Clinical characteristics and autoantibody pattern in newly diagnosed adult-onset autoimmune diabetes

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INTRODUCTION
Autoimmune diabetes in adults comprises a broad spectrum of clinical phenotypes. The aim of the study was to investigate clinical and biochemical features and anti-islet autoantibody pattern in adult patients with newly diagnosed autoimmune diabetes with regard to age and the number of autoantibodies detected at diagnosis.

OBJECTIVES

The studied age groups did not differ with respect to the majority of clinical and laboratory features (e.g., clinical presentation, metabolic status, or degree of insulin deficiency). Autoantibodies to islet cell cytoplasm and to glutamic acid decarboxylase 65 occurred more frequently in younger patients, while the prevalence of autoantibodies to intracytoplasmatic domain of the tyrosine phosphatase-like protein (IA-2A) was similar in both age groups. Single autoantibody positivity was observed more often in older patients. The most common isolated autoantibody in this group was IA-2A. The presence of multiple autoantibodies was associated with younger age, lower fasting and stimulated C-peptide levels, and shorter duration of symptoms.

CONCLUSIONS
The patient’s age at diabetes onset does not determine clinical and biochemical characteristics at diagnosis but is associated with different autoantibody status. IA-2A antibodies may be useful in diagnosing autoimmune diabetes in adult patients. The assessment of the immune profile at diagnosis may help identify patients at a higher risk of significant insulin deficiency.
with these characteristics, yet has detectable anti-islet autoantibodies and progresses rapidly to insulin therapy. This slowly progressing autoimmune diabetes is known as “latent autoimmune diabetes in adults” (LADA).

LADA is usually diagnosed at an older age; patients do not require insulin for at least 6 months after diagnosis and are positive for at least 1 known anti-islet autoantibody. Since LADA phenotype is not specific and often overlaps with type 2 diabetes, the final diagnosis can be based on the presence of anti-islet antibodies. However, there are some clinical features that suggest autoimmunity in phenotypically type 2 diabetic patients and enable to identify adults at a higher risk for autoimmune diabetes. The presence of these features, namely, younger age of onset, acute symptoms, non-obesity, lower C-peptide levels, lower β-cell response in the glucagon stimulation test, and personal or family history of autoimmune diseases, should incline the clinician to perform immunological tests. Diagnosis of autoimmune diabetes should be associated with treatment decisions. Early insulin intervention may help achieve tight metabolic control of the disease, and prevent or delay the progression of β-cell failure. However, despite the progress in treatment options and clear treatment targets defined in the recommendations, the optimal control of diabetes remains a challenge.

Considering the diversity of clinical presentation seen in adult-onset autoimmune diabetes, the purpose of this study was to investigate clinical and laboratory characteristics as well as anti-islet autoantibody pattern in adult patients with newly diagnosed diabetes and confirmed autoimmune background. These patients were analyzed with regard to their age and the number of detected autoantibodies. We investigated whether these factors provide any clinically relevant information at disease onset.

**FIGURE 1** Frequency distribution of patients according to age and sex

**PATIENTS AND METHODS** The study population comprised Caucasian patients with newly diagnosed diabetes admitted to the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poland, over 7 years (between 1 January 2005 and 31 December 2011). The inclusion criteria were: 1) diabetes diagnosed within ≤3 months prior to hospitalization; 2) age of onset ≥18 years, and 3) positivity for at least 1 of 3 anti-islet autoantibodies (ICA, GADA, IA-2A). The presence of anti-islet antibodies was tested when autoimmunity was clinically suspected.

Clinical and laboratory data were collected retrospectively from patients’ records. All laboratory parameters were measured in blood samples obtained on admission, before initiation of insulin therapy, using standard methods.

The presence of autoantibodies to ICA, GADA, and IA-2A was evaluated. Autoantibody analysis was performed as described earlier in the Immunopathology Laboratory at the Department of Pediatrics, Medical University of Lodz, Poland, which is a reference laboratory for islet antibody measurement and is a regular participant of international proficiency testing programs. ICA were detected using indirect immunofluorescence tests on cryostat sections of human pancreas of the donor with blood group O. Antibody titers were determined by serum dilution and expressed as Juvenile Diabetes Foundation (JDF) units based on reference sample of 80 JDF collected as a gift from the laboratory at Saint Vincent de Paul Hospital in Paris, France. GADA and IA-2A were measured by commercial enzyme-linked immunosorbent assay kits (EUROIMMUN GmbH, Germany).

