Scandinavian Journal of Clinical and Laboratory Investigation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iclb20

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To cite this article: Yu Fu, Chen Zhang, Yong Gu, Shibin Ge, Jianhua Li, Jianlin Feng, Li Zhang, Wei Liu & Heng Chen (2021): Establishing reference intervals for islet autoantibodies in Han Chinese type 1 diabetes, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: 10.1080/00365513.2021.2001564

To link to this article: https://doi.org/10.1080/00365513.2021.2001564

Published online: 15 Nov 2021.

SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION
https://doi.org/10.1080/00365513.2021.2001564

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ORIGINAL ARTICLE

Establishing reference intervals for islet autoantibodies in Han Chinese type 1 diabetes

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ABSTRACT

Currently, islet autoantibodies (IAbs) constitute the most reliable marker for detecting the autoimmune process of type 1 diabetes (T1D). However, there are no appropriate reference intervals (RIs) to interpret the results of IAbs in China. In this study, we aimed to establish the RIs of four common IAbs based on the Han Chinese population and evaluate their clinical diagnostic values in patients with T1D. We collected 177 blood samples from healthy volunteers to detect the levels of IAbs directed against insulin (IAA), glutamic acid decarboxylase-65 (GADA), insulinoma antigen 2 (IA-2A), and zinc transporter-8 (ZnT8A) using a chemiluminescence immunoassay. RIs were calculated using nonparametric 95th percentile intervals in accordance with the Clinical and Laboratory Standards Institute guidelines, and their clinical diagnostic values were evaluated by detecting the levels of IAbs of 140 blood samples from patients with T1D in a clinical setting. We defined 138 individuals as the apparently healthy population from the 177 healthy volunteers based on the exclusion criteria. No association between the levels of the four IAbs and gender ($p > .05$) and age ($p > .05$) were found in the apparently healthy population. The combined RIs for GADA, IA-2A, ZnT8A, and IAA were 0–1.78 IU/mL, 0–3.91 IU/mL, 0–2.36 AU/mL, and 0–0.58 COI, respectively. Overall, the diagnostic efficiency for the four IAbs, especially for GADA and IAA, were improved by using the RIs established in this study. The RIs for IAbs established in this study will be a valuable tool for disease diagnosis and the therapeutic management of T1D in a clinical setting.

ARTICLE HISTORY
Received 14 April 2021
Revised 1 September 2021
Accepted 3 October 2021

KEYWORDS
Reference intervals; islet autoantibodies; type 1 diabetes; apparently healthy population; diagnostic efficiency

Introduction

Type 1 diabetes (T1D) is a condition with peak incidence during childhood and adolescence that requires lifelong treatment using exogenous insulin [1,2]. The number of newly diagnosed cases of T1D has been increasing globally at an annual rate of 3–5% and has doubled in the past two decades [3–5]. T1D is caused by the autoimmune-mediated selective destruction of the pancreatic islet beta-cells and results in the appearance of islet autoantibodies (IAbs), which are directed against glutamic acid decarboxylase-65 (GADA), insulinoma antigen 2 (IA-2A), zinc transporter-8 (ZnT8A), and insulin (IAA), and appear years before overt clinical disease [6], and the presence of two or more islet autoantibodies is the specific biomarker of stage 1 of presymptomatic T1D [7]. Among patients with undifferentiated or ketosis-prone diabetes, the presence of IAbs may also help classify diabetes into its appropriate subtypes and predict beta-cell dysfunction, which is critical for timely and appropriate treatment [8,9]. Therefore, the measurement of IAbs in the peripheral blood is currently the most reliable marker to determine the autoimmune status of patients with T1D [10].

After extensive research, the radio-binding assay (RBA) has been well established as a current "gold standard" for measuring IAbs [11,12]. However, owing to several limitations of a standard RBA, including low sensitivity for early detection, the lack of ability to multiplex, and the possibility of radioactive contamination, other methods, such as chemiluminescence immunoassay (CLIA) [13], plasmon-enhanced fluorescence protein microchip assays [14], lateral flow immunoassay (LFIA) [15], and enzyme-linked immunosorbent assay (ELISA) [16,17], have been developed lately to measure IAbs. Among these methods, CLIA has been found to have high sensitivity and specificity, and have the advantage of low cost for large-scale screenings. Irrespective of the analytical method used in the laboratory, the test result, by itself, is of little to no value unless it is supported by appropriate information that could help in its interpretation. This information is usually in the form of reference intervals (RIs).

