Clin Chem Lab Med 2006;44(2):133–137 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2006.025

Review

Autoimmune diagnostics in diabetes mellitus[1)]

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Abstract

Type 1 diabetes results from a specific destruction of the insulin-producing β-cells of the pancreas. The disease is characterized by the appearance of specific autoantibodies against islet cell antigens. Autoantibodies to insulin, glutamic acid decarboxylase, tyrosine phosphatase IA-2 and cytoplasmic islet cell antibodies are useful markers for the differential diagnosis of type 1 diabetes when clinical and metabolic criteria alone do not allow definite classification. Autoimmune diagnostics is of particular importance in adults to discriminate between type 1 and type 2 diabetes and to assess the diagnosis of latent autoimmune diabetes in adults.

Keywords: autoantibodies; diabetes mellitus; glutamic acid decarboxylase; insulin autoantibodies; latent autoimmune diabetes in adults (LADA); type 1 diabetes; tyrosine phosphatase IA-2.

Introduction

Diabetes mellitus is defined as the dysregulation of glucose metabolism characterized by chronic hyperglycemia resulting from defects in insulin secretion, decreased insulin sensitivity or a combination of both. The clinical symptoms are polydipsia, polyuria, unexplained loss of body weight, weakness and susceptibility to certain infections.

According to etiopathogenic criteria, diabetes mellitus is subdivided into four major groups. The diagnosis should be made according to guidelines such as those of the German Diabetes Society using fasting blood glucose levels or 2-h postload glucose levels (glucose load containing 75 g glucose dissolved in water) during an oral glucose tolerance test (oGTT). The guidelines for the classification and the diagnosis of diabetes mellitus are summarized in Tables 1 and 2 (1).

Etiology and clinical features of type 1 diabetes

Type 1 diabetes is characterized by destruction of the insulin-producing β-cells of the islets of Langerhans in the pancreas (2–4). Although autoreactive T-lymphocytes are of major importance for the pathogenesis of type 1 diabetes and β-cell destruction, islet cell-specific autoantibodies play a major role in the diagnostics of the disease. Risk for type 1 diabetes is influenced by a genetic predisposition (HLA haplotypes), environmental factors and dysregulation of the immune system. The β-cell specific autoimmune process is silent over months to several years, until the number of β-cells is no longer sufficient to maintain normal glucose homeostasis. At manifestation, the clinical symptoms are correlated to absolute insulinopenia. The classic clinical presentation includes acute onset of symptoms in lean children, adolescents or young adults, hyperglycemia, ketoacidosis, loss of weight and different degrees of metabolic abnormalities, depending on the severity and duration of illness. During recent years it became apparent that the above-mentioned criteria alone are frequently not sufficient to discriminate type 1 diabetes from other forms of diabetes.

Large prospective studies in first-degree relatives of patients with type 1 diabetes and the general population, including autoantibody determinations and metabolic tests, revealed a large range of clinical pictures. Although type 1 diabetes peaks between the ages of 12 and 20 years, the disease can occur at any age. Acute onset of symptoms is typical for type 1 diabetes in children. However, the older the patient, the more moderate are the symptoms at the onset of the disease. In addition, diabetic ketoacidosis is not a condition sine qua non. Within screening programs, type 1 diabetes can be identified in an early phase before the appearance of severe metabolic abnormalities. Type 1 diabetic patients can also be overweight or obese with maintained residual β-cell function, leading to a misclassification as type 2 diabetes.

Autoimmune diagnostics

Clinically relevant autoantibodies in diabetes mellitus

The detection of autoantibodies to islet cell antigens provides evidence of an ongoing autoimmune pro-

[1)]The German version of this article has been published in LaboratoriumsMedizin 2005;29(4):246–50. The English version of the article is published in Clin Chem Lab Med with kind permission from the authors.

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Table 1 Classification of diabetes mellitus.

