IS TYPE 1 DIABETES OFTEN ASSOCIATED WITH OTHER AUTOIMMUNE DISEASES?

A CROSS-SECTIONAL STUDY IN BELGIUM

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INTRODUCTION

It has been known for more than 25 years that Type 1 diabetes is a chronic autoimmune disease characterised by self-destruction of the pancreatic beta-cells and the presence of autoantibodies directed against beta-cell components and endogenous insulin.[1,2] Such autoimmune aggression however is not limited to the pancreas: auto-antibodies against other endocrine systems (thyroid, adrenals) and non-endocrine tissues (gastrointestinal mucosa) were frequently found to be increased in Type 1 diabetic patients as compared to the non-diabetic population.[3-6]

The presence and titre of the antibodies are however not always a reflection of the degree of organ-destruction and are accompanied by a large spectrum of functional abnormalities. Thyroid autoimmunity can induce hypothyroidism as well as hyperthyroidism.[7] Gastrointestinal autoimmunity can generate anatomical lesions (atrophic gastritis, coeliac disease) but is also characterised by clinical syndromes induced by malabsorption of vital nutritional elements (pernicious and iron-deficiency anaemias).[8-12] Addison's disease can develop in Type 1 diabetes, but the presence of adrenal autoantibodies is more frequent than the full-blown clinical picture.[13] All these organ dysfunctions can interfere with the treatment and the prognosis of the Type 1 insulin-dependent diabetic patient.

Unfortunately, in contrast to thyroid disease in the general population,[14] little is known about the natural history of the associated autoimmunity in diabetic patients and the clinical significance of the presence of non-beta-cell antibodies.

For this reason, a large cohort of Type 1 diabetic patients was screened for the presence of organ-specific antibodies and by analysis of their characteristics a risk-profile useful to the clinician was defined so as to detect early system malfunction and to prescribe an adequate treatment.[15]

PATIENTS AND METHODS

Patients

A group of 783 Type 1 diabetic patients was studied, consisting of 286 children and 497 adults (male/female: 389/394) attending the out-patient diabetes clinic of the Antwerp University Hospital or registered in the Belgian Diabetes Registry. This group had a mean age (± SD) of 29·9 ± 16·6 years and a mean diabetes duration of 11·8 ± 10·1 years. Their fasting C-peptide level averaged 0·09 ± 0·08 pmol/ml (Nl: 0·25–1·00 pmol/ml). Still, 39% of patients were ICA+ (islet cell antibodies).

The diabetes profile and gender distribution are summarised in Table 1.

TABLE 1
Diabetes profile and gender distribution of the study population.

	Total	Males	Females	Statistics
Number	783	389	394	
Children/ Adults	286/497	137/252	149/245	
Age (y)	29·9 ± 16·6	30·1 ± 16·9	29·6 ± 16·3	n.s.
Duration (y)	11·8 ± 10·1	11·6 ± 10·1	12·0 ± 10·1	n.s.
Onset (y)	18·1 ± 12·7	18·4 ± 12·9	17·7 ± 12·5	n.s.
HbA1c (%)	8·1 ± 1·6	8·0 ± 1·7	8·2 ± 1·6	n.s.

