

Actual condition survey regarding mismatch of measurements between radioimmunoassay and enzyme-linked immunosorbent assay tests for anti-glutamic acid decarboxylase antibody in real-world clinical practice

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Keywords

Anti-glutamic acid decarboxylase antibody, Enzyme-linked immunosorbent assay, Radioimmunoassay

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J Diabetes Investig 2019; 10: 685–689

doi: 10.1111/jdi.12955

ABSTRACT

Anti-glutamic acid decarboxylase antibody (GADA) is an important islet cell-associated autoantibody for the diagnosis of autoimmune type 1 diabetes mellitus. In Japan, the GADA assay kit was recently changed from radioimmunoassay (RIA) to enzyme-linked immunosorbent assay (ELISA). Thereafter, a mismatched measurement between the two tests became apparent in clinical situations. The present study aimed to clarify the actual extent of mismatch between the two measurements on a larger-scale real-world clinical practice. In this cross-sectional non-local/non-hospital-based study, we collected anonymized data on GADA levels of 598 participants, who were simultaneously measured with GADA-RIA and GADA enzyme-linked immunosorbent assay tests. We found that 34% of the GADA-RIA-positive participants showed negative results in the GADA enzyme-linked immunosorbent assay test; the mismatch was predominantly observed in participants with relatively low GADA-RIA levels (<32 U/mL). This considerable mismatch might lead to physicians' confusion in diagnosing type 1 diabetes mellitus.

INTRODUCTION

Type 1 diabetes mellitus, considered to be caused by pancreatic β -cell destruction through islet cell autoimmunity, progresses to an insulin-dependent state in most cases¹. Anti-glutamic acid decarboxylase antibody (GADA) is one of the most important islet cell-associated autoantibodies for the diagnosis of type 1 diabetes mellitus. The presence of GADA in individuals with non-insulin-dependent diabetes strongly suggests a slowly progressive insulin-dependent type 1 diabetes mellitus (SPIDDM)² or latent autoimmune diabetes in adults.

As of December 2015, GADA measurement was shifted from radioimmunoassay (RIA) to enzyme-linked immunosorbent assay (ELISA) in Japan, and the RIA kit is now available only in a few countries. Compared with the RIA, the ELISA kit is

characterized by higher sensitivity and increased specificity for GADA detection (according to the technical information of the GADA autoantibody ELISA Kit; RSR Ltd., Cardiff, UK). However, recent studies showed that 17.4–25.5% of participants with SPIDDM, who were positive for GADA by the RIA (GADA-RIA) test (RSR Ltd.) showed a negative GADA result in the ELISA (GADA-ELISA) test (RSR Ltd.), particularly those with GADA-RIA levels of ≤ 20 U/mL^{3–5}. However, these findings were mainly obtained from hospital-based studies using a relatively low number of participants.

Usually, GADA tests are outsourced to several clinical laboratories throughout the various medical institutions in Japan. Because physicians include not only diabetes mellitus experts, but also many general physicians not specialized in diabetes mellitus, the purpose and/or timing of GADA measurements might vary among physicians in clinical practice. Furthermore,

Received 4 June 2018; revised 11 September 2018; accepted 7 October 2018

they encounter many patients with different clinical characteristics of diabetes mellitus. In addition, an interlaboratory coefficient of variation (CV) of GADA measurements existed among clinical laboratories. These various clinical situations might confound the results of GADA measurements in real-world clinical practice. Therefore, this survey aimed to clarify the actual extent of mismatch of measurements between these two GADA tests in real-world clinical practice by investigating a large number of participants through the non-local/non-hospital-based study.

METHODS

In the present cross-sectional observational study, we randomly and blindly selected 598 participants for whom the GADA-RIA and/or GADA-ELISA measurements were outsourced to the following five clinical laboratories between December 2015 and March 2016 throughout Japan: BML Inc. ($n = 135$), Health Sciences Research Institute Inc. ($n = 42$), FALCO Biosystems Ltd. ($n = 96$), LSI Medience Corporation ($n = 161$) and SRL Inc. ($n = 164$). The participants from each laboratory were randomly and blindly selected without prespecified selection criteria, including the number of participants in each laboratory. We then collected anonymized data on GADA levels, which were simultaneously measured by both RIA and ELISA tests using the remaining sera, and the concordance rate of positivity and negativity of the two tests was determined. Clinical information, including the presence or absence of a diabetes mellitus diagnosis or sex, was not obtained, indicating non-biased sampling. We confirmed that all samples did not overlap in each clinical laboratory.

