

The Predictive Significance of Autoantibodies in Organ-Specific Autoimmune Diseases

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Abstract Many organ-specific autoimmune diseases are preceded by a long pre-clinical phase, and several longitudinal cohort studies have shown that patients may carry autoantibodies many years before they manifest clinical symptoms. Detecting these antibodies in serum has been shown to have strong predictive value, depending on the particular autoantibody, test method, and disease at issue. This review examines the predictive value of various autoantibodies that are found in organ-specific autoimmune diseases, such as primary biliary cirrhosis, Addison's disease, Hashimoto's thyroiditis, type-1 diabetes, celiac disease, and Crohn's disease.

Keywords Predictive autoantibodies · Primary biliary cirrhosis · Addison's disease · Hashimoto's thyroiditis · Type-1 diabetes · Celiac disease · Crohn's disease

Introduction

The presence of specific autoantibodies constitutes an important criterion supporting the clinical diagnosis of many organ-specific autoimmune diseases. Several studies have demonstrated that the presence of many marker autoantibodies can precede the clinical symptoms of the disease by years. Indeed, the natural history of autoimmune diseases reveals that before the disease is clinically manifested with signs and symptoms that lead to a definite

diagnosis, disease onset is preceded by an asymptomatic phase which can last for many years and in which specific autoantibodies are already present in patients' sera [1, 2].

This review examines studies of various autoantibodies for which a role in predicting the development of an organ-specific autoimmune disease has been demonstrated. These include:

Anti-thyroid antibodies as predictors of hypothyroidism
Anti-mitochondrial antibodies as predictors of primary biliary cirrhosis

Anti-islet cell antibodies as predictors of type-1 diabetes

Anti-adrenal cortex antibodies as predictors of Addison's disease

Anti-*Saccharomyces cerevisiae* antibodies as predictors of Crohn's disease

Anti-tissue transglutaminase antibodies as predictors of celiac disease

Anti-Thyroid Antibodies and Hypothyroidism

Autoimmune thyroid diseases are marked by the presence of anti-thyroid peroxidase antibodies (anti-TPO). A long pre-clinical phase is characteristic, with the anti-TPO antibodies present even decades before the first clinical signs.

Turnbridge initiated a longitudinal study of the prevalence of thyroid disorders in the population of Great Britain [3]. The random sample of 2,779 adults was selected to parallel the age, sex, and social class of the general population. Subjects underwent detailed tests of thyroid function at the study's outset, including screening for anti-TPO and anti-thyroglobulin antibodies. Approximately 20 years later, 1,877 of the subjects were retested. The

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group which initially had had either anti-thyroid antibodies or raised serum thyroid-stimulating hormone (TSH) was associated with a significantly raised risk of developing clinical hypothyroidism [1]. Having only a raised serum TSH (>6 mU/l) meant an odds ratio of 8 (95% confidence interval, 3–20) for women and 44 (19–104) for men. Having positive anti-thyroid antibodies alone meant an odds ratio of 8 (5–15) for women and 25 (10–63) for men. Having both raised TSH and present anti-thyroid antibodies meant ratios of 38 (22–65) for women and 13 (81–370) for men.

Three other studies also found anti-TPO to be a strong predictor for hypothyroidism. In one, a 10-year prospective study, Huber et al. [4] showed that the strongest predictive factors, in order, were basal serum TSH, positive anti-TPO antibodies, and impaired thyroid reserve. Their study analyzed the spontaneous course of the development of overt thyroid failure in 82 female patients with subclinical hypothyroidism. The relative risk of developing overt thyroid failure increased by 15.6 times in the highest risk group when all three of these parameters were present.

In the second study, post-partum thyroid dysfunction (PPTD) was studied. Anti-TPO antibodies were shown to be a good predictor of PPTD by Kita et al. [5] in a study of 1,594 consecutive women within 3 days of their delivery. Eighty-three (5.2%) were found anti-TPO-positive and 74 were followed up for 1 year for clinical and biochemical evidence of PPTD. Approximately 38 of these (51.4%) developed a thyroid disorder: 18% developed hyperthyroidism alone, 40% developed hypothyroidism alone, and 42% developed hyperthyroidism followed by hypothyroidism. A high anti-TPO antibody level immediately postpartum could predict thyroiditis with 97% sensitivity and 91% specificity.

In the third study, a very large cohort of 308 anti-TPO-positive and 102 anti-TPO-negative pregnant women was studied at the University of Wales in Cardiff (UK) [6]. In the anti-TPO-positive group, 147 out of 308 developed PPTD, and 161 remained euthyroid. None of the 102 anti-TPO-negative women developed PPTD during the 35-week follow-up period. The sensitivity of anti-TPO antibodies in PPTD prediction was 100%, specificity was 62%, and the positive predictive value 48%. Furthermore, the likelihood of developing PPTD corresponded directly with the anti-TPO level.