As the essential components of reporting results, RIs play an important role in transforming numerical values into clinically meaningful information in a laboratory setting [18]. Appropriate RIs are vital for clinicians to arrive at a

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medical diagnosis, to determine appropriate therapy, and to aid in physiological assessment. Inappropriate RIs may cause misdiagnoses, false reassurances, and perhaps less aggressive treatment than what is warranted [19]. At present, the RIs for the measurement of IAbs used in China are mostly provided by manufacturers, and no publication about RIs for the measurement of IAbs can be referenced based on the Han Chinese population. Given that the incidence of T1D in China is higher among people in the 0–40 year age group compared to age group older than 40 and peaks during the ages 10–14 years [20], it is necessary to establish the appropriate RIs of IAbs by considering the age of these specific populations.

To provide appropriate RIs for their methodologies and the population they serve, the Clinical and Laboratory Standards Institute (CLSI) encourages laboratories to establish their own RIs [21]. In this study, we aimed to establish appropriate RIs of IAbs based on the Han Chinese population, which comprise roughly 20% of the global human population in the world, with a commonly used commercial kit available in China. To evaluate the accuracy of the measurements of the four IAbs obtained using our new established RIs, we performed further diagnostic tests by comparing our results to those obtained using the RIs provided by manufacture.

Materials and methods

Study population

A total of 177 healthy Han Chinese volunteers (aged 17–38 years) from East China were recruited for this study. After obtaining written informed consent under the guidance of experienced endocrinologists, each subject completed a questionnaire regarding personal details, including age, gender, menstrual status, current smoking habits, alcohol intake, physical exercise, and medication history. A physical examination was performed by professional physicians. Body mass index (BMI) was calculated as body weight (kg) divided by the height squared (m^2). After a mandatory 12-h fast, morning blood samples were collected using a standard venipuncture procedure performed by skilled phlebotomists. The blood samples were centrifuged, aliquoted, and frozen. Serum samples were stored at −80 °C and were used without subjecting them to freeze-thaw cycles. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

Laboratory measurements

The samples were analyzed once they reached room temperature (about 25 °C). The levels of GADA, IA-2A, ZnT8A, and IAA of the serum samples obtained from the 177 healthy volunteers and 140 patients were determined by skilled technologists using the method indicated by CLIA (Table 1). The technique was based on analysis using the iFlash 3000 platform (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China). The antibodies (acridine ester-labeled rat anti-human lgG) used in their reagents can be traced to WHO 97/550 International standard. The calibration range for GADA, IA-2A, and ZnT8A were 0–2000 IU/mL, 5–4000 IU/mL, and 0–3000 AU (Arbitrary unit)/mL, respectively, with a linearity of 5–250 IU/mL, 5–500 IU/mL, and 5–400 AU/mL, respectively. For precision which were conducted in duplicates by one operator, the declared with-run coefficient of variance (CV) and between-run CV (sample was re-examined for 40 times within 20 days) was lower than 10.0% and 15.0%, respectively. For accuracy, the relative deviation was in the ± 10.0% range for GADA, IA-2A, and ZnT8A, and 100% accuracy was obtained during the determination of IAA when 10 negative and 10 positive samples were tested. The lower limits of detection were 0.2 IU/mL, 0.7 IU/mL, and 1.0 AU/mL for GADA, IA-2A, and ZnT8A, respectively, whereas the manufacturer-declared reference values were $<$ 10.0 IU/mL for GADA and IA-2A, $<$ 10.0 AU/mL for ZnT8A, and $<$ 1.0 COI (cutoff index) for IAA. To screen the apparently healthy population by excluding the IAb-positive subjects, serum samples from the 177 enrolled healthy volunteers were simultaneously measured using RBA.

Apparently healthy population

The exclusion criteria for screening the apparently healthy subjects were as follows: (a) incomplete basic information or erroneous data resulting from negligence in data entry; (b) individuals testing positive for any of the following: GADA, IA-2A, ZnT8A, and IAA using RBA method (the "gold standard" for measuring IAbs [22]); (c) individuals with BMI $<$ 18.0 kg/m^2 or $>$ 25.0 kg/m^2; (d) individuals with a history of disease conditions, including thyroid disease, malignant neoplasm, and liver cancer, which influence insulin secretion; (e) individuals with a history of autoimmune diseases, including rheumatoid arthritis, sicca syndrome, and systemic lupus erythematosus; (f) serum samples exhibiting hemolysis and lipemia on visual examination; (g) alcohol consumption within 24 h prior to blood collection; (h) blood donation within 3 months of sample collection; (i) outliers. Outliers were removed based on the one-third ratio of the D/R rule [23], where D is the absolute difference between the highest (or lowest) observed value and the value it is numerically closest to; R is the range of all observed values. If the observed value of D was equal to or greater than one-third of R, then that value was rejected. Based on the responses in the questionnaire and laboratory measurements, 138 apparently healthy subjects of Chinese Han population were included in analysis, and 39 individuals were excluded, including 15 subjects with positive IAbs, 16 subjects with BMI $<$ 18.0 kg/m^2 or $>$ 25.0 kg/m^2, and 8 outliers.

