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ORIGINAL ARTICLE

Long-term follow-up of organ-specific antibodies and related organ dysfunction in type 1 diabetes mellitus

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ABSTRACT

Objective: Diabetes mellitus type 1 (DM1) is associated with other autoimmune disorders. To our knowledge, there are no longitudinal data considering the long-term clinical relevance of organ-specific antibodies (OS-Ab) in DM1 patients. We performed a long-term retrospective longitudinal study in order to investigate the presence and diagnostic accuracy (positive predictive value: PPV and negative predictive value: NPV) of OS-Ab in DM1 patients.

Research design and methods: In a retrospective longitudinal study, the presence of OS-Ab and related organ function were analysed in 396 DM1 patients (184 F/212 M, age 44±13 years, age at onset of DM1 21±13 years), with a median follow-up time of 23±10 years.

Results: OS-Ab frequencies at baseline were: antibodies against thyroglobulin (Tg-Ab) 4.3%, antibodies against thyroid peroxidase (TPO-Ab) 8.1%, Tg- and/or TPO-Ab 10.4%, antibodies against parietal cells (PCA) 5.8% and antibodies against adrenal cortex (ACA) 0.5%. The occurrence of (sub)clinical hypothyroidism was higher in patients with Tg-Ab (47%) or TPO-Ab (42%) than in those without these antibodies (6.2 and 5.1%, respectively, p<0.001). PPV and NPV for Tg-Ab were 0.60 and 0.88, respectively, for TPO-Ab 0.54 and 0.91. Also in patients with PCA, organ dysfunction occurred more often (61%) than in patients without PCA (9.7%, p<0.001). PPV for PCA was 0.61 and NPV 0.90. NPV and PPV for ACA could not be calculated because of the low prevalence.

Conclusion: Long-term follow-up of 396 DM1 patients shows that the presence of thyroid antibodies and/or parietal cell antibodies is clearly associated with dysfunction of the corresponding organ.

KEYWORDS

Type 1 diabetes mellitus, autoimmune antibodies, organ-specific antibodies, autoimmune thyroiditis, autoimmune gastritis; Addison's disease

INTRODUCTION

Type 1 diabetes mellitus (DM1) is associated with other immune-mediated disorders,[1,2] such as autoimmune thyroiditis,[3-6] Addison's disease[7] and pernicious anaemia.[8,9] In the past years, extensive research has been performed to predict the occurrence of autoimmune diseases by the presence of organ-specific antibodies (OS-Ab), as recently reviewed.[10]

Thyroid antibodies (Th-Ab) are directed against thyroglobulin (Tg-Ab) or against thyroid peroxidase (TPO-Ab). TPO-Ab prevalences in DM1 populations vary between 5.5 and 46.2% and in control populations between 0 and 27.0%.[5,9,11-32] Tg-Ab prevalences in DM1 populations vary between 2.1 and 40% and in control populations between 0 and 20%.[5,12-15,18-20,22,25,29,32,33] The prognostic significance of Th-Ab has been studied in several longitudinal non-diabetic populations.[34-36] The risk of developing overt hypothyroidism per year in TPO-Ab positive individuals is higher than in TPO-Ab negative individuals (4.3 and 2.6%, respectively).[34,37] Parietal cell antibodies (PCA) are directed against the parietal cells in the stomach,[38,39] chronically targeting H^+/K^+ ATPase, which can lead to atrophic gastritis, hypochlorhydria or achlorhydria, and a decline in intrinsic factor production, causing hypergastrinaemia, vitamin B12 malabsorption and ultimately pernicious anaemia.[39,39] Hypochlorhydria may also impair iron absorption which can lead to iron deficiency anaemia.[40-45] The PCA prevalences in DM1 populations

range from 3 to 34% and in control populations from 0 to 13%.[9,11,13,16,18-20,22,24,25,27,32,46-50] To our knowledge, no prospective studies have been published concerning PCA in DM1.

