LATEST NEWS ON AUTOANTIBODIES ASSOCIATED WITH TYPE 1 DIABETES IN CHILDREN: -SYSTEMATIC REVIEW-

Belhiba Ouijdane[1], Ahmed Aziz Bousfiha[1, 2], Farida Jennane [3]

[1.] Clinical Immunology Lab, Inflammation & Allergy LICIA, Faculty of Medicine and Pharmacy, King Hassan II[d] University, Casablanca, Morocco.   [2.] Clinical Immunology Unit, Pediatric Infectious Disease, Abderrahim Harouchi Children's Hospital, Casablanca, Morocco.   [3.] Pediatric Endocrinology Unit, Abderrahim Harouchi Children's Hospital, Ibn Rochd University Hospital, Casablanca, Morocco.

ABSTRACT :

Type 1 diabetes in children is a chronic autoimmune disease related to the destruction of islet cells β by an autoimmune process. The clinical symptoms are preceded by a pre-diabetic phase during which many autoantibodies can be detected in patients, such as anti-glutamic acid decarboxylase (GADA), anti-insulin (IAA), anti-tyrosine phosphatase (IA2-AAb), Langerhans anti-islet (ICA) and zinc transporter 8 (ZnT8-AAb).
Recently, they have reported tree new autoantibodies: elongation factor of eukaryotic translation 1 alpha 1 autoantibody (EEF1A1-AAb), autoantibodies 2L3 (UBE2L3-AAb), the enzyme involved in protein degradation, for ubiquitin conjugation, and autoantibodies GLIMA 38.
Our objective is to develop the latest news on autoantibodies specific for autoantigens expressed in pancreatic β cells, their targets, as also their prevalence and predictive values.

Keywords: Autoimmunity; Autoantibodies; Children; Diabetes type 1.

Corresponding Author:
Belhiba Ouijdane, **Ph.D**.
Address: Faculty of Medicine and Pharmacy, Hassan II[d] University, Casablanca, Morocco.
Tel.: (+212) 0681070988;
E-mail: ouijdane.belhiba@gmail.com

INTRODUCTION

Today, diabetes is a major public health problem; the World Health Organization (WHO) predicts that by 2030, diabetes will be the seventh most common cause of death in the world. [1]

The International Diabetes Federation (IDF) in their last report in 2017 reported that there are more than 425 million people with diabetes in the world, as well as a 48% increase by 2045, which will result in 632 million cases of diabetes in the world. [2]

Type 1 diabetes (T1D) is an organ-specific autoimmune disease characterized by the selective destruction of insulin-producing cells in pancreatic islets in genetically predisposed subjects until 80-90% of these cells are lost. [3]

The destruction of B cells is mainly caused by islet infiltration by cytotoxic CD8 T lymphocytes and thus by autoantibodies against pancreatic auto- antigens. This autoimmune process begins several years (5 to 10 years or more) before the appearance of the first clinical symptoms of diabetes.

The asymptomatic period of the disease is clinically quiet but biologically active, in which various autoantibodies are generated against several beta cell antigens. When the clinical presentation is typical of T1D with the absence of immunological markers, diabetes is classified as type 1B (idiopathic). [4]

The last forty years have been rich in discoveries, namely that of humoral immune- reactivity directed against the islets of Langerhans in 1974 by Gian Franco Bottazzo. [5]

The numbers of autoantibodies involved in the autoimmune process of T1D are increasing, allowing the pathophysiology of the disease to be clarified and the natural history of T1D to be understandable, such

as anti-glutamic acid decarboxylase (GADA), anti-insulin (IAA), anti-tyrosine phosphatase (IA2-AAb), langerhans anti-islet (ICA) and zinc transporter 8 (ZnT8-AAb).