Calculation of RIs for IAbs based on the apparently healthy population

RIs represent the central 95% of laboratory test values observed from the apparently healthy population which are

Table 1. Parameters declared by manufacture for IAbs detection.

Parameters	GADA, IU/ml	IA-2A, IU/ml	ZnT8A, AU/ml	IAA, COI
Calibration range	0–2000	0–4000	0–3000	\
Linearity range	5–250	5–500	5–400	\
Lower limit of detection	0.2	0.7	1.0	\
Reference value	10.0	10.0	10.0	1.0
Accuracy	±10.0%			100% accuracy for 10 negative samples and 10 positive samples
Within-run CV	<10.0%			
Between-run CV	<15.0%			
Traceability	WHO 97/550 islet cell antibodies, human serum			
Type of antibody	Acridine ester-labeled rat anti-human IgG			
Platform	CLIA-iFlash 3000			
Manufacture	Shenzhen YHLO Biotech Co., Ltd.			

IAbs: islet autoantibody; CLIA: chemiluminescence immunoassay; CV: coefficient of variation; GADA: glutamic acid decarboxylase-65; IA-2A: insulinoma antigen 2; ZnT8A: zinc transporter-8; IAA: insulin autoantibody; COI: Cutoff index; AU: Arbitrary unit.

free of diseases without influence of laboratory test result [18]. Age and gender were considered as two major factors to partition for RIs. When the results were not in favor of partitioning, data was combined and reevaluated for calculating RIs of IAbs using nonparametric 95th percentile intervals according to the recommendations of the CLSI EP28-A3C [21]. Because serum levels of IAbs below reference values are outside of clinical interest, the reference intervals were estimated using the one-sided upper 95th-percentile limit.

Calculation of diagnostic efficiency of the RIs for IAbs measurement

A total of 140 samples from T1D patients (aged 6–84 years) in a clinical setting were collected to evaluate the diagnostic performance of the newly established RIs. The clinical samples of these patients were handled following a procedure similar to the healthy participants in our study. To evaluate the diagnostic efficiency of CLIA when using the new established RIs, serum samples from the 140 clinical patients were also simultaneously measured using an RBA. Taking RBA as gold standard, the number of true-negative (TN), true-positive (TP), false-negative (FN), and false-positive (FP) results using CLIA were calculated; thus the sensitivity, specificity, and coincidence were deduced for the measured IAbs by CLIA using the following formulas: sensitivity(%) = TP/(TP + FN) × 100%; specificity(%) = TN/(TN + FP) × 100%; coincidence(%) = (TP + TN)/(TP + FN + TN + FP) × 100%.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 8.0 (GraphPad Prism Software, San Diego, CA). The Kolmogorov–Smirnov test was used to evaluate data distribution. Quantitative variables are presented as mean ± SD or percentiles. After logarithm transformation of the IAbs levels, the difference of IAbs levels between male and female were studied by applying Student's *t*-test. The relationship between IAbs levels and age were analyzed by Spearman's rank correlation. $p < .05$ was considered statistically significant.

Results

General characteristics of the apparently healthy and T1D population

A total of 138 apparently healthy individuals, including 51 men and 87 women, were screened from the pool of 177 participants (Table 2). The median age for men was 27.0 years and the range was 17–38 years; for women, the median age was 26.0 years and the range was 20–35 years. There were no significant differences between the patient characteristics of both sexes ($p > .05$) with the exception of the median height, weight, and BMI in men, which were 173.0 cm, 66.0 kg, and 22.5 kg/m^2, respectively; these were higher in men than the corresponding values in women, which were 163.0 cm, 54.0 kg, and 20.3 kg/m^2, respectively ($p < .001$). Considering that BMI can vary with age, the difference of BMI levels between male and female were compared by analysis of covariance, and showing that the difference disappeared after corrected by age ($p > .05$).

The T1D group included 80 men and 60 women with the median ages of 46.5 and 49.5, respectively (Table 2). Using the method of RBA, the total positive rate of GADA, IA-2A, ZnT8A, and IAA were 59.3%, 27.9%, 15.8%, and 45.0%, respectively; however, only the positive rate of IA-2A from female were higher than male (36.7% versus 21.3%, $p < .05$).