I. Type 1 diabetes
A) Immune-mediated
B) Idiopathic (rare in Europe, autoantibody negative)
II. Type 2 diabetes
III. Other specific types of diabetes
A) Genetic defects of β-cell function (e.g., MODY)
B) Genetic defects in insulin action
C) Diseases of the exocrine pancreas
D) Endocrinopathies
E) Drug- or chemical-induced forms
F) Infections
G) Uncommon forms of immune-mediated diabetes (e.g., stiff man syndrome, anti-insulin receptor antibodies)
H) Other genetic syndromes, sometimes associated with diabetes
IV. Gestational diabetes mellitus

cess. However, a negative result does not allow the complete exclusion of type 1 diabetes. Among the increasing number of autoantibodies described in type 1 diabetes, four autoantibody specificities have been shown to be relevant for the diagnosis (2–8):

- Autoantibodies against glutamic acid decarboxylase 65 (GADA);
- Autoantibodies against tyrosine phosphatase IA 2 (IA2-Ab);
- Insulin autoantibodies (IAA); and
- Cytoplasmic islet cell antibodies (ICA).

Autoantibodies against glutamic acid decarboxylase

In 70–80% of children and adults with type 1 diabetes, autoantibodies directed against an isoenzyme of glutamic acid decarboxylase with a molecular weight of 65 kDa (GAD65) have been described (5, 9–11). GAD65 antibodies (GADA) can be detected by commercial RIAs and ELISAs, with the highest sensitivity for RIAs using human recombinant GAD65. Besides type 1 diabetes, high levels of GADA have been reported for 70% of patients with stiff man syndrome, a rare neurological disease (12).

Autoantibodies against tyrosine phophatase IA-2

Autoantibodies to tyrosine phosphatase IA-2 (IA2-Ab) are present in 50–70% of children and adolescents and in 30–50% of adults at the manifestation of type 1 diabetes (6, 8, 10, 11). The most sensitive detection can be achieved by RIAs using human recombinant antigen. So far, the ELISAs available have lower sensitivity (Table 3).

Insulin autoantibodies

Autoantibodies against insulin (IAAs) are detectable in 50–70% of children at the onset of type 1 diabetes. In older patients, IAAs are present in only 20–30% (7, 8, 10, 13). The detection of IAAs should be carried out using RIA, because ELISAs have been shown to possess significantly reduced sensitivity and specificity (14). The sensitivity of the commercially available RIAs is slightly lower compared to competitive in-house RIAs used in clinical studies. After the start of insulin therapy, antibodies can be induced against exogenous insulin that cannot be discriminated from IAAs. Therefore, in patients treated with insulin, IAAs can no longer be detected.

Cytoplasmic islet cell antibodies

Cytoplasmic islet cell antibodies (ICAs) are detected by indirect immunofluorescence tests on cryostat sections of human pancreas (Figure 1) (15). ICAs were found to be positive in 80–90% of newly-diagnosed patients with type 1 diabetes (2, 8, 10, 11). The target antigens include GAD and IA-2. In some cases, ICAs may be directed to antigens that are as yet not identified. The advantage of the ICA test is that it can simultaneously detect autoantibodies to several antigens. The major disadvantages are that the test requires high-quality pancreas samples of human blood group O, provides only semi-quantitative results and is rather laborious. In addition, evaluation of ICA results requires an investigator of extensive experience (16). In routine diagnostics, the ICA test has been replaced by the detection of GADA, IA2-Ab and IAA.

Importance of cellular autoimmune diagnostics

The destruction of insulin-producing β-cells in type 1 diabetes is mediated by T-lymphocytes and antigen-presenting cells (macrophages, dendritic cells). There are practical and technical problems with the currently available T-cell assay for measurement of the cellular immune response (low frequency of autoreactive T-cells in peripheral blood, poor assay standard-

Table 2 Diagnostic criteria of diabetes mellitus.