Adrenocortical autoimmune disease, also called primary adrenal insufficiency or Addison's disease, is the result of humoral and cell-mediated inflammation of the adrenal cortex.[51] Adrenal cortex antibodies (ACA) are directed against 21-hydroxylase, a microsomal cytochrome P450 enzyme that converts 17-α-hydroxyprogesterone and progesterone into 11-deoxycortisol and 11-deoxycorticosterone.[52] The ACA prevalences in DM1 populations range from 0 to 4% and in control populations from 0 to 0.7%.[16,24,25,32,46-48,53-58] To date, only one longitudinal study has been performed that studied ACA: Betterle *et al*. performed a longitudinal analysis of 15 DM1 patients with organ-specific autoimmune disease who were positive for ACA: 40% developed Addison's disease during a mean observation period of 3.2 years.[59]

CLINICAL PROBLEM AND RESEARCH QUESTION

Early detection of antibodies and latent organ-specific dysfunction is important to alert physicians to take appropriate action in order to prevent full-blown disease.[60] Although from a clinical point of view it is of utmost importance to be able to determine the prognostic significance of OS-Ab, most studies so far have had a cross-sectional design. Obviously, longitudinal studies are needed to fill this gap in knowledge. Therefore, we performed a retrospective longitudinal study in order to investigate the prevalence and clinical relevance of thyroid antibodies, parietal cell antibodies and adrenocortical antibodies, and the prevalence of corresponding organ dysfunction during more than 20 years follow-up of 396 patients with diabetes mellitus type 1.

RESEARCH DESIGN AND METHODS

Research design

A total of 396 consecutive patients with DM1 from the Diabetes Outpatient Department of the Leiden University Medical Center were included in this retrospective longitudinal study between 1981 and 1998. We assessed the presence of OS-Ab and / or autoimmune thyroid disease, Addison's disease, or macrocytic, normocytic or microcytic anaemia during more than 20 years of follow-up.

ANTIBODY DETECTION METHODS

PCA and ACA were measured by indirect immunofluorescence using tissue slides of Scimedx (Denville, NJ, USA). Thyroid antibodies (TPO-AB and Tg-Ab) were measured by radioimmunoassay (DiaSorin, Saluggia, Italy). The Tg-Ab assay range is from 5 to 6500 kU/l, reference value <100 kU/l; the TPO-Ab reference range is <60 kU/l. Both assays had coefficients of variation of <10%. Monkey tissue was used to detect Th-Ab and ACA, whereas rat tissue was used to detect PCA. Both tissue slides were manufactured by SciMedex. Patients who were weakly positive or doubtfully positive for antibodies were not taken into account; only positive, strongly positive and negative patients were considered.

ENDOCRINE ASSESSMENTS

Serum thyroid-stimulating hormone (TSH) and FT4 were measured by time resolved fluoroimmunoassay and serum T4 and T3 by in-house radioimmunoassay methods. Reference values for T3 were 1.1 to 3.1 nmol/l, for T4 70 to 160 nmol/l, for free T4 10 to 24 pmol/l and for TSH 0.3 to 4.8 mU/l. Overt clinical hypothyroidism was defined as elevated TSH levels and T3, T4, or free T4 levels under the lower limit of normal. Subclinical hypothyroidism was defined as an elevated TSH level with normal T3, T4, or free T4 levels. Overt clinical hyperthyroidism was defined as both suppressed TSH levels and T3, T4, or free T4 levels above the upper limit of normal. Subclinical hyperthyroidism was defined as a suppressed TSH level with normal T3, T4, or free T4 levels.

Between 1978 and 1986, cortisol was measured by in-house radioimmunoassay with an interassay coefficient of variation of 10% and with a detection limit of 50 nmol/l. Between 1986 and 1994, a fluorescence energy-transfer immunoassay Syva-Advance (Syva Company, Palo Alto, CA) was used, with an interassay variation coefficient of 3.6 to 6.1% and a detection limit of 50 nmol/l. From 1994, cortisol was measured by fluorescence-polarisation assay on a TDx (Abbott Laboratories, Abbott Park, IL). The interassay variation coefficient is 5 to 6% above 500 nmol/l and amounts to 12% under 200 nmol/l. The detection limit is 20 nmol/l. The methods correlated well with each other, and therefore no correction factors were introduced for follow-up of patients. Reference values for morning cortisol were 0.20 to 0.60 µmol/l.

Adrenocorticotropic hormone (ACTH) has been measured since 1986 using an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with a detection limit of 3 ng/l. The intra- and interassay average variations ranged from 2.8 to 7.5% across the sample range observed. If Addison's disease had to be excluded because of the presence of ACA antibodies or because of a clinical suspicion, an ACTH stimulation test with 250 µg synacthen was used. The test was interpreted as normal when the cortisol level exceeded 0.55 umol/l at 60 minutes after stimulation; hypocortisolism was diagnosed when the cortisol level failed to reach this value.