Distribution of IAbs data in the apparently healthy population

The distribution of IAbs data, measured by CLIA, in the apparently healthy population are shown in Figure 1(A). The skewness and kurtosis of distribution curves of the data for GADA, IA-2A, ZnT8A, and IAA were all higher than 1.0 and the *p* values were all lower than .001 as determined using the Shapiro–Wilk test (Figure 1(B)). The normal quantile–quantile (*Q*–*Q*) plots showed departure in the chiasma frequency distributions from the normal distribution pattern of GADA, IA-2A, ZnT8A, and IAA (Figure 1(C)). Collectively, these results indicated that the data of GADA, IA-2A, ZnT8A, and IAA typically had a significant right-skewed distribution in our study.

Table 2. Clinical characteristics of the apparent healthy and T1D population.

Characteristics	Male	Female	Total
Apparent healthy population			
No.	51	87	138
Age, year	27.0 (25.0–32.0)	26.0 (25.0–27.0)	26.0 (25.0–28.0)
Height, cm	173.0 (171.0–176.0)	163.0 (160.0–166.0)***	165.0 (162.0–172.0)
Weight, kg	66.0 (62.0–72.0)	54.0 (52.0–57.0)***	57.0 (52.0–65.0)
BMI, kg/m^2	22.5 (20.8–23.4)	20.3 (19.5–21.5)***	21.0 (19.6–22.5)
T1D patients			
No.	80	60	140
Age, year	46.5 (28.0–60.0)	49.5 (24.3–61.3)	48.0 (27.3–60.0)
Positive rate of GADA (%)	55.0	65.0	59.3
Positive rate of IA-2A (%)	21.3	36.7*	29.7
Positive rate of ZnT8A (%)	12.5	20.0	15.8
Positive rate of IAA (%)	41.3	50.0	45.0

Non-normally distributed data are presented as median (25th–75th percentile). T1D: type 1 diabetes; BMI: body mass index. IAbs: islet autoantibody; positive rate of IAbs were evaluated by RBA. *$p < .05$, ***$p < .001$.

