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A comparison of current methods to measure antibodies in type 1 diabetes

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Abstract

Objectives: Type 1 diabetes (T1D) is a chronic autoimmune disease causing β-cell destruction, hyperglycemia, and lifelong insulin dependence that can lead to severe complications like ketoacidosis, with a 1% mortality rate in newly diagnosed patients. A significant breakthrough in T1D research was the identification of a long presymptomatic phase, characterized by disease-specific autoantibodies despite the absence of clinical symptoms. The aim of our study was to compare the results of different commercial assays for detecting anti-GAD, -IA-2, -ZnT8 antibodies and IAA to evaluate the state of the art of the current methods in a routine clinical laboratory setting.

Methods: We have analyzed 87 consecutive samples from patients screened for T1D and evaluated the agreement among four commercial assays (two chemiluminescence immunoassays and two immunoenzymatic assays) for detecting anti-GAD, -IA-2, -ZnT8 antibodies and IAA.

Results: The agreement among methods for all disease-specific antibodies measured by Cohen's kappa ranged from 0.514 to 1.000. The highest agreement was found for anti-GAD antibodies (0.923–0.963) and the lowest agreement for IAA (0.514–0.550). The average agreement was 0.796 (SD: 0.170) and it was statistically significant at $p<0.001$ for all comparisons.

Conclusions: Even though some differences exist among methods, our findings provide valuable insights into the use of new technologies for T1D diagnosis, demonstrating an overall consistent agreement among assays tested for all antibodies but IAA.

Keywords: type 1 diabetes; immunoassays; autoantibodies

Introduction

Autoimmune diabetes is a heterogeneous disease with clinical manifestations ranging from more aggressive phenotypes characterized by a rapid loss of β-cell function, as observed in early-onset "classic" type 1 diabetes (T1D), to milder and slowly progressing forms, such as latent autoimmune diabetes in adults (LADA) [1]. Genetic predisposition to the disease interacts with environmental factors to trigger pancreatic autoimmunity. The pathogenesis includes three phases that lead to a progressive loss of β-cell function: a pre-symptomatic period characterized by detectable immune changes and normoglycemia (stage 1); a period of asymptomatic dysglycemia (stage 2) when the percentage of functional residual mass of β-cells is too low to maintain blood glucose values within normal ranges; the complete loss of β-cells (stage 3). In people with classic T1D, progression from pre-symptomatic stages (1 and 2) to stage 3 is so rapid that in most cases asymptomatic dysglycemia is not diagnosed. Conversely, LADA is characterized by longer pre-symptomatic stages, allowing the diagnosis of dysglycemia in a non-insulin-dependent state. However, among people with LADA, some individuals will progress to an insulin-dependent state sooner (early insulin-dependent LADA) or later (late insulin-dependent LADA), although some people will maintain sufficient β-cell function and will not need insulin treatment (non-insulin-dependent LADA) [2].

The incidence of T1D has steadily increased over the past five decades in high-income countries, making it the most common chronic disease of childhood and adolescence today [3–5]. The disease requires lifelong management through insulin therapy and can lead to life-threatening

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complications like ketoacidosis, particularly in newly diagnosed patients, with a 1 % mortality rate. Therefore, early recognition is crucial to prevent severe outcomes [3, 4, 6].

Islet cell antibodies (ICA) were the first antibodies described in patients with T1D in 1974 [7]. Since then, other specific antibodies against pancreas antigens have been discovered. The disease-specific antibodies indicative of β-cell dysfunction and/or death are directed against four target antigens: 1) glutamic acid decarboxylase (GAD), a rate-limiting enzyme engaged in the synthesis of the neurotransmitter-aminobutyric acid from L-glutamate (GABA) that regulate β-cell proliferation and insulin release [8]; 2) insulin that regulates glucose levels in the blood and induces glucose storage; 3) islet antigen 2 (IA-2), whose physiological role is to regulate insulin secretory granule content and β-cell growth; and 4) zinc transporter 8 (ZnT8) that transports zinc ion crucial for the synthesis, processing and secretion of insulin [9, 10]. They can all be found even years before the clinical onset of the disease and are used in clinical practice for diagnosis, prediction and management of T1D [11]. Moreover, their detection can help distinguish LADA from non-autoimmune diabetes [1].

Two or more autoantibodies among anti-GAD, anti-IA-2, and anti-ZnT8 antibodies and IAA may be present in the first stage of the disease, with a 44 % and 77 % risk of T1D development within 5 and 10 years respectively [12]. To note, the number of positive autoantibodies rather than the type of autoantibody, is a stronger predictor of disease development [13].

