on ultrasound, we found a minimally non-significant association with cIMT ($p = 0.054$) with an odds ratio of 3.12. However, dividing the patients into two subgroups according to the reduced echogenicity on ultrasonography using Wilcoxon Mann-Whitney test, we found that patients with ultrasound features of HT had significantly higher cIMT ($p = 0.004$), which may also suggest the significance of the association of this factor with atherosclerosis. For other examined variables, the effects on cIMT were not significant. Multivariate logistic regression analysis showed that, of the studied parameters related to thyroid autoimmunity, there were no significant independent predictive factors for increased cIMT.

Discussion

Although CV risk factors have been studied quite extensively in diabetic groups, most of these data come from analyses of groups without sex stratification, especially in T1DM. The current study provides a unique opportunity to explore the new CV risk factors linked to thyroid autoimmunity in young women with T1DM.

As is well known, hyperlipidaemia is one of the most important risk factors responsible for the development of atherosclerosis. Interestingly, in our study there were no significant differences in lipid parameters between the aTPO-positive and aTPO-negative groups, and despite this, there were significant differences in cIMT thickness. We suspect that other factors related to thyroid autoimmunity may be the cause of subclinical atherosclerosis in this group of patients. In HT, anti-thyroid antibodies are a marker of autoimmunity, and among HT patients the prevalence of aTPO is about 90%, whereas aTG is 60–80% [18]. Their action outside the thyroid is not clear. Although our studied groups differed significantly in both antithyroid antibody levels, we found a significant, positive correlation with cIMT only for the aTPO antibody. However, in multivariate logistic regression analysis we did not confirm that aTPO is an independent risk factor for cIMT. In fact, antithyroid antibodies appear to have only an indirect relationship with the development of atherosclerosis, with unknown exact mechanisms explaining this relationship. It is well recognized that local thyroid autoimmunity is closely related to the activation of the inflammatory process, which probably affects other tissues, even in in the euthyroid stage [19, 20]. Interestingly, one study found a correlation between positive aTPO titres and increased oxidative stress and advanced glycation end products in serum in euthyroid HT patients, which may promote atherosclerosis [21]. Notably, patients with autoimmune thyroid disease present an imbalance between Th1/Th2 and Th17/Treg, causing activation of the inflammatory process and exacerbating thyroid damage [22]. Thus, it seems that polyautoimmunity may enhance this imbalance and the pro-inflammatory effect also in other tissue. In the present study, there was a significant positive correlation between ultrasound features of HT and increased cIMT, which confirms this hypothesis. Of interest, similarly to our study, Pittoco D et al. showed that in patients with two autoimmune diseases — T1DM and celiac disease — cIMT was greater than in those presenting only T1DM or celiac disease [23]. In addition, in the