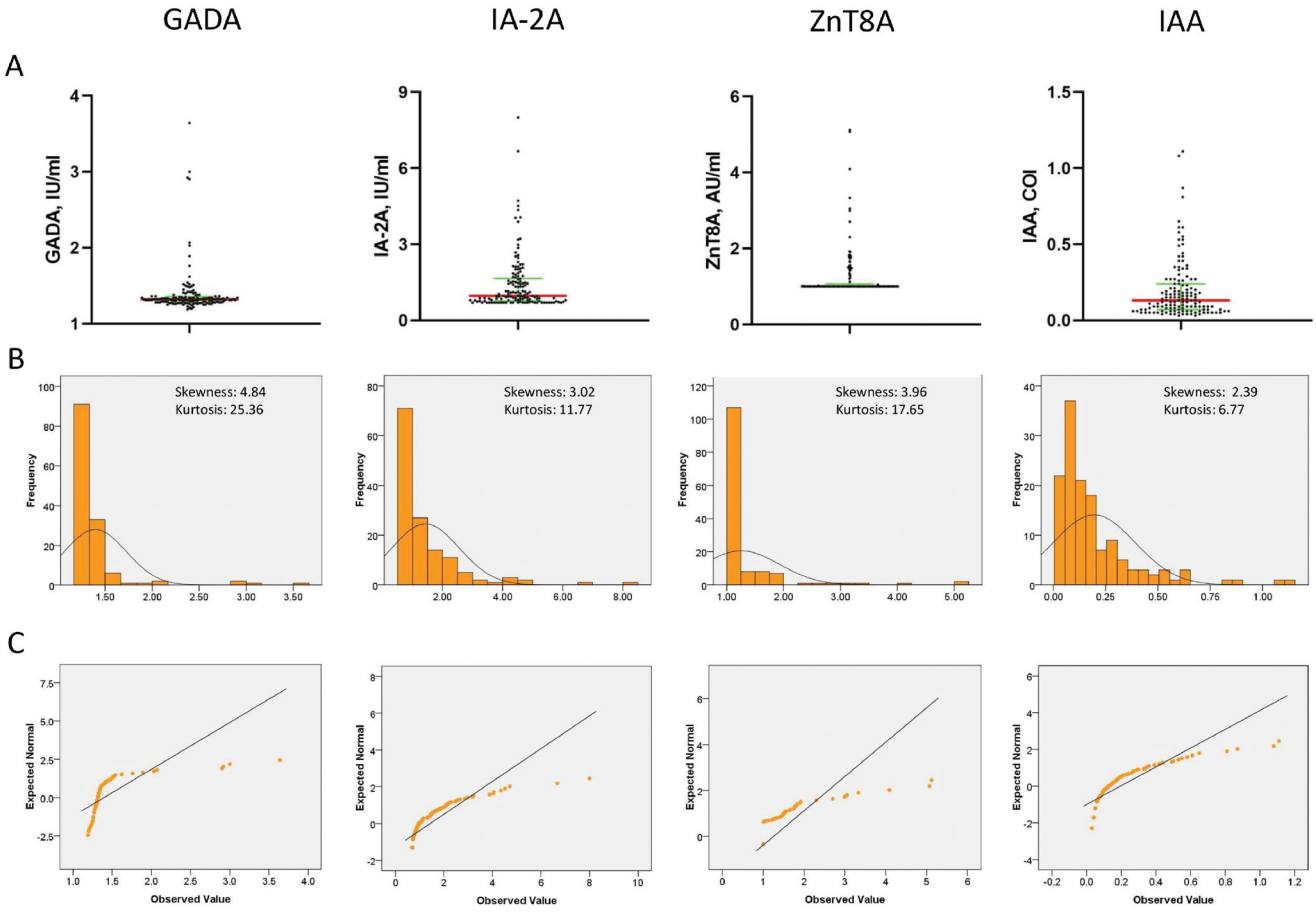

Figure 1. Distribution of IAbs data of the 138 apparent healthy population by CLIA GADA: Glutamic acid decarboxylase-65; IA-2A: Insulinoma antigen 2; ZnT8A: Zinc transporter-8; IAA: Insulin autoantibodies. The center lines represent the median; the top and bottom lines represent the 25th and 75th percentiles, respectively.

Levels of IAbs in healthy participants according to gender and age

According to gender and age, IAb levels after logarithm transformation are shown in Figure 2. No significant differences were observed in the GADA, IA-2A, ZnT8A, and IAA assays (p values of .961, .608, .282, and .841, respectively) using samples obtained from the male and female participants. Results of the linear regression showed that except for GADA, which showed a slight relationship with age ($r = 0.175$, $p = .040$), no other correlations between IA-2A, ZnT8A, and IAA levels and age were found in the healthy participants (correlation coefficients of 0.099, 0.099, and 0.083, respectively; $p > .05$).

RIs for IAb measurement in healthy Chinese individuals

Based on the distribution of the IAb data obtained from the apparently healthy population and our analysis (Figure 2), we established the combined RIs using nonparametric 95th percentile intervals without considering age and gender (Table 3). The combined RIs for GADA, IA-2A, ZnT8A, and IAA were 0–1.78 IU/mL, 0–3.91 IU/mL, 0–2.36 AU/mL,