A study examining first-degree relatives of individuals with T1D who eventually developed T1D, found that IAA or anti-GAD autoantibodies are typically the first to appear, followed by anti-IA-2 and anti-ZnT8 [14]. Anti-GAD is the most prevalent autoantibody in both acute T1D and LADA. However, the appearance of anti-GAD is followed by anti-IA-2 and anti-ZnT8 in the case of T1D, and by anti-IAA in LADA [15]. In contrast to anti-IA-2 and anti-ZnT8, anti-GAD are not considered a specific marker for pancreatic β-cell destruction [15] since they can be observed in other autoimmune diseases like antiphospholipid syndrome (APS) [16, 17] and Stiff-man syndrome [18]. Antibody prevalence in T1D decreases following diagnosis, but the speed of the decline differs based on the target antigen. Initial studies revealed that ICA decrease more quickly than anti-GAD and anti-IA-2 autoantibodies [19–21]. More recent studies show that anti-ZnT8 decrease more rapidly than anti-GAD or anti-IA-2 autoantibodies after diagnosis.

Studies investigating the molecular characteristics of autoantibodies, in particular their epitopic targets and relative affinity, have shown that the definition of these parameters may help to improve the identification of disease relevance in preclinical subjects. For example, full-length GAD65, typically used as a target protein to detect anti-GAD antibodies, has been shown to be less appropriate than 95-amino acid truncated GAD65 from the N-terminus in predicting T1D progression in preclinical cohorts of first-degree relatives. With regard to ZnT8 antigen the most specific and sensitive results were found using the C-terminal construct. A common polymorphism of the C-terminal construct in aa325 was found to be a key determinant of two main conformational epitopes with variants of arginine (R) or tryptophan (W) while the third known variant, glutamine (Q), is less relevant in individuals with recent onset of TD1. In addition, the use of the ZnT8WR dimer appears to be more effective in stratifying the disease progression. Regarding IA-2 antigen, the intracellular domain was found to be a highly reactive region in individuals with new-onset T1D [22].

Different detection methods such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and more recently, chemiluminescence immunoassay (CLIA) have been developed to detect diabetes specific antibodies. These methods also highlight the limitations of immunofluorescence assay (IFA) in the detection of ICA, namely that it is time-consuming, requires the use of human pancreatic tissue which is no longer permitted by current laboratory regulations, and does not allow quantitative results.

To achieve reduction in interlaboratory variability, the Diabetes Autoantibody Standardization Program (DASP) – a partnership between the U.S. Centers for Disease Control and Prevention and the Immunology of Diabetes Society – and its successor IASP (Islet Autoantibody Standardization Program), organized a series of international workshops with the aim to harmonize and standardize methods for the detection of autoantibodies against pancreas target antigens [23, 24]. However, despite numerous efforts to harmonize different methods, significant discrepancies among assays still persist [23, 25–30].

The aim of our study was to compare the results of different CLIA and ELISA commercial assays, which are currently available in clinical laboratories, in detecting anti-GAD, anti-IA-2, anti-ZnT8 antibodies and IAA in a patients cohort screened for T1D to evaluate the degree of harmonization of the diabetes-related antibodies methods.

Materials and methods

Eighty-seven consecutive serum samples from patients (F:M ratio 1.5:1, mean age=50 ± 14.8 years) screened for autoimmune diabetes-specific antibodies were tested using different commercial assays available to clinical laboratories.

The serum levels of anti-GAD autoantibodies were analysed by two different CLIA assays using MAGLUMI X3 (Snibe, Shenzhen, China) and iFLASH1800 instruments (Yhlo Biotech Co. Ltd, Shenzhen, China), and by two different ELISA assays using RSR Limited (Cardiff, UK) and Euroimmun (Lübeck, Germany) on DSX instrument. IAA were tested by two CLIA (Snibe and Yhlo) and by one ELISA (Orgentec, Mainz, Germany). Anti-IA-2 and anti-ZnT8 antibodies were assayed by two CLIA (Snibe and Yhlo) and by one ELISA (Euroimmun). The assays were performed following the protocols recommended by the manufacturers. The characteristics of each method are described in Table 1, including detailed information about the nature of antigens.

Clinical data obtained by retrospective medical records were reviewed only for patients whose sera have shown discordant results among assays.

This study meets and is in compliance with all ethical standards in medicine, and informed consent was obtained from all patients according to the Declaration of Helsinki and to Italian legislation (Authorization of the Privacy Guarantor No. 9, December 12, 2013).