According to the manufacturer's (RSR Ltd.) datasheets, the measurement intervals of GADA-RIA and GADA-ELISA are 1.3–156 U/mL (1 U/mL is equivalent to 25 U/mL in the National Institute for Biological Standards and Control (NIBSC) 97/550 (based on the manufacturer's datasheet of GADA-RIA kits; RSR Ltd.) and 5.0–2,000 U/mL (units are NIBSC 97/550), respectively. Thus, the GADA-ELISA value can be approximated by multiplying the GADA-RIA value by 25. The cut-off values of GADA-RIA and GADA-ELISA tests are 1.5 and 5.0 U/mL, determined based on Japanese⁶ and Caucasian control samples⁷, respectively. As with usual clinical practice, all GADA measurements were carried out using a single assay. Based on the manufacturer's instructions, the intra- and interassay CV for GADA-RIA are 3.6–3.7% and 5.5–6.9%, respectively, and those for GADA-ELISA are 3.5–8.5% and 5.2–6.4%, respectively, suggesting that a single assay possibly might not affect the analysis.

Ethics Statement

The present study protocol was in accordance with the Declaration of Helsinki and approved by the institutional review board of Saitama Medical University Hospital (Approval No. 858), which did not require informed consent because data on GADA levels were anonymously obtained.

RESULTS

The positivity and negativity concordance rates of the two tests were 52.7 (315/598) and 18.4% (110/598), respectively. Overall, 11 of the 598 participants (1.84%) showed negative results in the GADA-RIA (<1.5 U/mL) and positive results in the GADA-ELISA test (5.0–23.3 U/mL; open triangles in Figure 1). Given that all participants showed GADA-ELISA levels of <32.5 U/mL, equivalent to the lower GADA-RIA limit of 1.3 U/mL (=32.5/25), the discrepancy between the two measurements might reflect higher sensitivity of ELISA for GADA detection.

Meanwhile, a total of 162 participants (27.1%) showed positive results in the GADA-RIA (1.5–31.1 U/mL) and negative in the GADA-ELISA test (<5.0 U/mL) (open circles in Figure 1), which accounted for 34.0% of the GADA-RIA-positive participants ($n = 477$). This considerable mismatch rate is higher than those previously reported (17.4–25.5%)^{3–5}. Similar to previous reports^{3,5}, the mismatch was mainly observed in participants with relatively low GADA-RIA levels (1.5–32 U/mL). When considering lower GADA-RIA levels alone (1.5–32 U/mL), GADA-ELISA-negative participants accounted for 44.8% (162/362) of the GADA-RIA-positive participants. The ratio of GADA-RIA-positive and GADA-ELISA-negative participants ($n = 162$) to GADA-RIA-negative and GADA-ELISA-positive participants ($n = 11$) was 14.7 (=162/11).

A total of 39 (8.2%) of 477 GADA-RIA-positive participants and 56 (17.2%) of 326 GADA-ELISA-positive participants exceeded the upper limits of GADA-RIA (156 U/mL) and GADA-ELISA (2,000 U/mL) measurements, respectively. The frequency of participants exceeding the upper limit of the GADA-RIA test was significantly lower than that exceeding the upper limit of the GADA-ELISA test ($P < 0.01$ by χ^2 -test). This finding can be explained by the fact that the upper limit of the GADA-RIA value is equivalent to approximately 3,900 U/mL (156×25 U/mL) in NIBSC 97/550, which is higher than the upper limit of the GADA-ELISA test (2,000 U/mL in NIBSC 97/550).

DISCUSSION

The present study showed a considerable mismatch of measurements between GADA-RIA and GADA-ELISA, despite several limitations of the design, including a lack of clinical information on diabetes. According to previous studies^{3–5}, the majority of participants with diabetes showing positive GADA-RIA and negative GADA-ELISA results were those with SPIDDM. Thus, also in the present study, the majority of participants with this type of mismatch were considered to be individuals with SPIDDM, which warrants further investigation.

Previous observational studies regarding SPIDDM showed that diabetes in a non-insulin-dependent state with GADA-RIA values ≥ 10.0 U/mL is a higher risk for progression to an insulin-dependent state, as compared with cases with GADA-RIA values <10.0 U/mL^{8,9}. Furthermore, in a Tokyo study,