Measurements

The level of HbA1c (glycated haemoglobin) was determined by HPLC (VARIANT haemoglobin A1c, BIO-RAD, N: 4·8–6%). A mean of four annual determinations of HbA1c was used to give the degree of overall metabolic control. C-peptide levels were measured using C-PEPsp-RIA-CT kit (Biosource Technologies, Inc. Europe S.A.). ICA (pos ≥12 JDFU) were assayed using indirect immuno-fluorescence on cryosections of fresh-frozen human donor pancreas. Thyroid peroxidase antibodies (TPO) were measured by radiobinding assay (Henningtest, Brahms, Germany, NI <100 U/ml). Thyroid function was estimated by assay of TSH (Thyroid Stimulating Hormone, Wallac, Finland, Nl: 0·15–5·5 mU/L) and free T4 levels (free thyroxine, Amerlex, UK, Nl: 11–24 pmol/l). Parietal cell antibodies (PCA) were detected by indirect immunofluorescence on sections of rat gastric mucosa (MeDiCa kit, Medical Diagnostics California, US, Nl <1/20 dilution). The indirect immunofluorescence assay for PCA correlated well with the enzyme immunoassay for H+/K+ ATPase (Varelisa, Pharmacia & Upjohn, GmbH, Germany, pos >10 U/ml) ($r = 0{\cdot}85$; $p < 0{\cdot}0001$) in 175 patients. However, 13 patients had discordant results; four were PCA+ and had no H+/K+ ATPase antibodies and nine had these antibodies but were PCA-. Anti-adrenal antibodies (AAA) and anti-endomysium IgA (EmA-IgA) were determined by indirect immunofluorescence, using respectively unfixed frozen sections of monkey adrenal tissue and sections of monkey oesophagus as antigen-substrate (MeDiCa kit, catalogue nos 6001-AG and 6001-ES respectively, Nl <1/10 dilution). The value of AAA was compared with the use of 21-hydroxylase autoantibodies in 100 patients. Quantitative determination of antibodies against 21-hydroxylase using ^{125}I-radioassay (DLD, Diagnostika GmbH, catalogue no RA007/50, Hamburg) gave the same results as those obtained by determining AAA. Antibodies to intrinsic factor (AIF) were measured by radiobinding assay (Diagnostics Products Corporation, Los Angeles, US, Nl <1·1). Pernicious anaemia was defined as a megaloblastic anaemia with positive AIF and/or PCA.

Serologic HLA (Human Leucocyte Antigen) DR typing was performed in 280 adult patients using a two-colour fluorescence technique as described by Van Rood.[16]

Serum iron, total iron binding capacity, and a peripheral haemogram (Hb, Hct, MCV, MCH) were determined by routine laboratory tests. Serum gastrin was measured using RIA (radioimmunoassay) liquid technique (Eurodiagnostics, Malmö, Sweden, Nl <110 ng/L).

A gastroscopic and anatomo-pathological examination was performed in a subgroup of 88 patients (PCA+ vs. PCA- (46 vs. 42)) with gastric symptoms or in subjects suspected to have atrophic gastritis. At upper gastrointestinal endoscopy with the use of a fibreoptic endoscope (Olympus Videoscope), two tissue fragments from the corpus, two from the fundus, two from the antrum and two from the postbulbar duodenum were obtained and assessed for evidence of gastric atrophy as defined by the updated Sydney system.[17]

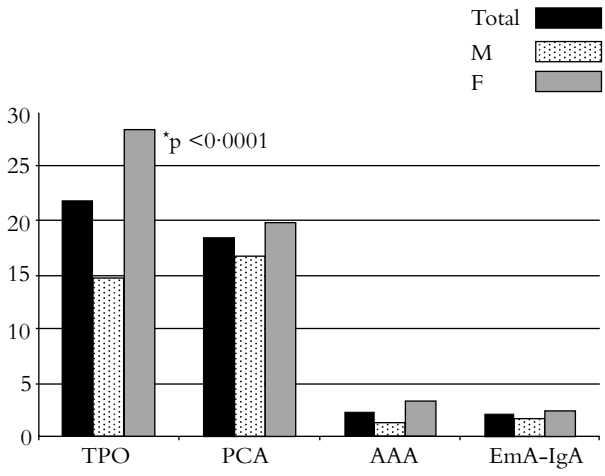
Statistical analysis

All data of this cross-sectional study were analysed using the statistical package SPSS version 8·0. Distributions of continuous data were tested for normality by the Kolmogorov Smirnov test. The unpaired t-test, Mann-Whitney U-test or ANOVA (analysis of variances) with Tukey Kramer as *post hoc* analysis was used to determine differences between groups. Differences in distributions of categorical data were investigated by Chi-square or Fisher's exact test when appropriate. Multiple logistic regression, stepwise forward, was used to assess the strength and independence of associations, and to describe an adequate predictive model. All tests were performed two-tailed. A p-value <0·05 was considered significant.

RESULTS

Of the 783 Type 1 diabetic subjects, 35·5% showed one or more organ-specific autoantibodies. TPO were present in 21·6%, PCA in 18·3%, AAA in 2·2% and EmA-IgA in 2·1% (Figure 1). In 45 individuals (5·7%) TPO and PCA were found simultaneously. Most patients showed the HLA DR3 and/or DR4 haplotype with similar gender distribution.

FIGURE 1
Prevalence (%) Auto AB.