Statistical analysis was performed using SPSS 28.0 software (SPSS, Chicago, IL). To evaluate the agreement between the different disease-specific antibodies, Cohen's Kappa coefficient was calculated assuming 0.01–0.20 as slight agreement; 0.21–0.40 as fair agreement; 0.41–0.60 as moderate agreement; 0.61–0.80 as substantial agreement;

Table 1: Characteristics of anti-islet cell antibody assays.

Manufacturer	Kit assay	Method	Instrument	Cutoff	Source of antigen	Dynamic range
GAD Ab						
Yhlo	iFlash-GADA	CLIA (sandwich immunoassay)	iFlash1800	10 IU/mL	Recombinant human GAD65	0.5–2,000 IU/mL
Euroimmun	Anti-GAD ELISA (IgG)	ELISA	ANALYZER I-2P	10 IU/mL	Recombinant human GAD65	0.59–2,000 IU/mL
RSR	ELISA RSR™ GADAb	ELISA	DSX	5 IU/mL	Recombinant human GAD65	0.57–2,000 IU/mL
Snibe	MAGLUMI® anti-GAD	CLIA	MAGLUMI X3	10 IU/mL	Not declared	5–2,000 IU/mL
IA-2 Ab						
Yhlo	iFlash-IA-2A	CLIA (sandwich immunoassy)	iFlash1800	10 IU/mL	Recombinant human IA-2	2–4,000 IU/mL
Euroimmun	Anti-IA-2 ELISA (IgG)	ELISA	ANALYZER I-2P	10 IU/mL	Recombinant human IA-2	1.04–4,000 IU/mL
Snibe	MAGLUMI® anti-IA-2	CLIA	MAGLUMI X3	10 IU/mL	Not declared	3.5–1,000 IU/mL
IAA						
Yhlo	iFlash-IAA	CLIA (indirect immunoassay)	iFlash1800	1.0 ± 0.1 COI	Recombinant human insulin	NA
Orgentec	Anti-insulin	ELISA	ANALYZER I-2P	10 U/mL	A mixture of highly purified preparation of bovine, porcine and recombinant human insulin	0.5–100 AU/mL
Snibe	MAGLUMI® IAA	CLIA	MAGLUMI X3	20 AU/mL	Not declared	8–175 AU/mL
ZNT8 Ab						
Yhlo	iFlash-ZnT8	CLIA (sandwich immunoassay)	iFlash1800	10 AU/mL	Recombinant human ZnT8	2–2,000 AU/mL
Euroimmun	Anti-zinc transporter 8 ELISA	ELISA	ANALYZER I-2P	15 UR/mL	Recombinant human ZnT8	1.2–2,000 UR/mL
Snibe	MAGLUMI® anti-ZnT8	CLIA	MAGLUMI X3	10 AU/mL	Not declared	5–500 AU/mL

ELISA, enzyme linked immunoassay; CLIA, chemiluminescence immunoassay; Ab, antibodies.

0.81–1.00 as almost perfect or perfect agreement. A p-value <0.05 was considered significant. To categorize the methods based on the qualitative results of the tests (positive or negative), hierarchical clustering was performed using the "pvclust" R statistical package [31]. Bootstrap resampling (n=10,000) and average cluster method were used to construct a cluster dendrogram. For each cluster, this package calculates p-values using a bootstrap resampling method, indicating how strongly data support the cluster. Two types of p-values are provided: BP (Bootstrap Probability) and AU (Approximately Unbiased) p-values. Clusters with p-value above 95 % were considered significant.

Results

The agreement among methods for all autoimmune diabetes-specific antibodies measured by Cohen's kappa ranged from 0.514 (Orgentec vs. Yhlo; IAA) to 1.000 (Yhlo vs. Snibe; IA-2) (Table 2). The average agreement was 0.796 (SD: 0.170) and it was statistically significant at p<0.001 for all comparisons. In particular, the average agreement was 0.536 for IAA (SD: 0.019), 0.738 (SD: 0.060) for anti-ZnT8, 0.821 (SD: 0.155) for anti-IA-2, and 0.943 (SD: 0.021) for anti-GAD. In the case of anti-IA-2 autoantibodies there was perfect agreement (100 %) between two CLIA systems (Yhlo and Snibe), with a moderate Cohen's k with ELISA (Euroimmun).

Table 2: Cohen's kappa agreement among methods for all anti-islet cell antibodies.