For analysis, patients were divided into 2 groups based on their age at diagnosis (<35 and ≥35 years). In some criteria of LADA, older age is defined as >35 years. It is because young age at onset is a relevant parameter associated with a rapid progression of autoimmune diabetes. Among older patients, especially those presenting with less marked symptoms of hyperglycemia, slower disease progression is more probable.

To test whether the characteristics of patients with autoimmune diabetes correlate with the number of anti-islet autoantibodies, we divided our study population into 3 groups according to the number of antibodies detected at diagnosis (1, 2, and all 3 antibodies present).

A statistical analysis was performed using the GraphPad Prism software. The t test, Mann–Whitney test, Fisher’s exact test, χ² test and one-way analysis of variance followed by the Tukey’s post-hoc test, or Kruskal–Wallis test followed by Dunn’s post-hoc test, were applied as appropriate. Differences were considered significant at a P-value of less than 0.05. The study was approved by the Ethical Committee of the Poznan University of Medical Sciences. The aims of the study were explained to the participants, who gave their written consent.

**RESULTS** The study group comprised 344 patients (224 men; 65.1%); mean age at diagnosis...
was 28.5 ±8.7 years. The population included 278 patients younger than 35 years of age and 66 patients aged 35 years and older. The proportion of male patients was higher in the younger age group and declined with age (FIGURE 1). In the younger age group, men constituted 67.6%, while male predominance was not so distinct in the older age group (54.5%) (TABLE 1). Interestingly, in our study, autoimmune diabetes affected mostly men in the age group of 35 to 45 years (male-to-female ratio, 1.8:1), while in patients above 45 years of age, it was more frequent among women (male-to-female ratio, 1.2). This difference was significant (P = 0.033).

Patients diagnosed with autoimmune diabetes under 35 years of age were similar in the majority of clinical and biochemical features in comparison with the older age group. Only few exceptions were observed (TABLE 1). First, infection foci were more prevalent among patients younger than
by patients (hospitalization time and insulin requirement at discharge) did not reveal any significant differences either.

We also analyzed immunological characteristics of autoimmune diabetes at diagnosis. Irrespective of age, GADA was the most common autoantibody (90.7%), followed by ICA (79.1%), and IA-2A (60.5%). GADA and ICA occurred more frequently among younger patients (92.4% vs. 83.3%, \( P = 0.019 \); 81.7% vs. 68.2%, \( P = 0.032 \), respectively), while the prevalence of IA-2A did not differ significantly between the age groups (61.5% vs. 56.1%, \( P = 0.484 \) (FIGURE 2). Single autoantibody positivity was more often observed in older subjects (27.3% vs. 16.5%, \( P = 0.053 \) (FIGURE 3). In patients with only 1 positive autoantibody, GADA was the most frequent in the younger age group (65.2%), while older patients had the highest prevalence of IA-2A (55.6%). Patients in the younger age group more often tested positive for 3 antibodies (52.2% vs. 34.8%, \( P = 0.014 \) (FIGURE 3). The most common two-antibody combination in both groups was GADA and ICA.

The analysis of patients divided according to the number of autoantibodies showed that the presence of multiple (3) autoantibodies was associated with a lower male-to-female ratio, earlier age of onset, lower C-peptide levels (both fasting and after stimulation), shorter duration of symptoms, and lower levels of total and low-density lipoprotein cholesterol (TABLE 2).

**DISCUSSION** In the majority of publications, patients with LADA were selected by testing for autoantibodies in a group of phenotypic type 2 diabetics.\(^{6,18,19}\) In our study, as in several others,\(^{14,20}\) the presence of anti-islet antibodies was tested when there was a clinical suspicion of autoimmunity (based on non-obesity and relatively low fasting and/or stimulated C-peptide levels). Such strategy is justified because, to date, no evidence supports routine autoantibody testing in patients with phenotypically evident type 2 diabetes.\(^{8,21}\) Regardless of antibody positivity, such patients should benefit from diet restrictions and oral hypoglycemic agents at the beginning of therapy, and lack of response to these regimens may guide the physician to the proper diagnosis.