TPO: Anti-thyroid peroxidase antibodies; PCA: Anti-pareietal cell antibodies; AAA: Anti-adrenal antibodies; EmA-IgA: Anti-endomysium antibodies IgA

Thyroid peroxidase antibodies were found in 16·1% of the paediatric group and 24·7% of the adults, with a female preponderance (p <0·0001) (Table 2). TPO+ patients were older (p <0·0001), had a longer duration of disease (p = 0·007) and had a higher age at onset of diabetes (p = 0·006) than TPO- subjects. The presence of TPO did not correlate with any HLA DR haplotype. TPO+ patients were more prone to have PCA than TPO- subjects (p = 0·0023). The role of gender, age, duration of disease, age at diagnosis of diabetes, HLA DR type, and PCA status as predictors of TPO status was assessed by logistic regression analysis. This analysis showed that gender (ß = -0·91; p <0·0001), age (ß = 0·02; p = 0·0008), and PCA status (ß = 0·50; p = 0·029) were independently associated with TPO status. A history of hypo- and hyperthyroidism was found in 3·8% and 4·2% respectively of all adult patients. Five children were dysthyroid. TPO+ individuals had a higher frequency of hypothyroidism (p = 0·0003) and of hyperthyroidism (p <0·0001) than TPO- subjects. Hypothyroidism, but not hyperthyroidism, was more prevalent in female than in male patients (OR: 4·04; 95% CI: 1·32–12·36; p = 0·0095). The area under the curve (AUC^{ROC}) for TPO to identify patients with dysthyroidism was 0·82 ± 0·04; meaning that 82% of cases with dysthyroidism have higher TPO values than euthyroid subjects. We also observed an association between the HLA DR5 haplotype and hypothyroidism (OR: 11·76; 95% CI: 3·41–40·53; p = 0·0006).

Parietal cell antibodies were present in 13·6% of children and in 20·9% of adults with Type 1 diabetes with a similar gender distribution (Table 3). Patients with PCA were older (p = 0·001), had a longer duration of disease (p = 0·004) and a higher age at onset of diabetes (p = 0·019) than PCA- individuals. In addition, PCA positivity was associated with the HLA DR5 haplotype (p = 0·0012) (Figure 2). Logistic regression was used to determine whether age, gender, duration of disease, age at onset, TPO status, and HLA DR type could predict PCA status. This analysis showed that only age (ß = 0·02; p = 0·0027) and TPO status (ß = 0·55; p = 0·0155) were independent risk factors for PCA.

We observed a higher prevalence of hypochromic microcytic anaemia in PCA+ than PCA- subjects (p = 0·0099). The mean serum iron level did not differ between patients with or without PCA at the moment of this study. This is because iron deficient patients, who were detected during an initial study,[18] were treated with iron supplements. As expected, women had lower serum iron concentrations than men (p = 0·0047). The presence of iron deficiency anaemia was determined by PCA status (ß = 0·81; p = 0·034), gender (ß = -0·99; p = 0·007) and duration of diabetes (ß = 0·06; p = 0·0005) when age, gender, duration, HbA1c, PCA status, and HLA DR type were tested as covariates. Mean levels of gastrin were higher in PCA+ than in PCA- patients (p <0·0001). Neither age nor gender influenced serum gastrin levels. Pernicious anaemia was present in 2·6% of all diabetic subjects, with more PCA+ than PCA- patients being affected (p <0·0001). In 84·6% of patients with this

TABLE 2
Profile of TPO-positive compared to TPO-negative diabetic children and adults.

	TPO +	TPO -	Statistics	Odds ratio (95% C.I.)
Total	169 (21·6%)	614		
Male/Female (n)	57/112	332/282	p <0·0001	2·31 (1·62–3·30)
Children/Adults (n)	46/123	240/374	p = 0·0051	0·58 (0·40–0·85)
Age (y)	34·3 ± 16·5	28·7 ± 16·4	p <0·0001	
Duration (y)	13·9 ± 10·9	11·3 ± 9·9	p = 0·007	
Onset (y)	20·7 ± 12·9	17·4 ± 12·6	p = 0·006	
TSH (μU/ml)	2·01 ± 1·54	1·62 ± 0·95	p = 0·007	
Hypothyroidism (n)*	12 (9·8%)	7 (1·9%)	p = 0·0003	5·67 (2·18–14·75)
Hyperthyroidism (n)*	16 (13·0%)	5 (1·3%)	p <0·0001	3·39 (2·53–4·53)
PCA+ (n)	45 (26·6%)	98 (16·0%)	p = 0·0023	1·91 (1·28–2·86)

*Tested in adult patients. Results are expressed as mean ± standard deviation.