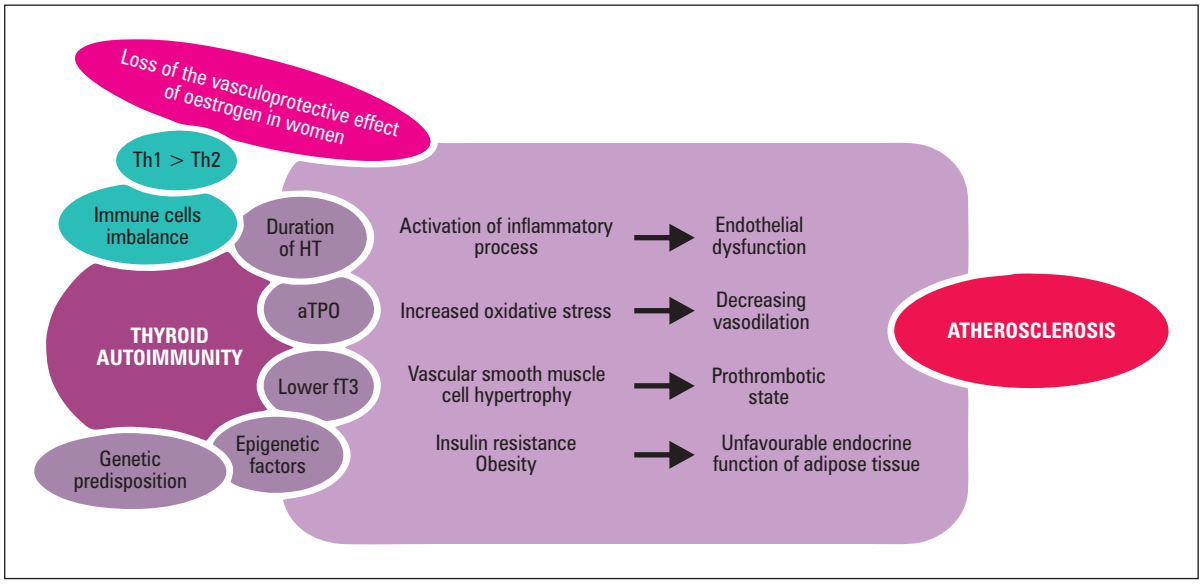

Figure 2. *Thyroid autoimmunity is possibly associated with activation of the pathways that promote atherosclerosis, which may contribute to the loss of vasoprotection in women, explaining why they are at higher cardiovascular risk. Own elaboration based on the data obtained in the study and [5, 6, 8, 21, 22, 29, 31, 38, 41]. Th — T helper cell; HT — Hashimoto's thyroiditis; aTPO — anti-thyroid peroxidase antibody; fT3 — free triiodothyronine*

study by Raterman et al. aTPO was associated with increased progression of cIMT in patients with another autoimmune disease — rheumatoid arthritis [15].

To date, only a few studies have presented data on cIMT thickness in patients with T1DM and coexisting thyroid autoimmunity in euthyreosis. In contrast to our study, the data obtained by Głowińska-Olszewska et al. showed no significant effect of the presence of HT on cIMT in the euthyroid T1DM population [14]. One explanation for these discrepancies may be that in this study the groups included both sexes. As we hypothesise, thyroid autoimmunity may have a stronger effect on cIMT in women because of the disruption of the anti-inflammatory effect of oestrogens likely to occur in people with autoimmune disorders [7]. Indeed, consistent with this view are the results obtained by Topaloglu et al. in euthyroid premenopausal women with HT but without T1DM [24], suggesting a link between thyroid autoimmunity and subclinical atherosclerosis in this group of patients. In fact, referring to previous studies in nondiabetic women with HT, thyroid autoimmunity was associated with chronic inflammation that may cause endothelial dysfunction [5], which promotes the development of atherosclerosis.

Of note, correlation analysis showed a significant association between the increase in cIMT and age and duration of HT in T1DM women. In fact, as demonstrated in previous studies, there is an increase in the intima-media complex with age [25], which is a well-known risk factor for atherosclerosis [26]. This is likely to be related to atherogenic remodelling in response to age and haemodynamic factors, including disruption of endothelial barrier integrity, stiffening of the intima, and deposition of fibronectin and collagen [27]. Notably, with age the structure of intima changes due to migration of smooth muscle cells from the media to intima and the degree of inflammatory cell infiltration correlates with the severity of atherosclerotic plaques [28]. It therefore appears that the presence of an autoimmune disease may favour the progression of inflammation in the tunica intima-media and my be more important with longer duration.

Similarly to our study, lower serum fT3 levels were associated with elevated cIMT, but in patients without diabetes [29] and with type 2 diabetes [30]. The anti-atherosclerotic effect of fT3 is probably due to its inhibition of angiotensin II-induced expression of pro-inflammatory cytokines and vascular smooth muscle cell hypertrophy, as well as by increasing vasodilation [31]. A negative correlation between fT3 levels and the fT3/fT4 ratio and the presence of peripheral artery disease was found among the euthyroid Chinese population [32], which also is in agreement with our results. In contrast, recent studies have revealed that a higher fT3/fT4 ratio is a significant predictor of insulin resistance and metabolic syndrome [33, 34], which can contribute to higher CV risk. However, in our study we found an inverse correlation of fT3/fT4 with cIMT. One reason for the observed differences may be the levothyroxine substitution used by some of our study patients, which has proved to decrease the fT3/fT4 ratio [35]. Therefore, the negative correlation of