At least 1 of these autoantibodies is present in 95% of individuals with T1D after detection of hyperglycemia. These autoantibodies can be used effectively for the prediction of T1D, and they can be used as early markers of T1D, due to their high value concerning persistence in patients' sera before the development of T1D, at the time of diagnosis and even after diagnosis. [6,7]

The purpose of this paper is to develop the latest news on autoantibodies specific for autoantigens expressed in pancreatic β cells, their targets, as also their prevalence and predictive values.

1. Epidemiology of type 1 diabetes worldwide:

More and more people have diabetes every day, and according to WHO studies, the number of cases of diabetes worldwide has quadrupled since 1990. [1]

The incidence of T1D is increasing worldwide, but there are significant differences between countries, with some regions recording much higher rates than others.

The reasons for this evolution are unclear, but there is a suspected interaction between genetic and environmental factors.

The incidence of T1D is increasing by about 3% per year worldwide, mainly among the youngest children, especially those under 15 years of age. [2]

From the International Society for Pediatric and Adolescent Diabetes (ISPAD), 2018 and the International Diabetes Federation (IDF) 2017, more than 96,000 children and adolescents under 15 years of age develop T1D, a figure that rises to more than 132,600 if the age is extended to 20 years. [2,8]

A total of 1,106,200 children and adolescents under the age of 20 are estimated to have T1D worldwide.

Europe and North America and the Caribbean have the highest number of children and adolescents with T1D under the age of 20.

Over a quarter (28.4%) of children and adolescents with T1D are living in Europe and more than one-fifth (21.5%) in North America and the Caribbean.

The United States of America, India, and Brazil have the highest incidence and prevalence of T1D among children and adolescents in both age groups, under 15 and under 20 years of age.

In the Middle East and North Africa, the prevalence of diabetes is 10.8%, the second highest percentage in the world, the number of people with diabetes is expected to increase by 110% by 2045.

In Africa, more than 69.2% of cases are undiagnosed with diabetes, and an estimated 50,600 children and adolescents under 20 years of age have T1D in the African region. [2] [9]

2. Clinical and biological diagnosis of childhood diabetes:

2.1. T1D Clinic:

Four clinical signs characterize the sudden and noisy onset of type T1D 1 mellitus: polyuria, polydipsia, weight loss, and polyphagia. When these signs appear, insulin secretion is already very strongly altered.

Clinical signs of the disease usually appear when 90% of the mass of β-langerhansian cells has disappeared, this disappearance being insidious, with no apparent clinical sign.

Any recovery becomes impossible, and insulin treatment is essential. From time to time, there may be a short remission ("honeymoon" in Anglo-Saxons), but recurrence usually occurs within a short period, leading to definitive insulin treatment. [3]

In the absence of medical intervention, this clinical situation can be complicated by ketoacidosis, which is an extreme manifestation of insulin deficiency, following the accumulation of ketone bodies in the blood. [10]

T1D is generally associated with other comorbidities such as autoimmune thyroiditis, celiac disease, and Addison's disease.

The severity of the disease lies in the appearance of severe or even fatal complications, such as kidney disease, retinopathy, neuropathy, dyslipidemia, and hypertension. [11]

2.2. Biological diagnosis of T1D:

The nosological classification of diabetes was published in 1997 by a group of experts under the responsibility of the American Diabetes Association (ADA), replacing the taxonomy developed in 1979 by the "National Diabetes Data Group" and approved by the WHO. [12]

According to the latest classification by ISPAD in 2018, T1D is defined as a complex metabolic disorder characterized by chronic hyperglycemia resulting from deficiencies in insulin secretion, insulin action, or both. Inadequate insulin secretion and reduced tissue responses to insulin in the complex pathways of hormonal activity occur in insufficient insulin action on target tissues. [8]

The diagnostic criteria for diabetes are based on blood glucose measurements, and the presence or absence of symptoms, the methods used to diagnose diabetes are fasting blood glucose, or random blood glucose, or glycated hemoglobin (HbA1c), or oral glucose tolerance test (HPGO) (**Table I**).