TABLE 3
Profile of PCA-positive compared to PCA-negative Type 1 diabetic patients.

	PCA+	PCA-	Statistics	
Total	143 (18·3%)	640		
Male/Female (n)	65/78	324/316	n.s.	
Children/Adults (n)	39/104	247/393	p = 0·0123	0·60 (0·40–0·89)
Age (y)	34·4 ± 16·9	28·9 ± 16·4	p = 0·001	
Duration (y)	14·2 ± 11·2	11·3 ± 9·8	p = 0·004	
Onset (y)	20·5 ± 12·6	17·6 ± 12·7	p = 0·019	
TPO+	45 (31·5%)	124 (19·4%)	p = 0·0023	1·91(1·28–2·86)
Serumiron (μg/dl)*	91·1 ± 36·3	91·5 ± 41·6	n.s.	
Gastrin (n = 158) (ng/L)	194·4 ± 226·0	96·6 ± 36·0	p <0·0001	
Hypergastrinaemia*	28 (26·9%)	28 (7·1%)	p <0·0001	4·80 (2·69–8·57)
Hypochr. anaemia*	16 (15·4%)	27 (6·9)	p = 0·0099	2·47 (1·27–4·77)
Pernicious anaemia*	11 (10·5%)	2 (0·5%)	p <0·0001	23·1 (5·04–106·1)

*Tested in adult patients. All parameters were analysed in all 783 patients unless otherwise indicated. Results are expressed as mean ± SD.

megaloblastic anaemia, PCA was present. A subgroup of 88 adult patients (PCA+ vs. PCA- (46 vs. 42); M/F: 51/37) demonstrating gastric symptoms or suspected to have atrophic gastritis underwent gastroscopy with biopsies. In the PCA+ group 26 (56·5%) of the patients had atrophic gastritis compared to five (11·9%) in the PCA- group (OR: 9·62; 95% CI: 3·20–28·93; p <0·0001). The area under the curve (AUC^{ROC}) provided by PCA to identify patients with atrophic gastritis was 0·80 ± 0·05. Patients with atrophic gastritis were more prone to have iron deficiency anaemia than those without atrophic gastritis (OR: 3·37; 95% CI: 1·22–9·29; p = 0·02). Gender did not influence the prevalence of autoimmune gastritis.

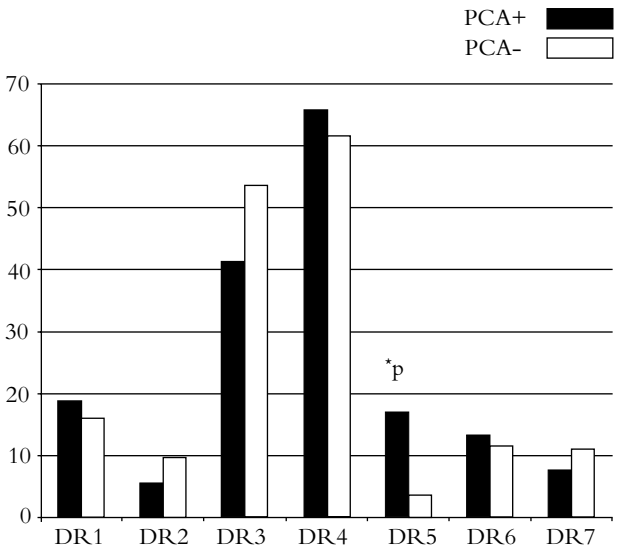
AAA were present in 2·2% and EmA-IgA in 2·1% of Type 1 diabetic subjects. Of the eight EmA-IgA+ patients six had the HLA DR3 haplotype. No patient was found to have Addison's disease, and coeliac disease was detected in three patients.

The prevalence of clinical thyrogastric associated disease is presented in Figure 3.

DISCUSSION

Type 1 diabetic children and adults show a high prevalence of organ-specific autoimmunity. In this study 35·5% of the patients were autoantibody positive. These antibodies were mainly targeted to the thyroid gland (21·6%) and the gastric

FIGURE 2
Prevalence HLA-DR (%).