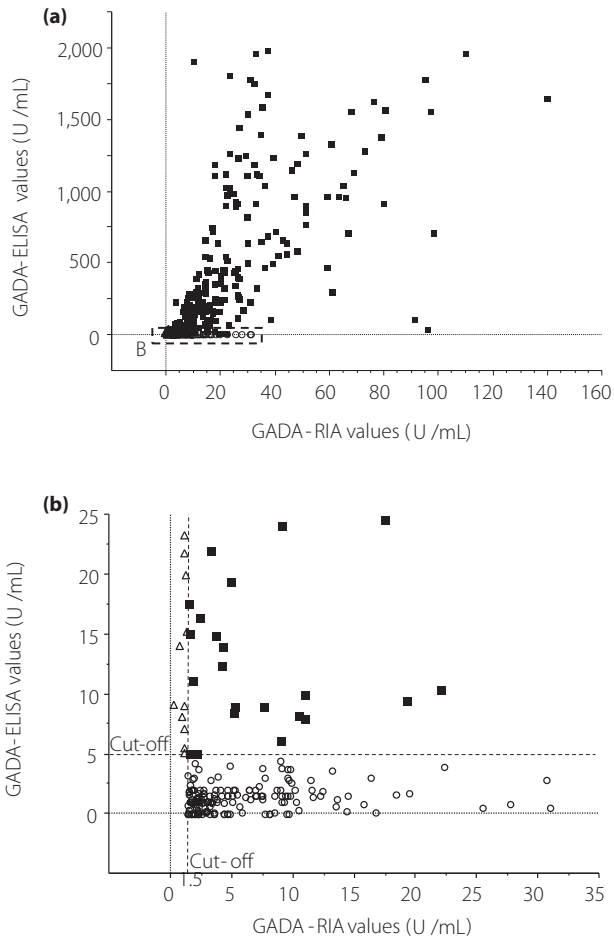


Figure 1 | Correlation between anti-glutamic acid decarboxylase antibody (GADA) levels obtained from GADA measurement by radioimmunoassay (RIA) and GADA measurement by enzyme-linked immunosorbent assay (ELISA) tests. (a) Dot-plots show a correlation between the two GADA levels of the two tests below the upper limits of GADA-RIA (156 U/mL) and GADA-ELISA (2,000 U/mL). (b) Enlarged figure of the dashed line square shown in (a). Dot-plots show a correlation between the two GADA levels of the two tests within the GADA-RIA level of <35 U/mL and GADA-ELISA level of <25 U/mL. Participants who showed negative results in both the GADA-RIA and GADA-ELISA tests are not shown. Filled square, GADA-RIA-positive and GADA-ELISA-positive participants; open circle, GADA-RIA-positive and GADA-ELISA-negative participants; open triangle, GADA-RIA-negative and GADA-ELISA-positive participants.

providing insulin therapy for non-insulin-dependent SPIDDM patients with GADA-RIA values ≥ 10.0 U/mL was recommended to prevent or delay progression to an insulin-dependent state¹⁰. Thus, it was important to clarify the relationship between a GADA-RIA value of 10.0 U/mL and GADA-ELISA positivity/negativity. An additional analysis showed that 23 (16.3%) of 141 participants with GADA-RIA values of 10.0–

32.0 U/mL showed negative results for the GADA-ELISA test. This suggests that a single application of the GADA-ELISA test might overlook some GADA-RIA-positive SPIDDM patients with GADA values ≥ 10.0 U/mL, who should be treated with insulin therapy in terms of evidence derived from the Tokyo study¹⁰. This is a clinically important issue.

Although the exact causes of mismatch remain unclear, the discrepant results between the two GADA tests might be due to differences in the assay methods; that is, solid phase (ELISA) versus liquid phase (RIA)^{7,11}. Another possible cause is the fact that GADA-RIA requires a single wash step, which might lead to a non-specific response and false positive result, particularly for the lower GADA range, in contrast to the three wash steps in GADA-ELISA^{7,11}.

Furthermore, the mismatch might be partly attributed to different GADA epitopes used in the two kits: a truncated recombinant protein lacking amino acids 2–45 in the N-terminal region in RIA and a full-length recombinant protein in the ELISA^{7,11}. Thus, the mismatch could occur based on a difference in epitope reactivity, as GADA might show distinct epitope recognition between individuals with acute-onset type 1 diabetes mellitus and SPIDDM¹². Further studies on epitope mapping of GADA based on the onset pattern of type 1 diabetes mellitus would resolve these issues.

Given that the sensitivity and specificity are higher in the ELISA than in the RIA assay, the GADA-RIA-positive and GADA-ELISA-negative results might represent a false positive result in the GADA-RIA test. However, according to our previous studies, most individuals with type 1 diabetes mellitus showing this type of mismatch are usually those with SPIDDM, not classic (acute-onset) type 1 diabetes mellitus^{3–5}. Thus, the mismatch cannot be explained by false positive results of GADA-RIA values alone. Recently, GADA-ELISA has been proposed to usually detect high-affinity over low-affinity GADA^{4,13}. Considering that high-affinity GADA is associated with type 1 diabetes mellitus progression^{14,15}, individuals with SPIDDM with high disease activity might harbor high-affinity GADA, leading to positive results in both tests, whereas those with low disease activity might have low-affinity GADA, and show positive and negative results in the GADA-RIA and GADA-ELISA tests, respectively. To confirm these speculations, further studies to reveal the relationship between GADA affinity and mismatch of measurements between GADA-RIA and GADA-ELISA are required.