	Anti-GAD Ab		
	Yhlo	**Snibe**	**RSR**
Euroimmun	0.963	0.961	0.963
Yhlo		0.923	0.927
Snibe			0.923
	Anti-IA-2 Ab		
	Yhlo		**Snibe**
Euroimmun	0.731		0.731
Yhlo			1.000
	Anti-ZnT8 Ab		
	Yhlo		**Snibe**
Euroimmun	0.792		0.750
Yhlo			0.673
	IAA		
	Yhlo		**Snibe**
Orgentec	0.514		0.550
Yhlo			0.544

When hierarchical clustering was performed, two clusters with a p-value <0.05 were identified: one including the IA antibody group and the second one with all the other antibodies (GAD, IA-2, ZNT8) (Figure 1). In Table 3, the discordant results for each disease-specific antibody among assays were analyzed according to the patients' history. Out of the 1,131 tests on 87 patients' sera, only 28 tests were discordant. Nearly all of the discordant sera have shown discordance for only one antibody, and only one patient was discordant for two different antibodies. Anti-GAD autoantibodies displayed three discordant cases; anti-IA-2 autoantibodies displayed six discordant cases, all positive only on Euroimmun ELISA and negative on both CLIA systems; ZnT8 autoantibodies displayed eight discordant cases while for IAA were eleven.

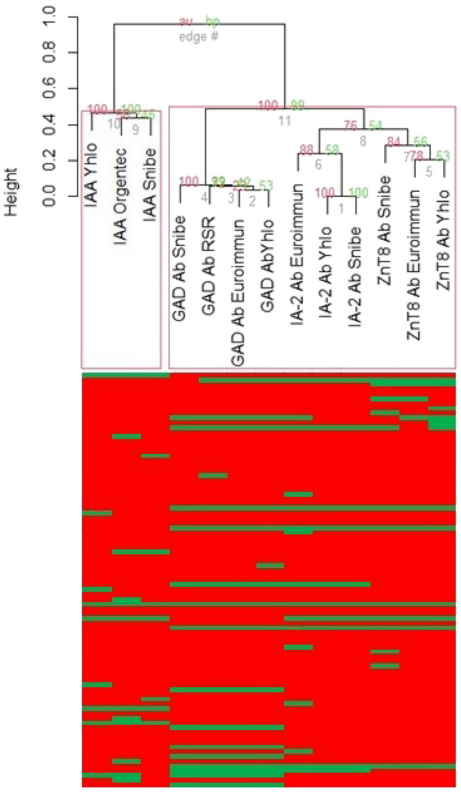

Figure 1: Hierarchical cluster analysis according to positive (green) and negative (red) results for the methods for anti-islet cell antibodies investigated. The dendrogram in the upper part of the Figure summarises the relationship of similarity among different methods. To identify the most similar assays to each other, read the dendrogram from bottom to top, identifying the first branch to join together. Two clusters with a significance level of <0.05 corresponds to AU/BP>95, are highlighted by the rectangles: one including the IAAs group and the second one with all the other antibodies.

Table 3: Discordant sera results among assays for anti-GAD, anti-IA-2, anti-ZnT8 antibodies and IAA: serological and clinical characteristics.

Sample ID	Anti-GAD Ab				Anti-IA-2 Ab			Anti-ZnT8 Ab			IAA			Clinical characteristics
	Euroimmun	Yhlo	Snibe	RSR	Euroimmun	Yhlo	Snibe	Euroimmun	Yhlo	Snibe	Orgentec	Yhlo	Snibe	
2	P	P	N	P	P	P	P	P	P	P	N	N	N	T1D
10	P	P	P	P	P	N	N	P	P	N	N	N	N	T1D
22	N	N	N	P	N	N	N	N	N	N	N	N	N	T1D
41	N	P	N	N	N	N	N	N	N	N	N	N	N	Screening
26	N	N	N	N	P	N	N	N	N	N	N	N	N	T1D
34	N	N	N	N	P	N	N	N	N	N	N	N	N	Screening
58	N	N	N	N	P	N	N	N	N	N	N	N	N	T1D
70	N	N	N	N	P	N	N	N	N	N	N	N	N	T1D
80	N	N	N	N	P	N	N	N	N	N	N	N	N	First degree familiarity
6	N	N	N	N	N	N	N	P	N	P	N	N	N	T1D
8	N	N	N	N	N	N	N	N	P	N	N	N	N	T1D
9	N	N	N	N	N	N	N	N	N	P	N	N	N	T1D
11	N	N	N	N	N	N	N	N	P	N	N	N	N	Hyperglycemia
12	P	P	P	P	P	P	P	N	P	P	N	N	N	T1D
59	N	N	N	N	N	N	N	N	N	P	N	N	N	T1D
62	N	N	N	N	N	N	N	N	N	P	N	N	N	T1D
14	N	N	N	N	N	N	N	N	N	N	P	N	N	Neurological disorder
18	N	N	N	N	N	N	N	N	N	N	N	N	P	T1D
30	N	N	N	N	N	N	N	N	N	N	N	P	N	Screening
46	N	N	N	N	N	N	N	N	N	N	N	P	N	Screening
48	N	N	N	N	N	N	N	N	N	N	P	N	N	Neurological disorder
66	N	N	N	N	N	N	N	N	N	N	N	P	N	T1D
69	N	N	N	N	N	N	N	N	N	N	N	N	P	T1D
73	N	N	N	N	N	N	N	N	N	N	P	N	N	Dysglycemia
82	N	N	N	N	N	N	N	N	N	N	P	N	N	T1D
85	P	P	P	P	N	N	N	N	N	N	P	P	N	T1D
86	N	N	N	N	N	N	N	N	N	N	P	N	N	T1D