HLA-DR distribution in PCA+ and PCA- patients
*p = 0·0012; OR 5·59 (95% CI: 2·05–15·32)

mucosa (18·3%), confirming previous findings.[3-6, 12, 15, 19-21] Type 1 diabetes mellitus is predominantly an autoimmune disease but the cause for this autoimmune response to the beta-cell and to other endocrine and gastrointestinal tissues remains to be clarified. However, not all Type 1 diabetic patients develop other autoimmune diseases and different pathogenetic mechanisms may exist between patients with isolated Type 1 diabetes and those with a more generalised autoimmune disease. In that case, it should be possible to identify a subgroup of diabetic patients who are at risk to develop other autoimmune disorders. We investigated whether the autoantibody status can be predicted by gender, age, duration of disease, age at onset of diabetes and HLA DR type.

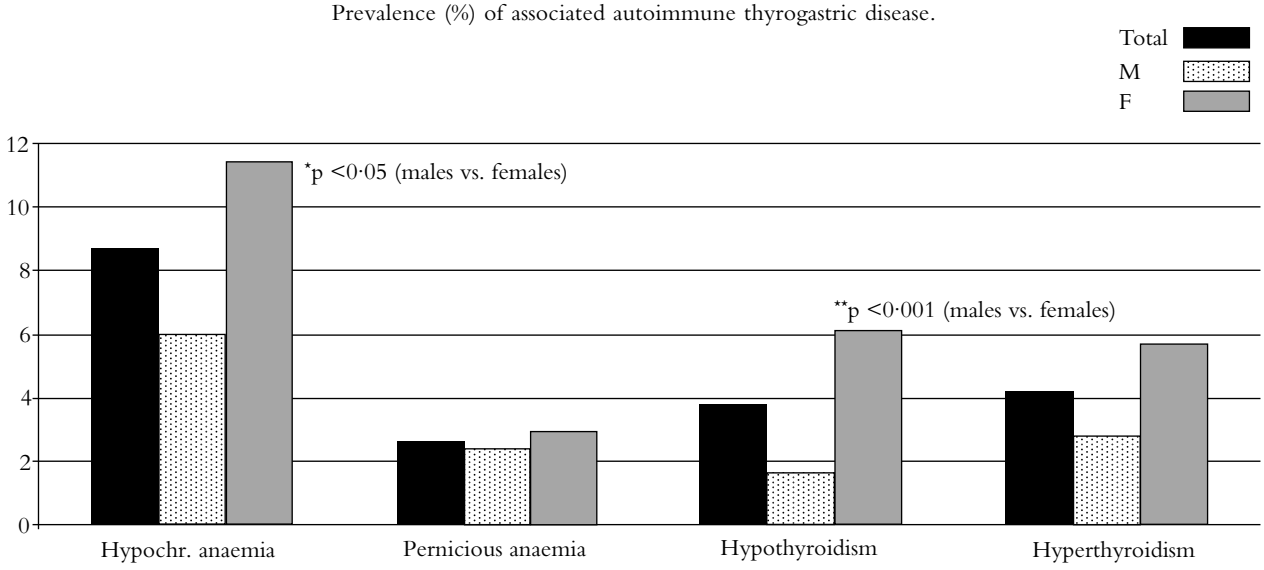
Thyroid autoimmunity was more prevalent in the female population, at higher age and in patients already showing PCA. Parietal cell antibodies were more prevalent with increasing age and in patients with TPO antibodies. Gender did not influence the presence of PCA. The trend for increased prevalence of thyroid and gastric autoantibodies with age is in line with the hypothesis of autoimmune disease being the final phase of a process starting with autorecognition, passing through immunity with appearance of autoantibodies, and finally cell damage and autoimmune disease.[22]

Different HLA type associations might partly explain the susceptibility to react to different organs as well. In the present study a positive association was demonstrated between PCA positivity and the HLA DR5, but not DR3 and/or DR4 haplotype. We must indicate however that the attributable fraction for the allele is low. We also observed an association between the rare HLA DR5 haplotype and hypothyroidism, confirming previous reports,[23, 24] but no associations were found between any HLA DR haplotype and hyperthyroidism, in contrast to literature data.[25, 26]

The timing of the screening also appears to be important. Indeed,when new-onset Type 1 patients are examined the presence of TPO antibodies is associated with islet cell autoimmunity (ICA) and the high risk haplotype HLA DQA1*0301-DQB1*0302, suggesting a more general immune response that could disappear[27] in some patients together with the positive ICA. Screening should start at diagnosis of diabetes, but should also be repeated at two to three year intervals after clinical onset, since age and duration of diabetes play an important role in the development of the antibodies.