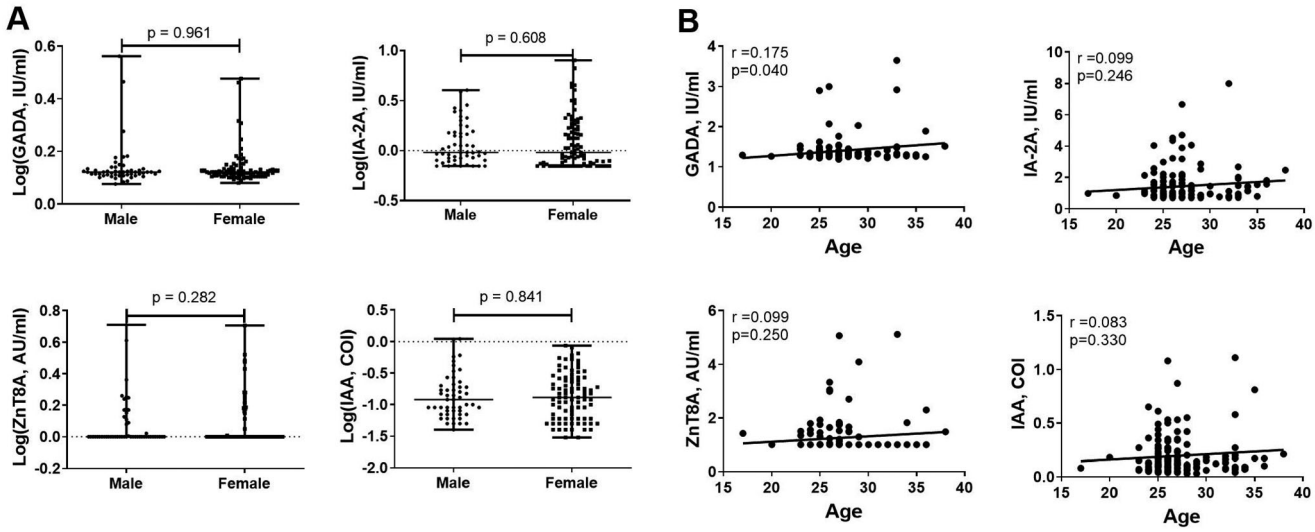

Figure 2. Relationship of gender and age to the levels of IAbs of the 138 apparent healthy population by CLIA. GADA: glutamic acid decarboxylase-65; IA-2A: insulinoma antigen 2; ZnT8A: zinc transporter-8; IAA: insulin autoantibodies.

Table 3. The RIs of IAbs based on Chinese Han population.

			RIs[a]		
Analytes	No.	Age	95th percentile	90% CI	RIs[m]
GADA, IU/ml	138	26.0	1.78	1.72–1.84	<10.0
IA-2A, IU/ml		(25.0–28.0)	3.91	3.72–4.10	<10.0
ZnT8A, AU/ml			2.36	2.24–2.48	<10.0
IAA, Col			0.58	0.55–0.61	< 1.0

Non-normally distributed data are presented as median (25th–75th percentile). RIs[a]: reference intervals established in this study; RIs[m]: reference intervals provided by manufacture; COI: Cutoff index; AU: Arbitrary unit.

and 0–0.58 COI, respectively. We also analyzed the RIs provided by the manufacturer (Table 3), which were 0–10.0 IU/mL for GADA and IA-2A, 0–10.0 AU/mL for ZnT8A, and 0–1.0 COI for IAA. Our analysis revealed that all RIs established in our study (RIs[a]) were lower than those provided by the manufacturer (RIs[m]).

Diagnostic efficiency of IAbs using the RIs[a]

The distribution of IAb data of the 140 T1D patients and the position of RIs[a] and RIs[m] were shown in Figure 3. As to the diagnostic efficiency of IAbs using the RIs[a], we found that the sensitivity, specificity, and coincidence of GADA were 85.5%, 98.2%, and 90.7%, respectively (Table 4). Among these, the sensitivity and coincidence were significantly higher than those of the RIs[m], which were 59.0% and 75.0%, respectively ($p < .001$). Although no significant differences were found for the IA-2A assay between RIs[a] and RIs[m], the sensitivity and the coincidence of the diagnostic efficiency in our study were found to be slightly improved compared to that calculated using the RIs[m]. For the ZnT8A assay, the sensitivity improved from 86.4% to 95.5%; however, the specificity and coincidence were decreased slightly compared to those when using the RIs[m]. The sensitivity was 74.6% for the IAA assay when using RIs[a]. The sensitivity was significantly higher using RIs[a] than that calculated using RIs[m] (57.1%; $p < .05$); however, no differences were found in terms of specificity and coincidence.

Discussion

Diagnostic test results play a critical role in a clinic setting because the medical decisions of almost 80% of physicians are based on the information provided by these laboratory reports [24]. The test results can be interpreted based on the RIs that are attached; thus, RIs serve as the decision-making tool for physicians to evaluate the disease condition of patients. Currently, IAbs are the most reliable biomarkers to determine the autoimmune status of patients with T1D; therefore, they play an essential role in predicting and diagnosing this condition. In this study, we established the RIs of four common IAbs based on a commonly used immunoassay platform in China. Moreover, we verified the diagnostic efficacy of the established RIs in the measurement of the four IAbs.

Apart from the RBA, the other methods used to determine the four major IAbs include radioimmunoassay (RIA) [25] and conventional ELISA [26], as well as newly developed methods based on ELISA [16,17], electrochemiluminescence (ECL) [13,27], plasmon-enhanced fluorescence protein microchip assays [14], and so on. However, the differences among platforms might lead to varied results among laboratories [11,28] and this variation could likely have a greater and direct impact on the interpretation of test reports when the RIs provided by laboratories are no longer appropriate. To avoid the impact of variation across platforms and to ensure consistency in the RIs, the first step we undertook in our study was to select a widely used platform based on CLIA that is commonly used in China as per the CLSI EP28-A3C guidelines [21].