P, positive; N, negative. Discordant sera results are highlighted in grey.

Discussion

Testing for autoantibodies against islet β-cells is commonly used in clinical practice to diagnose autoimmune diabetes. It has also taken on an important role in the clinical classification of T1D [32], in predicting insulin requirements [33], in identifying individuals at risk of developing the disease [34] and in clinical trials [35].

As the use of radioactive reagents has decreased significantly, several alternative methods such as ELISA and CLIA have been developed and introduced in clinical laboratories. The use of ELISA is progressively decreasing, being widely replaced by automated random-access methods such as CLIA [36]. The new methods avoid having to work in batches, making this diagnostics accessible to many clinical laboratories. In addition to being faster, automated platforms allow the simultaneous measurement of multiple antibodies. Furthermore, this has also favoured increasingly expanding antibody-based population screening programmes for T1D [37] and for the diagnostic process of LADA [1].

In the last decades, DASP and its successor IASP standardization programs provided blinded testing of large numbers of unselected sera from both healthy and diabetic individuals, allowing assessment of test performance in terms of sensitivity and specificity [25, 29]. DASP and IASP workshops showed a good performance of the diabetes-related autoantibody assays with the exception of IAA, where both RIA and the new methods performed poorly [23]. Our study aimed to compare the results of four commercial methods (two CLIA and two ELISA) from different manufacturers for detecting anti-GAD, anti-IA-2, anti-ZnT8 antibodies and IAA. Our data are broadly in line with the DASP results since all antibodies assays have a Cohen's kappa agreement ranging from substantial to almost perfect (0.673–1.000), except for IAA which have a moderate Cohen's kappa agreement (0.536). Anti-GAD antibodies assays revealed the best inter-assay comparability, with a Cohen's kappa always greater than 0.9 in all pairs of assays analysed. This is a relevant point because today anti-GAD is the most widely used test, in line with DASP data that since the first workshop in 2000 [25] has showed that anti-GAD antibodies assays, regardless of the method used, have consistently maintained high levels of sensitivity with a strong discrimination between healthy and disease conditions. Interestingly, results of the 2018 IASP workshop demonstrated that the majority of the novel non-RIA immunoassays showed a similar or better performance than RIA [26]. DASP proficiency evaluations have also revealed that many laboratories use commercial assays (RIA or ELISA), achieving levels of sensitivity and specificity equivalent to in-house RIA also for anti-IA-2 antibodies, with significant concordance between the two methods [23, 25]. The current study showed an average agreement for anti-IA-2 antibodies of 0.821 comparing both CLIA with ELISA.

Regarding IAA, DASP [24] and IASP workshops [29] both revealed that there is still a heterogeneous performance among assays, and that RIA still represents the gold standard for IAA detection. This is linked to the low performance of assays in classifying as positive sera when low levels of antibodies are present and to a possible modification of some insulin epitopes caused by the addition of tags (e.g. biotin residues in ECL) in the assay format or by post-translational modification of the antigen. Our data for IAA comparison revealed a moderate average agreement of 0.536, reflecting the lack of comparability. It would have been interesting to be able to compare the results of both CLIA and ELISA methods with the RIA, but unfortunately this latter method is no longer available in clinical laboratories, having fallen almost completely into disuse due to problems related to the radioactivity of the reagents.