The presence of autoantibodies may point to (sub)clinical organ dysfunction. The AUC^{ROC} for TPO and for PCA were 0·82 and 0·80 respectively, meaning that in over 80% of cases those with autoimmune thyrogastric disease have higher thyroid gastric antibody values. The natural history of thyroid disorders in TPO+ diabetic patients has not yet been established, but the progression to overt dysthyroidism seems very slow.[14] In the present study, 3·8% of adult Type 1 diabetic subjects had a history of overt hypothyroidism, with a preponderance of TPO+ and female patients, whereas 4·2% had a history of hyperthyroidism, but with a similar gender distribution. TPO+ men and TPO+ women had a similar

FIGURE 3
Prevalence (%) of associated autoimmune thyrogastric disease.



prevalence of thyroid dysfunction. These numbers are in line with previously reported prevalences.[28, 29] In the non-diabetic population an overt thyroid disorder is found in about 0·3% of subjects, which is ten times less frequent.[29] The appearance of thyroid pathology in Type 1 diabetic patients remains however underestimated. Screening of these patients for the presence of TPO and testing of the thyroid function is highly recommended by the American Diabetes Association.[30] This is to alert the physician in an early stage of thyroid pathology and, if necessary, to interfere with the natural progress. Hypothyroidism can cause suboptimal growth in diabetic children and increase the atherogenic risk due to hypercholesterolaemia, whereas hyperthyroidism can worsen the metabolic control.[29]

The clinical significance of PCA is demonstrated by their cytotoxicity to the gastric mucosa, eventually leading to autoimmune atrophic gastritis.[9] PCA specifically target the gastric proton pump, the H+/K+ ATPase,[31] resulting in a decreased gastric acid secretion and a decreased iron absorption.[32] This can cause iron deficiency anaemia, a common manifestation of atrophic gastritis.[10, 33] In the present study the presence of PCA was an independent risk factor for iron deficiency anaemia. Elevated levels of gastrin are also associated with autoimmune gastropathy. In addition, PCA inhibit the secretion of intrinsic factor, necessary for the absorption of vitamin B_{12}. This may lead to pernicious anaemia which is found in 2·5 to 4% of Type 1 diabetic subjects.[11] We observed pernicious anaemia in 2·6% of the subjects. PCA were present in 84·6% of those with pernicious anaemia, confirming published prevalences ranging from 75–90%.[34] The prevalences of atrophic gastritis and pernicious anaemia in the non-diabetic population are 0·1% and 1% respectively. In our study, atrophic gastritis was present in a high percentage of a subgroup of 88 adult patients, with PCA+ individuals being more frequently affected than PCA- patients. This percentage may be overestimated since only patients with gastric symptoms or those with suspected atrophic gastritis were studied.

Data from this study and the literature indicate that PCA are good markers of autoimmune gastric manifestations, such as iron deficiency anaemia, pernicious anaemia and atrophic gastritis. The well-known complications of these disorders can influence the prognosis of the patient. Iron deficiency anaemia can influence work capacity, cardiopulmonary status, immunity and intestinal function.[35] Pernicious anaemia can cause neurological complications and predisposes, as well as atrophic gastritis, to gastric adenocarcinoma.[36-38] Therefore, particularly in anaemic patients, screening for PCA, and in case of positivity additional evaluation, including gastroscopy with biopsy, are needed to take necessary steps in time.

The presence of AAA is associated with hypocorticism which, by reducing the insulin needs, can cause frequent attacks of hypoglycaemia.[13] The prevalence of EmA-IgA is rather low in this large cohort. This may be explained by the rather young age of the population, but a different diet and genetic background may play a role as well. EmA-IgA antibodies are a screening parameter for coeliac disease, which can lead to retarded growth and signs of malabsorption, undermining the metabolic control.[39]

In conclusion, the high prevalence of TPO (21·6%) and PCA (18·3%) positivity warrants their screening in Type 1 diabetic children and adults. On the basis of autoantibody positivity and in the absence of any other clinical sign of illness, functional tests or gastric biopsies showed that a rather large number of these diabetic patients had an autoimmune thyroid or gastric disease.

The actual database should enable the organisation of a systematic follow-up in a large number of patients and after ten or 20 years have a better idea about the natural history of associated autoimmune disease in Type 1 diabetes.

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