Table I: Criteria for the diagnosis of type 1 diabetes [8,25,26]

classic symptoms of diabetes or hyperglycemia, with plasma glucose concentrations ≥.11.1 mmol/L (200mg/dL)
Or
Fasting blood glucose ≥ 7.0 mmol/L (≥ 126 mg/dL)
Or
Hour post-load glucos after ingestion of 75 g oral glucose (oral glucose tolerance test (OGTT)) ≥ 11.1 mmol/L (200 mg/dL)
Or
HbA1c ≥ 6.5% (**≥48 mmol/mol**)

The detection of different autoantibodies in the serum of patients mainly: ICA, GADA, IA2, IAA, ZnT8 aims to affirm the autoimmune nature of diabetes before insulin therapy is started. [8]

3. The humoral biomarkers of T1D:

Like all autoimmune diseases, T1D is a multifactorial disease; however, the exact nature of causal factors such as genetic susceptibility, environmental factors, immune system and β-cells in the pathogenic processes underlying T1D continues to be the subject of debate. [13]

During the pre-diabetic phase of T1D, many autoantibodies are directed against several pancreatic auto-antigens (**Table II**).

TABLE II: AUTOANTIBODIES ASSOCIATED WITH T1D AND THEIR AUTO- ANTIGENS.

Auto-antibodies	Auto- antigens
IAA [6]	Insulin
GADA [6]	GAD 65
IA2-AAb [6]	Peptide fragments of 37/40 kDa
ICA [6]	Cytoplasm of Langerhans islets
ZnT8-AAb [20]	Transporter 8 of zinc
EEF1A1-AAb [21]	EEF1A1 is a factor involved in translating messenger RNA into protein
UBE2L3-AAb [21]	UBE2L3 is involved in protein degradation.
GLIMA 38-AAb [23]	38 kDa Glycoprotein of the tetraspanin family

These are serological markers of β-cell autoimmunity, including GADA, IAA, IA2-AAb, ICA, and ZnT8-AAb.

These autoantibodies are detected at the moment of diagnosis, and they can be present long before the symptomatic onset of T1D, as well as they, can persist long in patients' sera after diagnosis [14], they offer the opportunity to detect individuals at risk and to follow the destructive process. [15]

The expression of these antibodies is age-related, IAA and ZnT8-AAb are more frequently expressed in children under 10 years of age, while GADA and IA-2-AAb are associated with the older generation. [16]

3.1 Anti- Islet cell autoantibody (ICA):

These autoantibodies are the first ones to be detected by indirect immunofluorescence on sections of the human pancreas. They are present in 75 to 90% of patients at diagnosis (**Table III**).

Table III: Predictive value of autoantibodies associated with T1D [6,21,23]

Autoantibodies	Prevalence (%)	Detection before diagnosis	Persistence after diagnosis
ICA	75-90	-	from a few weeks to a few months
GADA	70-80	8 years	8 years (90%), 25 years (20%)
IA2-AAb	30-80	5 years	12 years (50%), 25 years (20%)
IAA	50-70	-	-
ZnT8-AAb	25 -50	-	5 years (40%), 25 years (7%)
EEF1A1-AAb	29.5	-	-
UBE2L3-AAb	35.8	-	-
GLIMA38	30	-	-

ICAs are present long before the onset of diabetes, but they tend to decrease or even become undetectable a few weeks to a few months after the beginning of the disease[17] . It has been shown that ICA disappears a few years after diabetes diagnosis. They suggest that the ICA may be more closely related to beta cell damage. Their dosage could be replaced by the combined dosage of GADA, IA2-AAb/IAA. [6, 7]

3.3 Anti-Insulin autoantibody (IAA):

Insulin antibodies are targeted against insulin and its precursor, proinsulin. During diabetes, the prevalence of antibodies against these molecules is slightly lower than for ant GADA, about 50%.