It is also important to consider that distinguishing IAA autoantibodies from insulin antibodies produced by exogenous insulin injection is difficult; according to the recommendations, patients are considered positive for IAA if insulin antibodies are present in insulin-naïve patients or in those within two weeks from the start of insulin therapy [9, 38]. A good average agreement (0.738) was obtained for anti-ZnT8 antibody tests even if the DASP workshop in 2011 identified immunoprecipitation-based ZnT8 assays to achieve a high degree of sensitivity and specificity [39]. Genetic polymorphism may cause heterogeneity of immunogenic epitopes thus causing inter assays variability [40, 41]. This may explain the lower agreement of anti-ZnT8 antibodies compared to anti-GAD and anti-IA antibodies found in our study. The introduction of a common international standard for anti-ZnT8 antibodies might help to improve the agreement of results but, unfortunately, the international WHO standard serum for antibodies anti-GAD65 and anti-IA-2 [42, 43] is negative for anti-ZnT8 antibodies (A. Williams and P. Achenbach, unpublished observations).

The presence of different epitopes for the same antigen might partly explain the discordance observed in our tests and the variability of the patients' clinical phenotypes. The main epitopic targets of islet autoantibodies have been studied to facilitate both early diagnosis of the disease and understanding the underlying autoimmune response mechanisms but the identification of epitope variants is not always possible. Commercially certified methods do not currently detect all possible disease-specific epitopes of autoantibodies involved in the pathogenesis of T1D. The analysis of the clinical data on the discordant results allows us to

interpret only partially the discrepancies among the different assays because these antibodies may appear many years before the clinical onset of the disease. It is important to note that the discordant results refer to only one antibody per patient and that only one sample shows discordance for more than one antibody.

Although the detection of a single islet autoantibody positivity, confirmed in a second sample is an important indicator to support the diagnosis of autoimmune diabetes in patient with recent-onset disease and compatible clinical phenotype, the clinical significance of single islet autoantibody positivity in the context of early-stage T1D risk assessment remains a subject of ongoing investigation [44].

Progression rates to clinical T1D in individuals exhibiting a single islet autoantibody are heterogeneous and influenced by the specific autoantibody involved, the individual's chronological age, and their underlying genetic susceptibility [45].

In adults, the repertoire of detectable islet autoantibodies available for the identification of early-stage T1D is diminished. For example insulin autoantibodies (marker in childhood) are infrequently detected in adults, and the autoantibody profile often consists primarily of single anti-GAD autoantibodies. The 2024 Consensus of the American Diabetes Association and of the European Association for the Study of Diabetes establish that frequency of monitoring can be based on the stage at which an individual with islet autoantibody positivity is diagnosed.

Patients with a single T1D antibody with dysglycemia should be monitored more frequently than those with normoglycemia. Additional risk stratification may also be possible based on other characteristics, such as age, or modifiable factors, such as abdominal obesity. More frequent monitoring is proposed also for individuals with multiple autoantibodies if they are diagnosed with stage 2 compared with stage 1 [46].

In contrast, in screening settings involving individuals without clinical diabetes, the presence of two or more autoantibodies is required to define a preclinical stage of T1D and confirmation using a different assay platform is recommended [45]. Consensus reported also recommendation for monitoring children. Individuals with the presence of two or more autoantibodies are at very high risk for progression to stage 3 T1D within 15 years and their detection should be confirmed within three months. Conversely, although loss of confirmed multiple autoantibodies is rare and may be associated with reduced risk of progression to T1D, the monitoring should not be discontinued [46].

Finally, from a laboratory point of view the diagnosis of early-stage T1D is critical and requires the application of multiple sequential tests; for these reasons accurate criteria are vital to avoid over- or underdiagnosis.

Our study offers valuable insights into the detection of specific antibodies for diabetes diagnosis using new technologies, but several limitations should be noted. First, our sample size was limited, particularly within the pediatric group. Second, RIA was excluded from our comparative study because it was unavailable. However, a key strength of this study is the number of different commercial assays analyzed for the detection of four diabetes-related antibodies.

Future studies with larger cohorts of patients with detailed clinical information are needed for a more robust clinical performance evaluation of these assays and the introduction of international standards for other islet cell antibodies to improve the harmonization of the results.

Research ethics: This study meets and is in accordance with all ethical standards in medicine, and informed consent was obtained from all patients according to the Declaration of Helsinki and to Italian legislation (Authorization of the Privacy Guarantor No. 9, December 12, 2013).
Informed consent: Not applicable.
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