Anti-Mitochondrial Antibodies and Primary Biliary Cirrhosis

Three studies of anti-mitochondrial antibodies (AMA) show its high value as a predictor for primary biliary cirrhosis (PBC). In 1986, Mitchison et al. [7] reported a group of 29 patients who were positive for AMA, the disease-specific marker for PBC, but who had normal liver

function test results and no symptoms of liver disease. Seven of the patients had been referred to the liver clinic for investigation of an incidental finding of AMA positivity during investigations for other diseases. The other 22 were found during a general antibody screening, in which individuals with positive AMA were selected. Twenty-six of the 29 AMA-positive patients had biopsy samples that were diagnostic of or compatible with PBC, two had nonspecific hepatitis, and one had normal liver histology. About 10 years later, the same investigators reviewed the records of these 29 patients and collected clinical and biochemical data [8]. Five had died (although none of the deaths was attributable to liver disease); 22, including two of the five who died, had become symptomatic, and 24 had developed persistently abnormal liver function. The median time from first positive AMA test to persistently raised alkaline phosphatase was 5.6 years. Repeated liver histology was done for ten patients: in nine, original and follow-up biopsies were diagnostic for PBC; in one patient, both biopsy samples were normal, even after the development of raised alkaline phosphatase and γ -glutamyl transpeptidase. This study provided clear evidence of the predictive link between AMA positivity and the development of clinical signs of PBC. It showed that almost all subjects testing positive for AMA already had histological lesions compatible with PBC, or would develop clinical evidence of it, even in the absence of clinical or biochemical signs of hepatic damage at time of antibody testing.

A prospective study by Prince et al. [9] of 770 patients, followed for 28 years, also showed that development of PBC symptoms is almost assured for subjects testing positive for AMA. At the time of initial testing, 61% of the subjects (496 individuals) were asymptomatic for liver disease. All 770 patients had been diagnosed either with probable PBC, on the basis of either AMA positivity or abnormal hepatic function, or with definite PBC, on the basis of three simultaneous results: AMA positivity, abnormal hepatic function, and histology. Approximately 50% of the patients developed symptoms after 5 years and 95% after 20 years. The Kaplan–Meier analysis clearly showed that the asymptomatic phase of PBC can be regarded as a temporary phenomenon. If patients survive for a long enough period of time, virtually all will develop one or more symptoms of PBC.

Kisand et al. [10], in a screening study of the prevalence of autoimmune diseases in the Estonian population, tested 1,958 individuals aged between 15 and 99 years. They used two immuno-enzymatic (enzyme-linked immunosorbent assay) tests, one for antibodies to native pyruvate dehydrogenase complex (PDC) and the other to recombinant PDC-E2 fusion protein. There were 14 persons with at least one of the two antibodies who were asymptomatic. Long-term follow-up was possible on eight of these subjects. By the

ninth year of follow-up, three out of the eight had developed abnormal biochemical liver test results.

Together, these studies show that AMA may be present well before the clinical manifestation of PBC and that they have a very high positive predictive value.

Anti-Islet Cell Antibody and Type-1 Diabetes

Four different autoantibodies are predictive markers of type-1 diabetes [insulin-dependent diabetes mellitus (IDDM)]: pancreatic islet cell antibodies; antibodies to insulin, to 65-kD glutamic acid decarboxylase (GAD), and to tyrosine phosphatase-like protein (IA-2). They represent the autoimmune response against pancreatic islet cells and are already present during the pre-clinical phase of the disease.

In the study by Bingley et al. [11], the presence of islet cell antibodies (ICA), GAD, IA-2, and insulin antibodies was determined at study entry in 3,655 nondiabetic first-degree relatives of patients with type-1 diabetes. After a median follow-up of 3 years (range 0.6–11.3), 26 family members developed insulin-requiring diabetes. Overall, 18% had four positive markers in the baseline sample, 27% had three markers, 35% had two markers, 8% had a single marker, and 12% had no markers. Individuals with two or more antibodies had a 50% overall risk of developing diabetes within 10 years. Combining autoantibody markers identified higher levels of risk and achieved higher sensitivity than any single marker. Similar results were observed in studies by Verge et al. [12] and Ziegler et al. [13].

An unselected population of 755 siblings of children with IDDM was studied by Kulmala et al. [14] to evaluate the predictive value of GAD, IA-2, anti-insulin and ICA antibodies, and combination of these markers. About 32 siblings progressed to IDDM within 7.7 years of the initial sample taken at or close to the diagnosis of the index case (median follow-up, 9.1 years). The positive predictive values of ICA, IA-2, GAD, and anti-insulin antibodies were 43, 55, 42, and 29%, and their sensitivity 81, 69, 69, and 25%, respectively. The risk for IDDM in siblings with four/three, two, one, or no antibodies was 70, 25, 2, and 0.8%, respectively.