Our study confirmed that adult-onset autoimmune diabetes is generally more prevalent among men. Of note, the proportion of female patients increased with age. The results indicate that sex distribution in autoimmune diabetes is age-dependent. Studies of childhood-onset type 1 diabetes proved that the incidence is similar in male and female children, while in young adults male predominance is clear.\(^{22,24}\) The male preponderance in young adults with autoimmune diabetes is in contrast with other immune-mediated diseases, which are characterized by female prevalence. The explanation of this phenomenon is unknown, but sex differences in obesity, insulin resistance, and sex hormones are the most common hypotheses.\(^{25}\) Our finding that antibody-positive
In contrast to clinical and laboratory characteristics, the autoantibody pattern at diagnosis was different in the 2 age groups in our study. In line with the available studies, we observed a higher prevalence of single autoantibody positivity in older patients. It suggests that autoimmune processes might be less severe in this group. A slowly progressing destruction of β-cells could explain the manifestation of diabetes at an older age. On the other hand, the higher prevalence of multiple autoantibodies in younger patients may be one of the reasons for disease onset earlier in life.

Similarly to other reports, GADA and ICA were the most common antibodies in both age groups in our study, which makes them both useful in identifying adult patients with autoimmune diabetes. As for IA-2A screening, most studies reported no additional value of their measurement because they are detected in the minority of subjects with adult-onset type 1 diabetes and LADA. In contrast to clinical and laboratory characteristics, the autoantibody pattern at diagnosis was different in the 2 age groups in our study. In line with the available studies, we observed a higher prevalence of single autoantibody positivity in older patients. It suggests that autoimmune processes might be less severe in this group. A slowly progressing destruction of β-cells could explain the manifestation of diabetes at an older age. On the other hand, the higher prevalence of multiple autoantibodies in younger patients may be one of the reasons for disease onset earlier in life.

Interestingly, we observed a correlation between the number of autoantibodies detected at diagnosis and sex distribution. The proportion of women was the highest in the group with multiple autoantibodies. This may suggest that, although adult-onset autoimmune diabetes is more prevalent among men, women are prone to develop a more severe autoimmune process.

Our data show that the patient’s age at onset of autoimmune diabetes does not determine the clinical and biochemical presentation at diagnosis. Both age groups had similar characteristics with regard to clinical presentation, metabolic status at diagnosis, and degree of insulin deficiency. The few parameters that differed significantly are either of undetermined importance (infection foci) or are known to change with age in the general population (eGFR, ESR, and total cholesterol). These results indicate that rapidly progressing autoimmune diabetes is a common phenotype also at an older age.
To our knowledge, this study is the first to show such a high prevalence of IA-2A-positive patients among adult-onset autoimmune diabetics. Moreover, our study revealed that the presence of IA-2A in adults does not show any significant age-dependence, while, according to the available data, IA-2A prevalence decreases with age at diagnosis.\textsuperscript{7,8,10,27} Additionally, IA-2A are considered to occur in combination with other antibodies in adult-onset autoimmune diabetes.\textsuperscript{14,15,22} Interestingly, this study demonstrated a high percentage of isolated IA-2A positivity in the older age group. In these patients, it was the only detectable marker of autoimmunity. These results should be confirmed in a larger population of patients, but they clearly point to the relevance of IA-2A in adult-onset autoimmune diabetes.

To date, GADA still remains the most sensitive (and hence recommended for screening) autoantibody marker of autoimmunity. In the light of this study, IA-2A determination (together with GADA) may help identify an additional group of adult patients with autoimmune diabetes.

In line with numerous reports,\textsuperscript{6,27,28,32,34} our study confirmed that the presence of multiple autoantibodies in comparison with single autoantibody positivity is associated with an earlier age of onset, lower C-peptide levels, and more severe symptoms of hyperglycemia. Based on these results, it can be hypothesized that the number of autoantibodies detected at diagnosis correlates with the pace and intensity of autoimmune β-cell destruction and with the degree of insulin deficiency.