The presence of IAA is not specific to T1D; it is also present in certain autoimmune diseases, polyendocrinopathies, and viral infections and in about 1% of the general population. IAAs predisposing to T1D do not have the same specificity as those associated with other pathologies or encountered in controls.

If these antibodies are detected before any insulin therapy, then they are associated with early juvenile type 1 diabetes. [18]

After age 15-years-old, they become much rarer. They could also be the first antibodies to appear in children with T1D, the prevalence of IAAs decreases with age: 60 to 90% < 4 years, 5 < 40-75% < 9 years, 10 < 35-60% < 14 years.

IAA-AAb appear after insulin injection (anti-diabetic treatment), those associated with the development of T1D recognize proinsulin, human, porcine and bovine insulins and are associated with the HLA DR4-DQ8 haplotype. [6,7]

IAA-AAbs not related to the development of T1D identify only human insulin and not animal insulins.

3.3 Anti-glutamic acid decarboxylase autoantibody (GADA):

In 1982, autoantibodies (AAbs) against a 64 kDa protein were detected by immunoprecipitation in 80% of newly diagnosed diabetic children, a high prevalence confirmed by many later studies showing the presence of these AAbs during the asymptomatic phase until 8 years before the clinical onset of diabetes (**Table III**).

Their persistence is more prolonged than that of ICAs, especially in subjects over six years of age, in whom GADAs are still present five years after diagnosis in 90% of cases. [17]

Human GAD is a protein consisting of a sequence of 585 amino acids; it is synthesized in two forms of 65 and 67 kDa, GAD catalyzes the decarboxylation of glutamic acid to 7-aminobutyric acid (GABA) with the CO_2 release

GABA has antidiabetic effects by acting on both islet ß cells and the immune system, producing membrane depolarization and Ca2+ influx, leading to the activation of growth and survival-dependent PI3-K / Akt pathways.

In severely diabetic mice, GABA has an essential role in regulating islet function and glucose homeostasis, restoring ß cell mass and reversing disease. Also, GABA suppresses insulin and the systemic production of inflammatory cytokines. [19]

3.4 Anti- tyrosine phosphatase autoantibody (IA2-AAb) :

They are circulating antibodies directed against peptide fragments of 37/40 kDa derived from islet homogenates trypsinization, IA2 is a glycoprotein belonging to the tyrosine phosphatase family.

Many reactive antibodies have been proposed whose reactivity varies with the different fragments of the juxta-membrane and intracellular IA2 domain.

IA2-AAb is detected in 78% of people with T1D at the time of diagnosis. The combined detection of IA2-AAb with that of ICA-AAb and/or GADA provides a predictive value of 75 to 100% over the next 5 years in at-risk populations (**Table III**). [20]

Tyrosine Phosphatase 1B (PTP1B) is an intracellular protein that is primarily expressed in the body, including the brain, liver, muscles and fat tissue, and is increasingly regulated in obesity, type diabetes and breast cancer. [21]

The PTP1B is a negative regulator of the insulin signaling pathway; it has a crucial role in pancreatic cells in the regulation of cell proliferation and apoptosis.

3.5 Anti-transporter 8 zinc autoantibody (ZnT8-AAb):

The microarray screening of β cell lines with sera from diabetic patients revealed a new immunoreactivity directed against a transporter involved in cation output.

Pancreatic β cells have the highest zinc levels compared to other cells in the body. The protein SLC30A ZnT8 helps to transport zinc ions from the cytoplasm into insulin secretory vesicles in the β cells of the pancreas. [17]

Zn T-8 or Slc30A8 is a transporter that regulates the movement of zinc, a cation whose activity on the stabilization of the insulin molecule is also known. It also protects pancreatic cells from destruction induced by cytokines found in patients with T1D.

