Diabetes

Antibodies in diabetes mellitus

Analyte Information

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Introduction

DM type 1, previously known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic ß-cells. Autoimmune destruction of ß-cells is thought to be completely asymptomatic until 80 - 90% of the cells are lost. This process may take years to complete and may occur at any time. During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to ß-cell antigens. These autoantibodies are present years before the onset of DM type 1. Early studies utilized the immunofluorescence test for islet-cell antibodies (ICA), which has been difficult to standardize. It has been replaced by a combination of several immunoassays for antibodies against specific ß-cell antigens, such as insulin (IAA), glutamic acid decarboxylase (GAD) and tyrosine phosphatase ICA 512 (IA2).

Antibodies are typically divided into three categories:

a) Antibodies against cellular structures (e.g. cell membranes, enzymes, etc.: antibodies against glutamic acid decarboxylase – GAD, anti-IA2)

b) Antibodies against hormones produced by the gland (anti-insulin antibodies)

c) Antibodies against receptors functionally exhibiting either inhibitory or stimulatory effects on glandular secretion

Till now, more than 30 auto-antigens and corresponding autoantibodies were described in patients with DM type 1 and their relatives. Most of them have not been exactly characterized yet and their role in the disease pathogenesis and particularly in the prediction of DM type 1 development is being verified.

Anti-insulin autoantibodies (Anti-insulin; IAA)

Antibodies against insulin represent immune response to a single antigen specific for ß-cells. In pre-diabetic stage or in the first grade relatives who were never treated with exogenous insulin, these antibodies react with endogenous insulin. Their occurrence is significantly associated with HLA-DR4, which is found in 15 – 80% at the time of type 1 DM diagnosis and in 2 - 5% in the first grade relatives.

Clinical application

IAA appears to be inversely correlated with age for both new-onset DM type 1 and their high-risk non-diabetic relatives.

Children in onset DM type 1 are more commonly IAA positive than adults. Anti-insulin autoantibodies are present in more than 80% of children who develop DM type 1 before age 5, but in less than 40% of individuals developing diabetes after age 12, and only in 30% of adults. Their frequency in healthy people is very low - 0.5%.

The predictive value of IAA is considerable as well. The risk in positive relatives is quite high, it is reported that in about 40 - 50% diabetes develops within 5 years.

Very important is also fact that insulin antibodies develop after insulin therapy, even in those persons who use human insulin. Insulin antibodies (IA – which were developed as a response to therapy with exogenous insulin) can be find 5 - 7 days after insulin introduction; the concentrations induced by exogenous insulin are often ≥ 10-fold than insulin autoantibodies (IAA).