In a similar study, Knip et al. [15] observed a group of 57 siblings of IDDM patients, who had tested positive either for ICA or anti-insulin antibodies, or for both, in their initial blood sample taken within 6 months after the diagnosis of the index case. Approximately 17 siblings (29.8%) presented with IDDM during the observation period, which averaged 47 months (range, 0.5–69 months).

In a long-term prospective study in the general population, La Gasse et al. [16] tested autoantibodies to ICA, GAD, IA-2, and insulin in 4,505 schoolchildren. Overall,

12 (0.3%) had multiple antibody positivity, 102 (2.3%) had a single antibody, and 4,391 (97.5%) were negative for all antibodies. About 8 years later, 3,000 (67%) subjects were re-contacted: 6 subjects among the 12 who were positive to multiple autoantibodies had developed diabetes. Of the others (those with either only one antibody-positive result or with ICA and one antibody positivity or those with no antibody positivity), none developed diabetes. The positive predictive value for multiple antibody positivity and diabetes occurrence was 50%, and sensitivity was 100%.

The Diabetes Autoimmunity Study in the Young (DAISY) study followed 1,972 children who were identified by human leukocyte antigen (HLA) screening as at risk for diabetes [17]. During the follow-up (mean 4 years; range, 0.2–9) 162 children were found positive for at least one autoantibody. However, 31% (50 of 162) of the children were false positive (autoantibodies were negative twice on blinded duplicate aliquots), and none of them developed IDDM. Of those confirmed positive, in 50 children (31%) the positive result was transient (confirmed on blinded duplicate aliquots but negative on follow-up), and in 62 (36%), the result was persistently positive. None of the transiently positive children developed diabetes. In contrast, in the persistently positive group, 24 have progressed to diabetes, while 38 were nondiabetic.

Ferber et al. [18] studied 184 women with gestational diabetes mellitus and found that HLA typing and GAD antibodies could predict the development of post-partum IDDM. Predictive sensitivity increased to 92% when testing for both HLA alleles (DR3, DR4) and autoantibodies was performed in combination. Combining the determination of susceptible HLA alleles (DR3, DR4) with autoantibodies measurement increased the sensitivity of identifying women with gestational diabetes developing post-partum IDDM to 92%. In this study, GAD-antibody presence was the strongest independent predictor of IDDM.

In general, on the basis of the aforementioned studies and on numerous other prospective studies effected on both diabetic patients and nonselected populations, it has been proven that antibody tests give elevated but not absolute predictive specificity and sensitivity (about 90%) and that the risk of diabetes progressively grows with the duration of the follow-up and with the number of positive autoantibodies present in the subject.

Anti-Adrenal Cortex Antibodies and Addison's Disease

The predictive value of adrenal cortex autoantibodies (AcA) was first demonstrated in 1983 by Betterle et al. [19] who studied 284 healthy subjects and 1,045 individuals with other organ-specific autoimmune diseases. Among the healthy subjects, the only one who had AcA

later developed Addison's disease. In the group of patients with organ-specific autoimmune diseases, four of the five who had AcAs and normal adrenal function developed overt Addison's disease during the 20-month follow-up.

In a subsequent study, the same authors measured AcA by indirect immunofluorescence (IIF) in 8,840 adult patients [20] and in 808 children [21] with organ-specific autoimmune diseases without adrenal insufficiency. Among the adult patients, 67 (0.8%) were AcA-positive. Forty-eight of these AcA-positive patients and 20 AcA-negative individuals were further enrolled into a prospective study. In these individuals, antibodies to steroid 21-hydroxylase (21-OH), steroid 17 α -hydroxylase and cytochrome P450 side-chain enzyme were measured by immunoprecipitation assay. During the study, overt Addison's disease developed in 21% and subclinical hypoadrenalism in 29% of the AcA-positive patients, while 50% of the AcA-positive patients maintained normal adrenal function. Progression to Addison's disease was more frequent in patients with subclinical hypoadrenalism, high titers AcA and high levels of 21-OH antibodies. None of the AcA-negative subjects developed the disease.

In the group of children, the authors found 14 (1.7%) who were AcA positive. Ten AcA-positive and 12 AcA-negative children were followed up for 10 years by evaluating the adrenocortical function (ACTH test) and autoantibody status. Overt Addison's disease developed in 9 (90%) of the AcA-21-OH-positive children, and the one remaining child had subclinical hypoadrenalism. None of the AcA-negative control children developed Addison's disease.