The antibodies against Zn T-8 are detected in 60 to 80% of T1D cases, compared to only 2% in controls and 3% in type 2 diabetes. This prevalence can vary according to age, type of diabetes and origin of the population studied. [22]

Zn T8-AAb has been shown to be more frequently expressed in children under 10 years of age, compared to GAD-AAb or IA2-AAbs. [16]

Before the appearance of clinical symptoms of the disease, the concentration of ZNT8-AAbs in patients' sera is at its highest, and the level of these antibodies begins to decline in the months following diagnosis. [22]

ZnT8 is also associated with CD4+ and CD8+ self-reactive T cells in human T1D. This combination can help to develop therapeutic or preventive agents that target ZnT8-specific T cells and stop the progression of the disease.

The determination of ZnT8-AAbs could replace the search for ICAs by indirect immunofluorescence.

3.6 Eukaryote translation elongation factor 1 alpha 1 autoantibody (EEF1A1-AAb) and ubiquitin-conjugating enzyme 2L3 auto-antibody (UBE2L3-AAb):

Recently, they have reported two new autoantibodies found through the exploration of T1D autoantibody repertoires using a high-density fluorescence-based protein microarray. These two autoantibodies, elongation factor of eukaryotic translation 1 alpha 1 autoantibody (EEF1A1-AAb) and autoantibodies 2L3 (UBE2L3-AAb), the enzyme involved in protein degradation, for ubiquitin conjugation.

The prevalence of EEF1A1-AAb and UBE2L3-AAb is respectively 29.5% and 35.8%, they have been detected in 40% of T1D patients who are GADA negative.

EEF1A1-AAb and UBE2L3-AAb are detectable only in T1D patients <40 years of age, their prevalence increases with the age of onset of the disease.

These two autoantibodies are more frequently present than ZnT8 in T1D patients who are GAD negative. Lastly, UBE2L3 autoantibodies were detectable in 18% of patients with a fulminant form of T1D and were not detectable in LADA patients. [23]

Since age can be associated with positivity of autoantibodies in T1D subjects, a Korean team looked as if age affects the levels of these two antibodies in non-diabetic subjects; they reported that in control subjects, there is no significant correlation between age and EEF1A1-AAb and UBE2L3-AAb levels. [24]

Glima 38:

A new antibody has been discovered very recently, GLIMA 38, an antibody against a 38 kDa glycoprotein from the tetraspanin protein family TSPAN7, the prevalence of GLIMA 38 is significantly lower than other autoantibodies (< 30%). [25]

4. Screening for type 1 diabetes:

The existence of the preclinical phase, during which autoantibodies associated with diabetes emerge sequentially, provides the opportunity to identify these individuals at risk before the clinical manifestations of the disease. [2]

The risk of T1D can be estimated by examining the familial history of T1D, the sex of the family members with T1D and the age at which they developed diabetes, and by determining the patient's immune profile and genetic markers. [26]

The loss of pancreatic beta cells associated with the development of T1D can be detected reliably in first and second degree parents of people with T1D by the presence of autoantibodies against pancreatic beta cells in the serum. [11]

If there is a clear association between T1D and some HLA haplotypes, the presence of these haplotypes is not enough to develop T1D. [26]

In combination with the detection of autoantibodies in first-degree relatives of diabetic subjects, they increase the positive predictive value of the latter (**Table III**).

The search of autoantibodies remains the only first-line recommended for the screening of first-degree relatives, HLA genotyping should only be performed in the second-line if the autoantibodies are positive.

There are clinical studies underway testing different strategies to prevent T1D at an early phase in cases of positive autoimmunity or to reverse its progression.

Conclusion:

The autoantibodies associated with type 1 diabetes are very important to confirm the clinical diagnosis of the disease; they are a biological biomarker allowing the follow-up of the destructive process of the Langerhans islet.

These autoantibodies can be used effectively for the prediction of T1D, and they can be used as early markers of T1D, due to their high value concerning persistence in patients' sera before the development of T1D, at the time of diagnosis and even after diagnosis.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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