Some researchers are concerned that the cut-off value of GADA-ELISA (5.0 U/mL) might be high, particularly in the Japanese population, and could lead to a false negative result. Therefore, the original cut-off value of GADA-ELISA should be confirmed using Japanese control samples. For reference, the cut-off value of GADA-ELISA test was determined based on the 99th percentile of Caucasian control samples⁷. However, the definition of control was not clearly defined⁷. According to the datasheet of the GADA-ELISA kit, a small portion of non-diabetic individuals with Graves' disease, Hashimoto's

thyroiditis or systemic lupus erythematosus show positive results in the GADA-ELISA test. Therefore, there might be a need to re-evaluate the cut-off value of the GADA-ELISA test using true and clearly defined control samples.

Participants with values that deviated widely from the average ratio of the two GADA values (i.e., outliers) might provide a useful clue to the mismatched results between the two GADA tests, and clinical information on the participants is absolutely essential to clarify the mechanism underlying the mismatch. Further studies focusing on such outliers are required.

Next, to clarify the factor(s) affecting the mismatch, we additionally analyzed the concordance of positivity and negativity of the two GADA tests according to the clinical laboratories. As a result, there was a statistically significant difference in the concordance among the five laboratories ($P < 0.01$ by χ^2 -test; Table S1). In particular, the frequency in participants with GADA-RIA-positive/GADA-ELISA-negative results was lower in the BML laboratory than the others. This might be partially attributed to the finding that the GADA-RIA values in the BML laboratory were distributed within a relatively high range (Figure S1). These findings suggest that a laboratory bias affecting the degree of the mismatch does exist and could be an important limiting factor in this study.

Finally, we did not investigate the interlaboratory CV of GADA measurements among the five clinical laboratories, which might lead to some of the measurement mismatch between GADA-RIA and GADA-ELISA. Meanwhile, the present study aimed to clarify the actual mismatch in real-world clinical practice, where many factors might confound the results of GADA measurements; for example, difference in the clinical characteristics of patients with diabetes mellitus; difference in the purpose and/or timing of GADA measurements among physicians; difference in the type of medical institution, such as university hospitals and medical clinics; and the possible existence of interlaboratory CV of GADA measurements. We believe that the present study showed the actual mismatch condition between the two GADA measurements.

In conclusion, the present cross-sectional non-local/non-hospital-based study showed that considerable mismatched measurements between GADA-RIA and GADA-ELISA were observed in the real-world clinical practice, suggesting that the mismatch might affect a precise diagnosis of type 1 diabetes mellitus, and could cause physicians' confusion on diagnosis. Therefore, the cause for the mismatch should be promptly identified.

ACKNOWLEDGMENTS

The authors thank Nobuyuki Shihara (Japan Association for Diabetes Education and Care) for the clerical support. No specific funding or grant was received for this work.

DISCLOSURE

YS received consultation and/or speaker fees from Eli Lilly Japan K.K.; Sanofi K.K.; Novo Nordisk Pharma Inc.;

GlaxoSmithKline K.K.; Taisho Pharmaceutical Co. Ltd.; Taisho Toyama Pharmaceutical Co. Ltd.; Astellas Pharma Inc.; Becton, Dickinson and Company; Nippon Boehringer Ingelheim Co. Ltd.; Johnson & Johnson; and Takeda Pharmaceutical Company Limited. YS received clinical commissions/joint research grants from Taisho Toyama Pharmaceutical Co. Ltd., Nippon Boehringer Ingelheim Co. Ltd., Eli Lilly Japan K.K. and MSD K.K. AS has received lecture fees from Novo Nordisk Pharma Inc., Sanofi K.K. and Eli Lilly Japan K.K. YO, TK and MK declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Number of participants according to positive and negative results of anti-glutamic acid decarboxylase antibody tests obtained from measurement by radioimmunoassay and measurement by enzyme-linked immunosorbent assay in each clinical laboratory.

Figure S1 | Distribution of anti-glutamic acid decarboxylase antibody (GADA) levels obtained from measurement by radioimmunoassay (GADA-RIA) test according to clinical laboratories (box-and-whisker plot; GADA-RIA range of 1.5–32.0 U/mL).