The haemoglobin (Hb) levels and mean corpuscular volume (MCV) were determined with an automated analysis system (Coulter Counter; Coulter Electronics, Hialeah, Florida). Reference values for Hb were 7.5 to 10 mmol/l for women and 8.5 to 10 mmol/l for men; the reference value for MCV was 80 to 100 fl for both sexes.

Serum levels of vitamin B12 were determined using the Dual Count Solid Phase No-Boil Assay (Diagnostic Products Corp., Los Angeles, California). Vitamin B12 deficiency was defined as serum vitamin B12 levels lower than 150 pmol/l.

Parietal cell dysfunction was diagnosed when atrophic gastritis, macrocytic anaemia or pernicious anaemia was present. Macrocytosis was defined as a high MCV without anaemia. Pernicious anaemia was defined as anaemia with a high MCV in the presence of atrophic gastritis. Microcytic and normocytic anaemias were also taken into account, since achlorhydria can cause iron deficiency and subsequent microcytic anaemia, which can result in normocytic anaemia when combined with macrocytic anaemia.

STATISTICAL ANALYSIS

Patients' data were analysed using SPSS 16.0 (ANOVA and Chi Square). Prevalence of organ dysfunction was compared between antibody positive and negative DM1 patients. Positive predictive value (PPV) was calculated as the number of patients with organ dysfunction divided by the total number of patients with OS-Ab for whom organ function was tested. Negative predictive value (NPV) was calculated as the number of patients with organ dysfunction divided by the total number of patients without OS-Ab for whom organ function was tested.

RESULTS

The age at the time of the study of the 396 patients (184 females and 212 males) was 44±13 years, age at onset of DM1 was 21±13 years. Median time from referral to final assessment was 21 (8 to 70) years and was comparable for antibody positive and negative patients (*table 1*).

Table 1. *Prevalence of organ-specific antibodies and corresponding organ dysfunction in 396 DM1 patients*

Antibodies	**Tg-Ab**		**Hyperthyroidism**		**Tg- and/or TPO-Ab**			**PCA**			**ACA**	
	-	+	-	+	-	+		-	+		-	+
N (total)	333 (84.1%)#	17 (4.3%)	308 (77.7)#	32 (8.1%)	295 (74.5)#	41 (10.4%)		362 (91.4)#	23 (5.8%)		392 (98.9)#	2 (0.5%)
% F	42%	71%**	42%	78%**	41%	76%**		45%	70%*		46%	100%
Age (baseline)	43.4 ±12.9	45.8 ±10.7	43.2 ±12.9	45.3 ±10.5	43.1 ±13.0	45.4 ±11.1		43.6 ±12.5	43.4 ±17.7		43.6 ±12.8	59.0 ±17.0
DM duration (baseline)	22.4 ±10.0	22.6 ±10.0	22.5 ±10.0	21.7 ±11.1	22.4 ±10.1	22.5 ±11.2		22.7 ±10.2	21.9 ±12.2		22.7 ±10.4	27.5 ±26.2
Organ dysfunction (total)	11.7%	60.0%	9.4%	53.4%	9.1%	52.9%		9.7%	60.9%			
Subclinical hypothyroidism	0.8%	13.3%	0.9%	11.5%	0.9%	14.7%	Macrocytosis	1.4%	4.3%	Hypocortisolism	2.4%	0
Clinical hypothyroidism	5.5%	33.3%	4.3%	30.8%	3.6%	29.4%	Macrocytic anaemia	0.3%	4.3%	Hypercortisolism	4.9%	0
Hyperthyroidism	3.1%	0	1.7%	3.8%	1.8%	2.9%	Pernicious anaemia	0.3%	8.7%			
Graves	2.3%	13.3%	2.6%	7.7%	2.7%	5.8%	Normocytic anaemia	5.1%	26%			
							Microcytic anaemia	2.6%	17.4%			
Diagnostic accuracy	NPV 0.88	PPV 0.60	NPV 0.91	PPV 0.53	NPV 0.91	PPV 0.53		NPV 0.90	PPV 0.61			
AB+ vs AB -	p<0.001		p<0.001		p<0.001			p<0.001			NS	

Data are presented as mean ± SD unless stated otherwise * p<0.05 ** p<0.01, # total patient numbers do not add up to 396 since weakly positive patients were left out of the analysis; Tg-Ab = antibodies against thyroglobulin; TPO-Ab = antibodies against thyroid peroxidise; PCA = antibodies against parietal cells; ACA = antibodies against adrenal cortex; F = female; DM = diabetes mellitus; hyperthyroidism = hyperthyroidism without thyroid stimulating antibodies; Graves = Graves' disease; PA = pernicious anaemia; Addison = Addison's disease; PPV = positive predictive value; NPV = negative predictive value; AB^+ vs AB^- = level of significance for the difference in organ dysfunction frequency between AB-positive and AB-negative patients.