To our knowledge, this study is one of a few to evaluate autoimmune diabetes at the time of diagnosis. Its potential limitations include differences in the size of the age groups. Although our department serves as a reference center for all new cases of autoimmune diabetes in the Polish region of Great Poland, a fraction of older patients, especially presenting with less severe diabetes symptoms, may be misdiagnosed as type 2 diabetes cases and treated ambulatory without any immunological tests performed. This could lead to their underrepresentation in this study. Nevertheless, we are convinced that the difference in age group sizes reflects mostly the disease epidemiology itself.

Several conclusions can be drawn from the present study. First, patient's age at autoimmune diabetes onset does not determine the clinical and biochemical presentation at diagnosis. Second, the number of anti-islet antibodies reflects severity of autoimmune process. Patients with multiple autoantibodies seem to be at higher risk of having more pronounced destruction of β-cells and insulin deficiency. Finally, to our knowledge, this is the first study to show such a high prevalence of IA-2A antibodies in adult-onset autoimmune diabetics. IA-2A positivity was equally frequent in younger and older patients, and for some patients, it was the only anti-islet antibody detected. These results definitely highlight the potential importance of IA-2A in screening for autoimmunity in adult patients with newly diagnosed diabetes.

REFERENCES


Charakterystyka kliniczna oraz profil przeciwciał u dorosłych pacjentów z nowo rozpoznaną cukrzycą o podłożu autoimmunologicznym

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SŁOWA KLUCZOWE
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-cukrzyca o podłożu autoimmunologicznym
-cukrzyca typu LADA
-cukrzyca typu 1
-przeciwciała przeciwyspowe

WPROWADZENIE

Cukrzyca o podłożu autoimmunologicznym u osób dorosłych obejmuje szerokie spektrum obrazów klinicznych.

CELE

Celem badania była ocena parametrów klinicznych, biochemicznych oraz profilu przeciwciał przeciwyspowych u dorosłych pacjentów z nowo rozpozaną cukrzycą o podłożu autoimmunologicznym w zależności od wieku i liczby wykrytych autoprzeciwciał podczas diagnozy.

PACJENTI I METODY

Oceniono retrospektywnie 344 pacjentów (wiek ≥ 18 lat) z nowo rozpozaną cukrzycą oraz wykrywalnymi przeciwciałami przeciwyspowymi. Pacjentów podzielono w zależności od wieku (<35 i ≥35 lat) lub liczby wykrytych przeciwciał (1, 2 lub 3).

WYNIKI

Badane grupy wiekowe nie różniły się pod względem większości parametrów klinicznych i laboratoryjnych (takich jak obraz kliniczny, status metaboliczny czy stopień niedoboru insuliny). Przeciwciała przeciwko antygenom cytoplazmatycznym wysp trzustkowych oraz przeciwko dekarboksylazie kwasu glutaminowego 65 występowały częściej u osób młodszych, podczas gdy częstość przeciwciał przeciwko białku podobnemu do fosfatazy tyrozynowej (IA-2A) była podobna w obu grupach wiekowych. U starszych pacjentów obserwowano częściej obecność jednego przeciwciała. Najczęściej występującym pojedynczym przeciwciałem w tej grupie pacjentów były IA-2A. Obecność wielu typów przeciwciał wiązała się z młodszym wiekiem, mniejszymi stężeniami C-peptydu na czczo i po stymulacji oraz krótszym okresem występowania objawów przed rozpoznaniem.

WNIOSKI

Wiek pacjenta przy rozpoznaniu cukrzycy nie determinuje cech klinicznych i laboratoryjnych w czasie rozpoznania, ale wiąże się z występowaniem różnych profilów przeciwciał. Przeciwciała IA-2A mogą być przydatne w diagnoście cukrzycy o podłożu autoimmunologicznym u osób dorosłych. Ocena profilu przeciwciał przy rozpoznaniu może pomóc w identyfikacji pacjentów ze zwiększonym ryzykiem znacznego niedoboru insuliny.

STRESZCZENIE

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SŁOWA KLUČOWE

cukrzyca o podłożu autoimmunologicznym, cukrzyca typu LADA, cukrzyca typu 1, przeciwciała przeciwyspowe