	Plasma glucose		Whole blood glucose	
	Venous	Capillary	Venous	Capillary
Diabetes				
Fasting	≥7.0 mmol/L (126 mg/dL)	≥7.0 mmol/L (126 mg/dL)	≥6.1 mmol/L (110 mg/dL)	≥6.1 mmol/L (110 mg/dL
oGTT 2-h value	≥11.1 mmol/L (200 mg/dL)	≥12.2 mmol/L (220 mg/dL)	≥10.0 mmol/L (180 mg/dL)	≥11.1 mmol/L (200 mg/dL)
IGT				
oGTT 2-h value	≥7.8 mmol/L (140 mg/dL)	≥8.9 mmol/L (160 mg/dL)	≥6.7 mmol/L (120 mg/dL)	≥7.8 mmol/L (140 mg/dL)

oGTT, oral glucose tolerance test; IGT, impaired glucose tolerance.

Table 3 Prevalence of diabetes-specific autoantibodies at the manifestation of type 1 diabetes.

Autoantibody	Prevalence, %	
	Children	Adults
GADA	70–80	70–80
IA2-Ab	60–70	30–50
IAA	50–70	20–30
ICA	80–90	70–80
GADA or IA2-Ab or IAA	95–100	70–80

GADA, antibodies to GAD65; IA2-Ab, antibodies to tyrosine phosphatase IA-2; IAA, insulin autoantibodies; ICA, cytoplasmic islet cell antibodies.

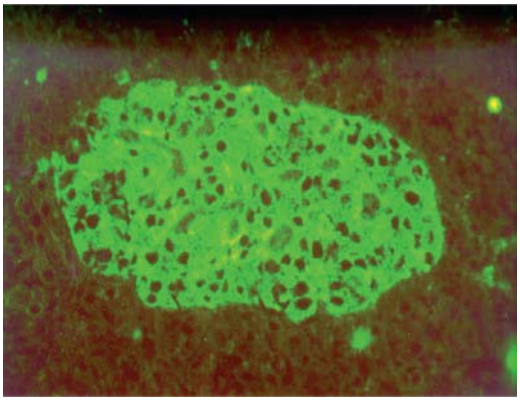

Figure 1 Characteristic ICA staining in the indirect immunofluorescence test on cryostat sections of human pancreas tissue. Intracytoplasmic bind of IgG autoantibodies on all cells of the islets of Langerhans.

ization) (17, 18). At present, cellular assays are not important in the diagnosis of type 1 diabetes.

Strategies for differential diagnosis

Autoimmune diagnostics in type 1 diabetes

When typical symptoms of type 1 diabetes (lean patient, proneness to ketosis) are present, autoimmune diagnostics may be indispensable. For problems in differential diagnosis [e.g., maturity onset diabetes of the young, other congenital or secondary diabetes types (Table 1), early diagnosis without insulin dependency], immune diagnostics can be very helpful. Combined screening for IAA and GADA (age <10 years) or IA2-Ab and GADA (age >10 years) is the recommended first-line analysis (Figure 2) (7, 8, 10, 11, 19). The detection of one autoantibody provides evidence of an ongoing autoimmune process. A negative result in a child makes the diagnosis of type 1 diabetes unlikely. Because diabetes-specific autoantibodies are not present in all cases with type 1 diabetes, negative autoantibodies do not completely exclude the presence of type 1 diabetes (idiopathic type 1 diabetes). It is important to note that in adults approximately 20–30% of patients presenting absolute insulin dependency at diabetes onset are antibody-negative. In these cases, determination of residual β-cell function (fasting or glucagon-stimulated C-peptide levels) and/or genotyping may help to confirm the diagnosis.