Elevated anti-insulin antibodies levels

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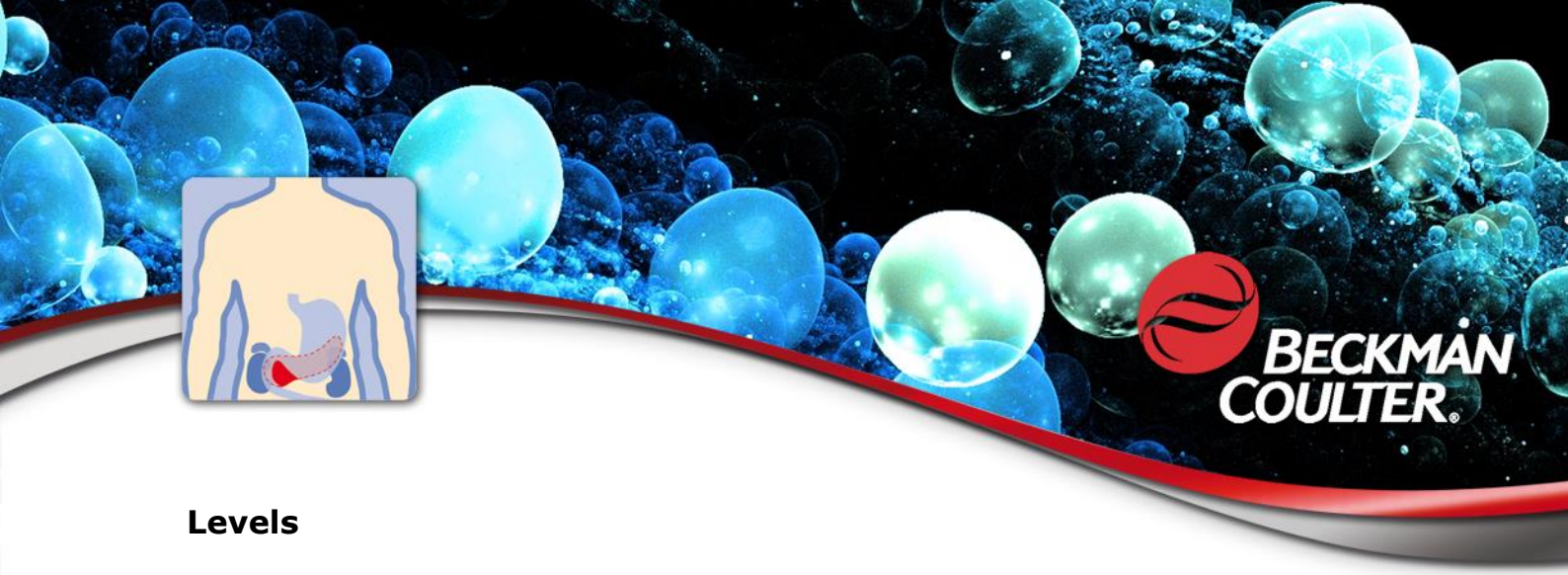
- immune insulin resistance, pre-type 1 DM
- polyendocrine autoimmune disease
- treatment with exogenous insulin



Levels

The determined levels of anti-insulin antibodies differ significantly in dependence on particular assay. For each assay, relevant reference values are given in the appropriate Instructions for Use (IFU). Anti-insulin levels presented in Tab.1 were obtained using Beckman Coulter RIA kit (cat. No. A36473 and A36474).

Tab.1: Anti-insulin antibodies levels

Specimen (serum)	**Reference interval** (U/mL)
Healthy individuals	**0 – 0.4**
Positive	**> 0.4**

Anti-GAD autoantibodies (anti-GAD)

Glutamic acid decarboxylase (GAD) is a neuronal enzyme involved in the synthesis of the neurotransmitter gamma-aminobutyric acid (GABA).

GAD occurs in two isoforms, 65 and 67 kDa. The 65 kDa form predominates in the ß-cells of the pancreas and the type 1 diabetes autoantibodies are directed against it.

Antibodies directed against the 65-kDa isoform of GAD (GAD_{65}) are also seen in a variety of autoimmune neurologic disorders including Stiff-man (Moersch-Woltman) syndrome, autoimmune cerebellitis, brain stem encephalitis, seizure disorders, neuromyelitis optica and other myelopathies, myasthenia gravis, Lambert-Eaton syndrome, and dysautonomia.

Clinical application

GAD antibodies are the major pancreatic islet antibody and an important marker of predisposition to DM type 1. Autoantibodies against GAD are present in ~60% of recently diagnosed patients, and in 5 - 13% of the first grade relatives. Positivity of anti-GAD can be found up to 10 years before onset of clinical DM type 1. GAD antibodies may be used to identify patients with apparent DM type 2 who will be subsequently recognized as patient with DM type 1.