In a study by Ketchum et al. [22], AcA testing by IIF was performed in three groups: 1,675 patients with insulin-dependent diabetes; 2,032 relatives of IDDM patients, and 2,543 clinically normal subjects. AcA was found in 30 (1.8%) of the IDDM group, 28 (1.6%) of relatives group, and 16 (0.6%) of the normal group. Adrenal function was studied in 30 asymptomatic patients (13 who presented with adrenal cortex antibodies and 17 without the antibodies): the mean plasma levels of ACTH and plasma renin activity were significantly higher in those with the antibodies than in subjects with no antibodies ($P < 0.01$), indicating initial adrenal dysfunction.

Finally, studying a large group of 3,020 patients with one or more endocrine autoimmune diseases, Laureti et al. [23] identified 26 AcA-positive subjects. Approximately 19 of these patients were followed for 2–4 years: eight patients developed clinical Addison's disease, six showed a deterioration of adrenal function without development of clinical Addison's disease, and five had remission of adrenal dysfunction during the follow-up period.

Taken together, these studies show that AcA may precede disease onset by many years and that the positive predictive value of AcA presence is about 70%.

Anti-*Saccharomyces cerevisiae* Antibody and Crohn's Disease

Israeli et al. [24] were the first to document the predictive value of anti-*Saccharomyces cerevisiae* antibodies (ASCA) as markers for Crohn's disease. Serum samples from 32 subjects with Crohn's disease and from 8 patients with ulcerative colitis (taken at the time the subjects were serving in the Israeli Defense Force before they were clinically diagnosed) were tested for ASCA. About 95 samples from matched controls were also studied. ASCA were present in 10 of 32 (31.3%) patients with Crohn's disease before clinical diagnosis, compared with 0 of 95 controls ($P < 0.001$). None of the eight patients with ulcerative colitis was ASCA positive. The mean interval between ASCA detection and diagnosis was 3 years.

Anti-Tissue Transglutaminase Antibodies and Celiac Disease

Anti-tissue transglutaminase (anti-tTG) and anti-endomysial (EMA) antibodies have a high diagnostic sensitivity and specificity for celiac disease (CD). Their predictive value for disease onset has been documented by Maki et al. [25]. Serum samples collected from 3,654 Finnish students (age range, 7 to 16 years) were screened for EMA and anti-tTG antibodies. About 56 subjects (1.5%) tested positive for the antibodies. Of these, 10 had already received a diagnosis of CD, 10 refused further investigation, and of the other 36 subjects who agreed to undergo an intestinal biopsy, 27 had evidence of CD.

Hoffenberg et al. [26] tested for the haplotype HLA-DR3 (DQB1*0201) in a population-based screening study of 22,346 newborns in Denver, Colorado. About 987 positive babies were selected for a prospective study. Participants were followed for as long as 7 years with serial testing for serum IgA anti-tTG antibodies and for evidence of CD. Overall, 40 children tested positive for IgA tTG autoantibody on at least one occasion, and 19 had evidence of CD (10 by biopsy, 9 by persistent seropositivity).

Simmel et al. [27] studied preserved serum samples of children previously recruited for the Finnish Type-1 Diabetes Prediction and Prevention Project. Among the 41,127 original participants, 1,101 were determined to be susceptible for CD because they carried HLA-DQB1*02. These individuals were selected for further study. During up to 8 years of follow-up, 35 children developed anti-tTG antibodies. However, 18 of these 35 anti-tTG-positive children (51%) subsequently lost anti-tTG without any dietary manipulation. In addition, among the 21 children who underwent a duodenal biopsy (19 IgA anti-tTG positive and 2 IgA-deficient and IgG anti-gliadin positive),

only 13 (62%) had villous atrophy. This study clearly showed that, in young children, transient anti-tTG positivity is not unusual and that the predictive value of anti-tTG antibodies is about 50–60%.

Conclusions

It has been clearly demonstrated that many autoantibodies have the ability to predict the development of an organ-specific autoimmune disease in asymptomatic persons. It is also clear that the progression towards a given autoimmune disease, and its severity, can be predicted from the type of antibody, the antibody level, and the number of positive antibodies. However, the results of laboratory tests depend on the diagnostic accuracy of the analytic method used; therefore, it is important to select assay methods with high diagnostic specificity to minimize the number of false positives and obtain a high positive predictive value [28].

Clinical and ethical implications of autoantibody presence in asymptomatic subjects remain an area for further discussion. In cases where the predictive value of an antibody is extremely high (say, above 90%, such as in primary biliary cirrhosis), ethical questions become acute: should subjects be alerted to their likelihood of developing a disease? The answer would be “yes” if lifestyle behavior or early therapeutic intervention could delay disease onset or mitigate the severity of the disease. If not, the risk of a negative psychological effect could largely exceed any potential benefit of informing the patient [29]. To make an ethical decision, a physician must weigh the patient's personal preferences, risk tolerance, and general psychological health, along with the confidence level in the diagnostic specificity and predictive value of the tests used for the disease in question [30]. This becomes a complicated subjective equation requiring a significant familiarity with the patient, but it is a question that must be confronted in each patient's case.

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