In addition, because selecting clinically relevant populations is very important in establishing appropriate intervals [29], another important step for laboratories to establish appropriate RIs is to screen the apparently healthy population. Generally speaking, the reference population can be considered to be free of diseases that influence laboratory test results. To reduce interference resulting from the pre-existing conditions in a seemingly healthy reference

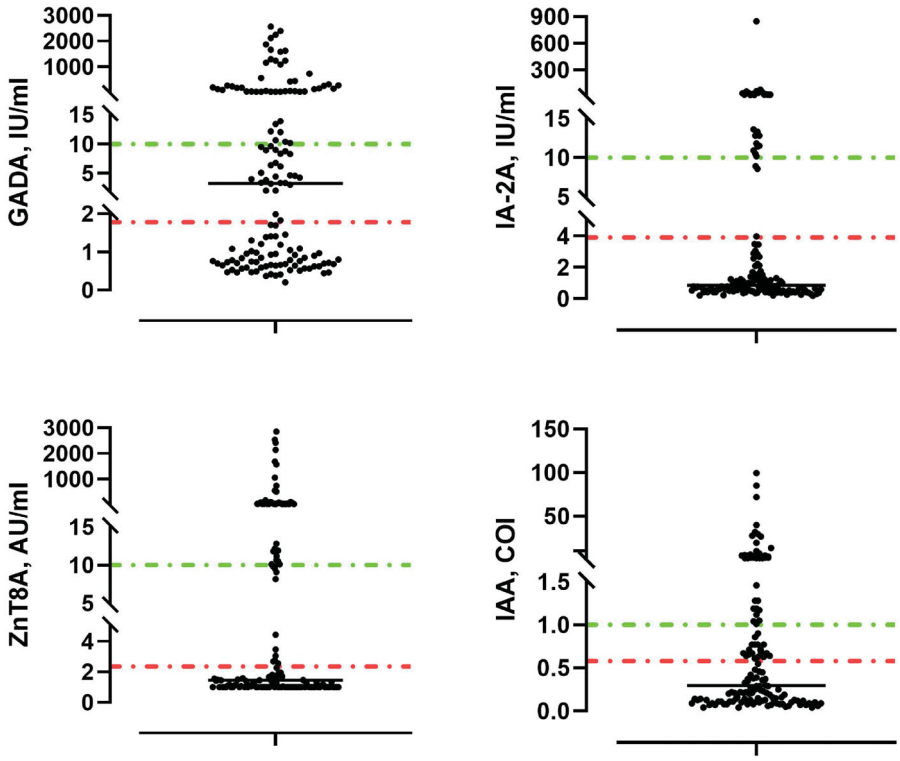

Figure 3. Scatter plot graph of the distribution of four IAbs in 140 T1D patients by CLIA RIsa: Reference intervals established in this study; RIsm: Reference intervals provided by manufacture; GADA: Glutamic acid decarboxylase-65; IA-2A: Insulinoma antigen 2; ZnT8A: Zinc transporter-8; IAA: Insulin autoantibodies. The solid lines represent the median of IAbs levels; the top and bottom dotted lines represent RIsm and RIsa thresholds intersecting IAbs levels.

Table 4. Diagnostic efficiency for the 140 T1D patients using RIs based on CLIA-iFlash 3000 platform.

Analytes	CLIA		RBA Positive	RBA Negative	RBA Total	Sensitivity (%)	Specificity (%)	Coincidence (%)
GADA	RIs^m	Positive	49	1	50	59.0	98.2	75.0
		Negative	34	56	90			
	RIs^a	Positive	71	1	72	85.5***	98.2	90.7***
		Negative	12	56	68			
IA-2A	RIs^m	Positive	15	11	26	38.5	89.1	75.0
		Negative	24	90	114			
	RIs^a	Positive	17	11	28	43.6	89.1	76.4
		Negative	22	90	112			
ZnT8A	RIs^m	Positive	19	24	43	86.4	79.7	80.7
		Negative	3	94	97			
	RIs^a	Positive	21	34	55	95.5	71.2	75.0
		Negative	1	84	85			
IAA	RIs^m	Positive	36	4	40	57.1	94.8	77.8
		Negative	27	73	100			
	RIs^a	Positive	47	11	58	74.6*	85.7	80.7
		Negative	16	66	82			

RBA: radio-binding assay, the current "gold standard" for measuring IAbs; CLIA: chemiluminescence immunoassay; RIs^a: reference intervals established in this study; RIs^m: reference intervals provided by manufacture. *$p < .05$, ***$p < .001$.

population, we first excluded subjects with basic conditions, such as hypertension and autoimmune diseases. Next, we detected IAbs using RBA and excluded subjects who were positive for one or more IAbs. Moreover, because T1D is mainly prevalent in the younger population [20], our cohort consisted of a reference population comprising individuals aged 40 years and younger. After establishing the combined RIs of the IAbs, we found that the RIs for GADA, IA-2A, ZnT8A, and IAA were all lower than the corresponding values of RIs provided by the manufacturer, although both RIs were established based on the CLIA-iFlash 3000 platform and the Chinese Han population. The primary reason may be attributed to the operational variation while using this platform as well as the exclusion criteria considered in the selection of the apparently healthy population.