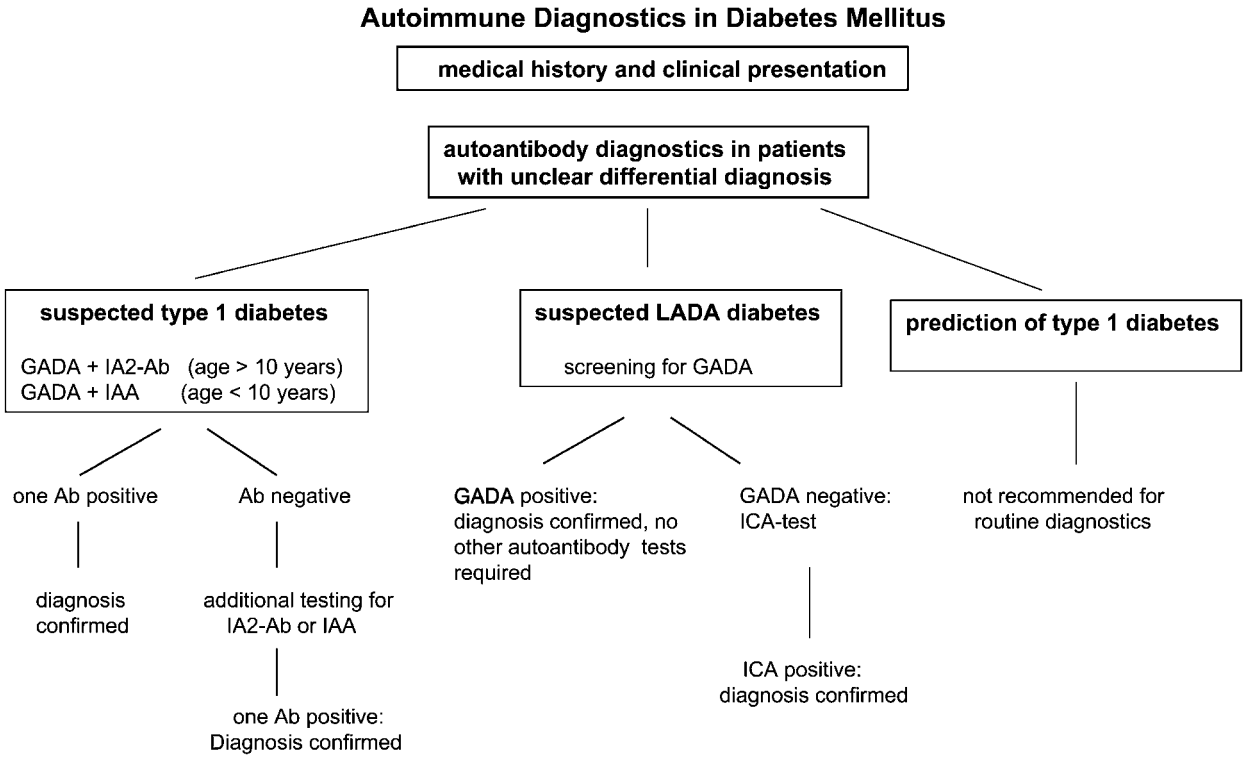

Figure 2 Flow chart of the autoimmune diagnostics in diabetes mellitus. Ab, antibodies; ICA, cytoplasmic islet cell antibodies; GADA, antibodies to GAD65; IA2-Ab, antibodies to tyrosine phosphatase IA-2; IAA, insulin autoantibodies.

Diagnosis of LADA diabetes

A special form of type 1 diabetes is the so-called latent autoimmune diabetes in adults (LADA) (20–22). These patients typically develop diabetes after the age of 30 years and have a slowly progressing autoimmune process. Therefore, endogenous insulin secretory capacity is only slightly decreased at the manifestation of the disease. Due to clinical features, these patients are often misclassified as suffering from type 2 diabetes and are treated with oral antihyperglycemic drugs. After several months or a few years, the patients develop absolute insulin deficiency and require insulin treatment. In young and middle-aged adults, the frequency of this form of diabetes is relatively high (approx. 20% of patients with type 2 diabetes in the age range 25–44 years).

The detection of LADA should be made by primary screening for GADA (11, 22–24). In the case of a negative result, additional determination of ICA is recommended, because some LADA patients are only positive for ICA directed to an as-yet unknown antigen (11, 24). The detection of GADA and/or ICA indicates autoimmune pathogenesis of diabetes. The measurement of IA2-Ab and IAA in LADA patients is of no relevance.

Prediction of type 1 diabetes

It is well known from various prospective studies that the risk for the development of type 1 diabetes in children and adolescents can be predicted with high sensitivity and specificity by a combined screening for diabetes-specific autoantibodies (25–28). However, due to the lack of effective therapeutic intervention strategies, general autoantibody screening is not recommended. Screening programs should only be performed in the context of controlled studies aiming at the further improvement of risk estimation or the evaluation of novel prevention trials.

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