ANTIBODY PREVALENCES AND ORGAN DYSFUNCTION

Altogether, 396 patients were tested for Th-Ab, PCA and ACA. All patients had islet cell antibodies (ICA), since this was obligatory for the diagnosis of DM1. Of the patients tested for Tg-Ab, 4.3% were positive. 60.0% of the Tg-Ab-positive patients tested had organ dysfunction (PPV 0.60, NPV 0.88). In patients positive for Tg-Ab, the occurrence of organ dysfunction was significantly higher than in patients negative for those antibodies (60.0 *vs* 11.7%, p<0.001).

Of the patients tested for TPO-Ab, 8.1% were positive; 53.4% of the TPO-Ab positive patients tested had organ dysfunction (PPV for hypothyroidism was 0.53, NPV 0.91). This was significantly higher than in patients negative for TPO-Ab (53.4 *vs* 9.4%, p<0.001).

Of the patients, 10.4% were positive for either TPO-Ab, Tg-Ab, or both. Of these patients, 52.9% had organ dysfunction at testing (PPV 0.53, NPV 0.91), which was significantly higher than in patients negative for these antibodies (52.9 *vs* 9.1%, p<0.001).

Of the patients tested for PCA, 5.8% were positive; 60.8% had organ dysfunction (PPV 0.61, NPV 0.90). In patients positive for PCA, the occurrence of organ dysfunction was significantly higher than in patients negative for those antibodies (60.9 *vs* 9.7%, p<0.001).

Of the patients tested for ACA, two were ACA positive. None of them had signs of adrenal dysfunction.

Fifteen patients had multiple antibodies: nine had Th-Ab (either TPO-Ab, Tg-Ab, or both) and PCA, two had Th-Ab and ACA and four had Th-Ab (and, like all included patients, ICA). However, none of these patients had the combination of different types of organ dysfunction leading to the clinical diagnosis of one of the polyglandular syndromes.

Table 1 shows antibody prevalences and organ dysfunction in all patients tested. *Figures 1 and 2* show the occurrence of different types of organ dysfunction in patients positive for thyroid or parietal cell antibodies, compared with patients negative for those antibodies.

There was a female predominance for Tg-Ab and TPO-Ab (p<0.001), and for PCA a tendency towards female predominance (p=0.06). The two ACA positive patients were female.

DISCUSSION

As shown in our recent review,[10] most studies performed in the past to investigate the relevance of organ-specific antibodies in DM1 used a cross-sectional design; no longitudinal studies have been performed to date. In order to investigate the predictive value of these OS-Ab in

Figure 1. *Difference in prevalence of thyroid dysfunction in patients negative (Th-AB-) and positive (Th-AB+) for thyroid antibodies (p<0.001)*