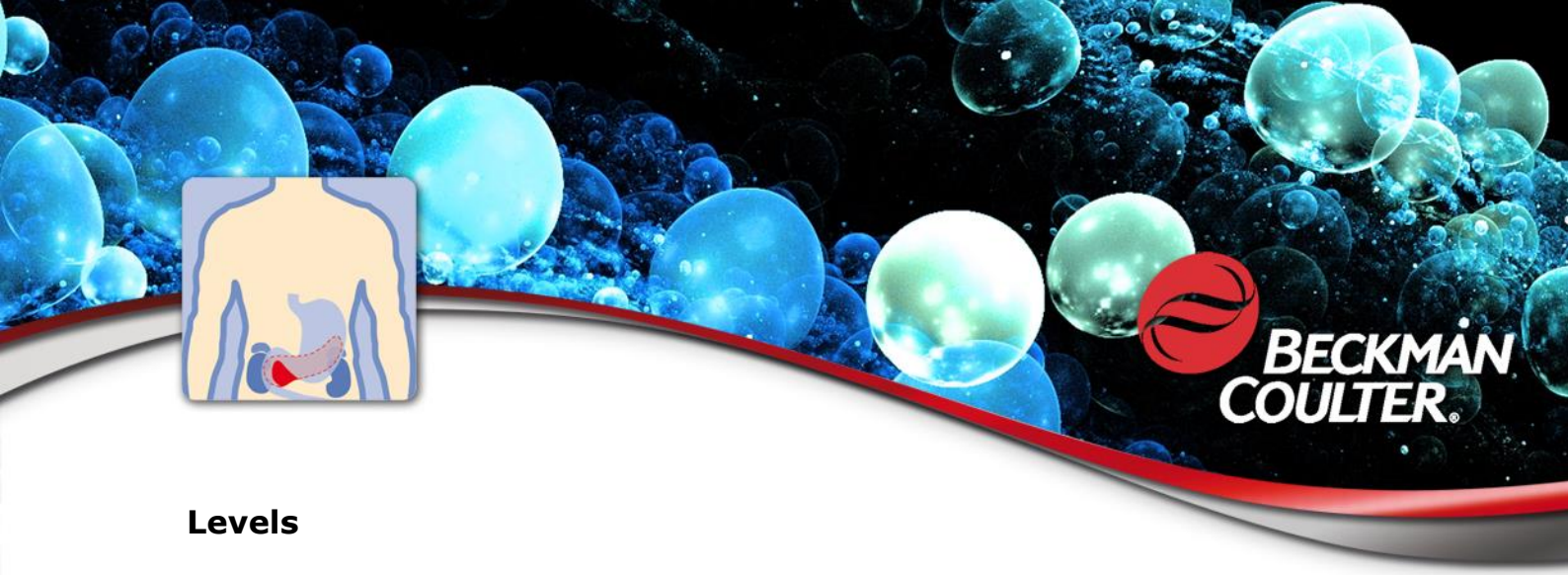
In comparison with other autoantibodies, GAD antibodies are more sensitive but also less specific marker (positive frequently in Stiff-man syndrome, in other

endocrine glands immunopathy, or generally in autoimmune diseases). In order to increase the specificity, the determinations of other antibodies should be carried out (anti-insulin, anti-IA2).

GAD autoantibodies also serve as a marker of predisposition to other autoimmune disease that occurs with DM type 1, including thyroid disease (e.g., thyrotoxicosis, Graves' disease, Hashimoto's thyroiditis, hypothyroidism), pernicious anemia, premature ovarian failure, Addison's disease, (idiopathic adrenocortical failure) and vitiligo.

Elevated anti-GAD levels

- diabetes melitus typ 1
- LADA (latent autoimune diabetes of adulthood)
- Stiff-man (Moersch-Woltman) syndrome
- thyroid diseases
- autoimmune cerebellitis
- brain stem encephalitis
- seizure disorders
- Lambert-Eaton syndrome
- dysautonomia
- pernicious anemia
- premature ovarian failure
- Addison's disease
- vitiligo



Levels

The determined levels of anti-GAD antibodies differ significantly in dependence on particular assay. For each assay, relevant reference values are given in the appropriate Instructions for Use (IFU). Anti-GAD levels presented in Tab.2 were obtained using Beckman Coulter RIA kit (cat. No. IM3650 and IM3651).

Tab.2: Anti-GAD levels

Specimen (serum)	**Reference interval** (U/mL)
Healthy individuals	**0 – 1.0**
Positive	**> 1.0**

Anti-Islet antigen-2 autoantibodies (Anti-IA2)

The IA2 protein (insulinoma associated antigens IA-2A and IA-2ßA), originally called ICA 512, is a 979 amino acid polypeptide belonging to the tyrosine phosphatase family, it is localized in the dense granules of pancreatic ß-cells. The anti-IA2 autoantibodies are directed against the 37 - 40 kDa fragments obtained after trypsinization of the islets.