the fT3/fT4 ratio with cIMT in our study may indirectly indicate an association with levothyroxine supplementation and thereby the insufficient hormone production resulting from thyroid autoimmunity. Similarly, the positive correlation between levothyroxine dose per week and an increase in cIMT shown in our study does not mean that the hormone replacement favours the formation of atherosclerotic changes, but only indirectly indicates the relationship with the degree of progression of thyroid insufficiency. Increased cIMT has been also shown in women with HT, regardless of thyroid function and other CV risk factors, but only if connected with overweight or obesity [36]. This is because thyroid autoimmunity probably has some effect on adipose tissue even in euthyroidism [37]. Although all the women in our study were non-obese, the BMI in the T1DM aTPO+ group was slightly higher than in the T1DM aTPO– group. Interestingly, in a study by Liu et al. aTPO levels were associated with chronic inflammation and insulin resistance in patients without obesity [38]. These findings may suggest that aTPO promotes endocrine dysfunction of adipose tissue also in non-obese people, which may promote atherosclerotic plaque formation.

Our results also indicate that a positive family history of HT may be a risk factor of increased cIMT, which could suggest the influence of genetic factors. Relevant studies have shown that human leukocyte antigen (HLA), autoimmune regulator (AIRE), lymphoid protein tyrosine phosphatase (LYP), forkhead box protein P3 (FOXP3), and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) confer susceptibility to the development of autoimmune thyroiditis and T1DM [39]. What is important, these factors associated with autoimmunity are also important in regulating the inflammatory processes that underlie CV complication [40]. Interestingly, epigenetic factors may mediate the link between autoimmunity, adipose tissue, and the atherosclerotic plaque formation [41], but the exact mechanism of these relationships remains unclear and requires further study. Figure 2 shows the possible mechanisms involved in the association between factors related to thyroid autoimmunity and atherosclerosis in women.

The strength of our study was to qualify a homogeneous group of young asymptomatic women with T1DM to eliminate CV risk factors such as obesity, hypertension, or hyperlipidaemia. Moreover, for the first time we performed a thorough analysis of factors associated with thyroid autoimmunity in young T1DM women. However, there were also some limitations in our study. First, the number of patients in this study was relatively small. Second, data on diabetes and associated complications were assessed based on medical diagnoses recorded from interviews and past medical records, implying a potential misclassification. Moreover, in view of the possible long interval between the appearance of subclinical changes in the CV system and the first manifestation of CV disease, prolonged follow-up of women with T1DM aTPO+ is needed.

Conclusions

The original findings of our study are that not only the co-occurrence of aTPO, but also the duration of antibody exposure, severity of HT, and genetic predisposition independent of thyroid function may be associated with subclinical atherosclerosis. However, parameters associated with thyroid autoimmunity are not independent risk factors for increased cIMT in women with type 1 diabetes. In fact, thyroid autoimmunity probably has no harmful effect per se but may be an indicator of an immune imbalance that indirectly induces pathways involved in atherosclerotic plaque formation. We suspect that intima-media thickening may be mediated by autoimmunity, which may be related to sex differences. Overall, future research on a larger group of patients is needed for a better understanding of the mechanisms leading to atherosclerosis in T1DM women, which may contribute to personalized and gender-specific treatment of vascular complications.

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Conflict of interests
None of the authors have any potential conflicts of interest associated with this research.

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Contributors
M.Ł.T. contributed to the conception and design of the study and data collection, researched and analysed the data, and wrote the manuscript. J.Z. contributed to the conception and statistical data analysis and the critical revision of the manuscript. B.M.R., E.F., and L.C. contributed to the conception and design of the study and the critical revision of the manuscript. All approved the final version of the manuscript.

Ethical approval
The study was approved by the Ethics and Surveillance Committee for Research in Human and Animal Sciences at the Central Clinical Hospital of the Ministry of Internal Affairs (no. 22/2018 of 09.05.2018).

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