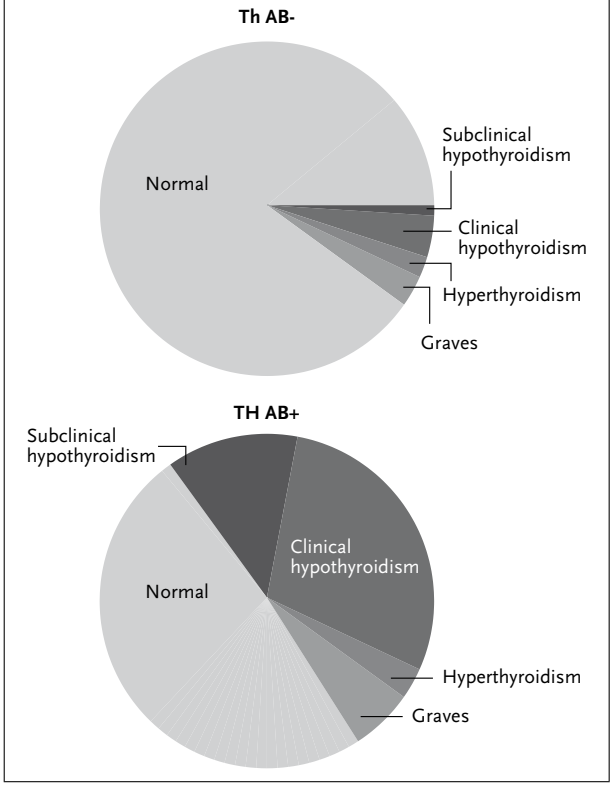
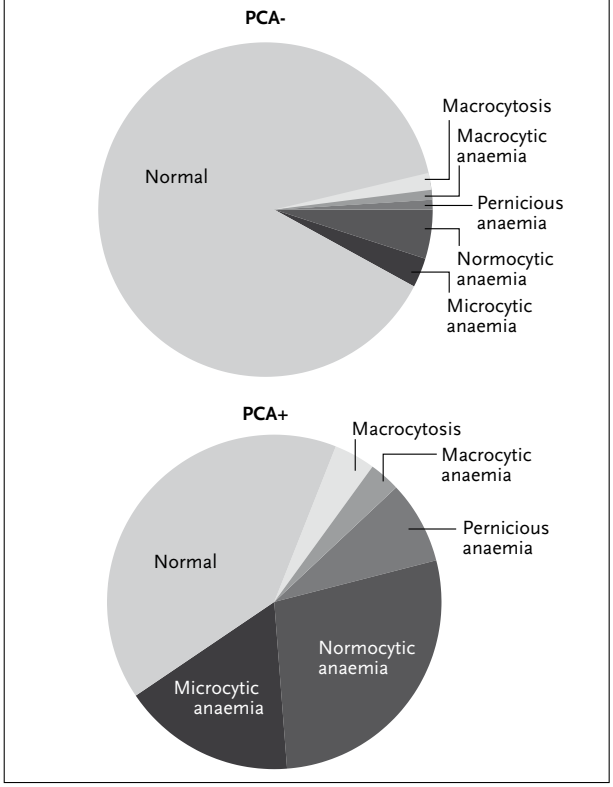


Figure 2. *Difference in prevalence of macro-, normo- and microcytic anaemia in patients negative (PCA-) and positive (PCA+) for parietal cell antibodies (p<0.001)*



DM1 patients, we performed a retrospective longitudinal study on the prevalence and clinical relevance of thyroid antibodies, parietal cell antibodies and adrenocortical antibodies. We report on the presence of OS-Ab and on the development of corresponding organ dysfunction during more than 20 years follow-up of 396 patients with DM1, median follow-up being comparable for antibody positive and negative patients.

As expected, the frequency of (subclinical) hypothyroidism, macrocytic haematological profile and different types of anaemia was significantly higher in DM1 patients with than in DM1 patients without thyroid and gastric antibodies.

Among our population of DM1 patients, the organ most frequently affected by OS-Ab was the thyroid gland. Of all the patients, 10.4% tested were positive for thyroid antibodies, which was within the range of prevalence found by other authors.[5,9,11-31] TPO-Ab were more frequent than Tg-Ab, which is also in accordance with the literature. The prevalence of hypothyroidism was significantly higher among Th-Ab positive patients than among Th-Ab negative patients and this was true for both Tg-Ab and TPO-Ab.

The PCA prevalence in our DM1 population was 5.8%, which was within the range of prevalence found by other authors.[11,13,20,22,24,25,27,46-50] Of 23 PCA-positive patients, 9% had a macrocytic blood picture, 9% pernicious anaemia and 43% had normocytic or microcytic anaemia, which was significantly higher than in PCA-negative patients.

In accordance with previous studies, we found a low prevalence of ACA (0.5%) in our DM1 population. Only two patients were ACA positive, both without signs of adrenal dysfunction. The low prevalence of ACA in our population makes it impossible to determine the predictive value of these antibodies, but high positive predictive values have been reported in the literature.[10,16,46,55,58,59,61]

In summary, this study is the first to investigate the long-term clinical relevance of organ-specific antibodies in DM1 patients in a longitudinal manner. The presence of thyroid and parietal cell antibodies is associated with an increased risk of developing (sub)clinical hypothyroidism and different types of anaemia.

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