Clinical application

Antibodies against IA2 (IA-2A) are present in up to 80% of children and adolescents at diagnosis of DM type 1, and in 2 - 5% of the first grade relatives. Anti-IA2 generally develops later in the process leading to DM type 1 and is therefore associated with more rapid progression into manifested diabetes – the risk in the positive relatives is 82% within 5 years. In comparison, especially with anti-GAD, they are markedly more specific and are found less frequently in other autoimmune diseases.

These antibodies do not persist as long following diagnosis as anti-GAD, and are less common in patients who are diagnosed with type 1 diabetes over the age of 30 years. They are therefore less useful than anti-GAD for characterising diabetes in longer term or older patients.

Elevated anti-IA2 levels

- diabetes melitus typ 1
- LADA (latent autoimune diabetes of adulthood)

Levels

The determined levels of anti-IA2 antibodies differ significantly in dependence on particular assay. For each assay, relevant reference values are given in the appropriate Instructions for Use (IFU). Anti-IA2 levels presented in Tab.3 were obtained using Beckman Coulter RIA kit (cat. No. IM3652 and IM3653).

Tab.3: Anti-IA2levels

Specimen (serum)	**Reference interval** (U/mL)
Healthy individuals	**0 – 1.0**
Positive	**> 1.0**

Diagnostic utility – practical applications

The simultaneous measurement of different types of antibodies is very important part of clinical practice and improves diagnosis of DM type 1 and LADA significantly.

Specific classification in DM type 1 and LADA

Positive finding of one or more antibodies at the time of DM manifestation makes the DM type 1 diagnosis very presumable or certain. The determination is of great importance for autoimmune component identification namely in individuals who were not insulin-dependent at the time of diagnosis. Approximately 10 - 12% of white adult patients who have been diagnosed as DM type 2 patients also have islet cell autoantibodies, particularly to GAD. This condition is termed latent autoimmune diabetes of adulthood (LADA – which is an specific form of DM type 1 in adults, often with a slower course of onset)[3].

DM type 1 prediction in the first grade relatives

Experience gained with individual autoantibodies shows that determination of anti-GAD exhibits the highest sensitivity (up to 90 %), followed by anti-insulin (up to 80%), and anti-IA2 (85%). The sensitivity increases when two autoantibodies are determined at the same time: the combination anti-insulin + anti-GAD yields 98% sensitivity, IA-2 + anti-GAD 92%, when 3 different autoantibodies are determined the achieved sensitivity is nearly 100%.

Autoantibodies – prevalence in DM type 1 - summarization:

Autoantibody type	**Prevalence in DM type 1**
Anti-GAD	70 - 90%
Anti-insulin	30 - 50% (in children up to 80%)
Anti-IA2	50 - 85%
Combined sensitivity of presence autoantibodies	
Anti-insulin + Anti-GAD	~ 98%
Anti-IA2+ Anti-GAD	~ 92%
Anti-insulin + Anti-IA2+ Anti-GAD	almost 100%

In diabetic patient families, first determination before 6 years of children's age