Many studies have reported age and gender as the two main factors affecting test results [30–33]; therefore, these parameters should be taken into account while establishing age- or gender-specific RIs [29]. No significant differences were found between the male and female reference population enrolled in our study with respect to the four IAbs. Moreover, no correlation between age and IAbs was found,

with the exception of GADA. Thus, we did not further divide the reference population into subgroups to establish age- or gender-specific RIs.

Apart from age and gender, the ethnic background should be also considered as a crucial factor that affects test results [29]. Previous findings suggest that the establishment of racial/ethnic-specific RIs may have a significant clinical and public health implication for a more accurate diagnosis and in the determination of appropriate treatment modalities to improve the quality of patient care [34,35]. Unfortunately, in the case of assays used for the determination of IAbs, the majority of RIs provided by manufacturers are based on limited data obtained from individuals chosen at random from a certain region without specifically taking race into account. Thus, it is the responsibility of individual laboratories or laboratory networks to use RIs that are appropriate for their methodologies and the population they serve.

To evaluate the diagnostic efficiency of the newly established RIs for the four IAbs in our study, we performed a diagnostic test and evaluated 140 clinical samples. Our results indicated varied performances of the four IAbs based on our RIs. For the GADA assay, the sensitivity and the specificity were both higher than 85.0% with a coincidence of 90.7%, indicating the excellent diagnostic efficiency of our established RIs. In the IAA assay, except for the sensitivity which was slightly less than 80%, both the specificity and the coincidence were higher than 80.0%, suggesting an acceptable performance. The specificity of the IA-2A assay was higher than 80.0%, and the sensitivity for ZnT8A was higher than 95.0%, showing excellent diagnostic efficiency; however, when using our established RIs the sensitivity for IA-2A was still less than 50.0%, indicating relatively poor diagnostic efficiency.

Next, we compared the diagnostic efficiency of IAbs using the RIs provided by the manufacturer versus those established in the current study. Our findings suggested that the sensitivity for GADA and IAA, and the coincidence for GADA were significantly improved when using RIs established in this study as opposed to those provided by the manufacturer. In addition, the sensitivity for ZnT8A and the coincidence for IA-2A and IAA showed a slight improvement when the RIs established in this study were used; however, the coincidence for ZnT8A slightly decreased when using the new established RIs. We also found that compared to the sensitivity of IA-2A determined using the RIs provided by the manufacturer, the values determined using the method established in this study was superior. Overall, the diagnostic efficiency for the four IAbs, especially for GADA and IAA, was improved when the RIs established in this study were used.

It may be worth mentioning that after insulin treatment IAA assays cannot be relied upon to detect the autoimmunity of subjects as exogenous insulin drives their production. Our study has some limitations. First, we did not enroll subjects younger than 15 years of age; thus, further studies that include this age group are warranted. Second, the levels of IA-2A and ZnT8A in some subjects were lower than 0.7 IU/mL and 1.0 AU/mL, respectively, the limit that could be detected using the CLIA-iFlash 3000 platform. Therefore, it is important for the manufacturer to improve the limit of detection for IA-2A and ZnT8A. Third, the RIs established in this study were based on the Chinese Han population and the CLIA-iFlash 3000 detection system; thus, the RIs established in our study may not be suitable for use in other laboratories. However, these RIs can be "transferred" to other laboratories after they have been verified [24].

Conclusion

In this study, we have established the RIs for GADA, IA-2A, ZnT8A, and IAA in the healthy Chinese Han population based on the CLIA method. These RIs can improve the diagnostic efficiency, especially for GADA and IAA, compared to the RIs provided by the manufacturer. Therefore, these RIs for IAbs provide a valuable tool to improve the classification of diabetes in the Han Chinese, and are valuable to clinicians for medical diagnosis and determining an optimal therapeutic approach for patients with T1D.

Acknowledgments

The authors thank all the volunteers that participated in this program.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work is supported by the Open Program of NHC Key Laboratory of Nuclear Medicine and Jiangsu Key Laboratory of Molecular Nuclear Medicine (Item number: KF201902). The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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