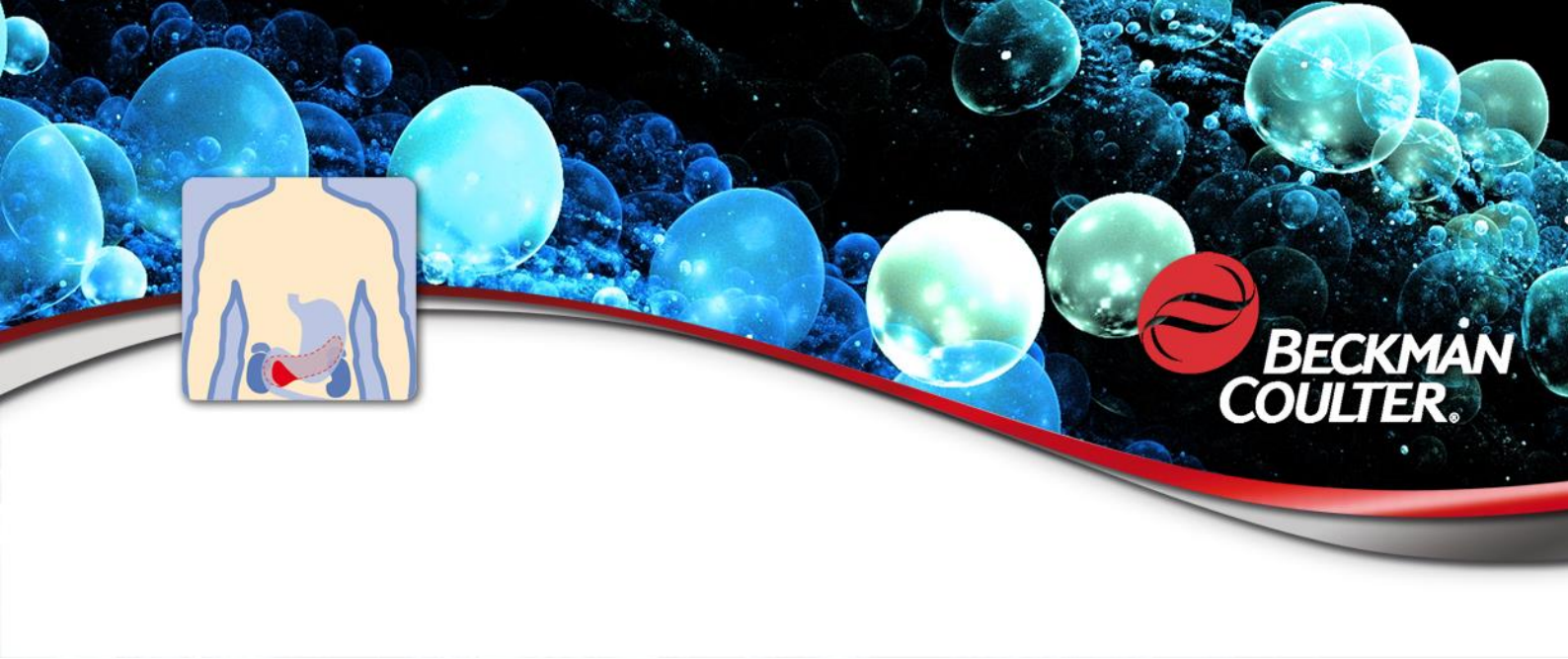
The risk of diabetes development within 5 years in DM type 1 patient family members, who were found to be positive in one autoantibody, is as follows: for anti-GAD and anti-insulin between 50 - 60%, for IA2 almost 80%. In parallel positive results in two or more autoantibodies, the risk of diabetes development within 5 years increases to 65 - 100%, and the highest risk is probably associated with the combination anti-insulin and anti-IA2 (almost 100% in all studies). However, data presented in different studies are often difficult to compare, they evaluate the cumulative risk in a different time horizon and different combinations of autoantibodies.

It is recommended to start screening in diabetic patient families before 6 years of child's age. The main reasons for it are as follows: antibodies appear early, they have a considerable predictive value - opportunities for intervention in initial insulitis phases, DM type 1 incidence manifestation peak is achieved around 6 - 7 years of age and in the period of puberty. In screening, it is recommended to start with anti-GAD + anti-IA2 determination, other antibody determination is advisable in positive cases. In case of a positive single autoantibody, repeated examinations are recommended.

Risk of diabetes development within 5 years in relatives of DM patients with positive antibodies- summarization:

Autoantibody type	Risk of diabetes development within 5 years in relatives of DM patients
Anti-GAD	~ 50%
Anti-insulin	~ 50%
Anti-IA2	~ 80%

Positive results in two or more antibodies: 65 - 100% (combination anti-insulin and anti-IA2 almost 100%)



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3. Burtis C.A., Ashwood E.R., Bruns D.A.: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th edition, Elsevier Saunders, Philadelphia, 2006, 856.