

IgG4-IgE complex interferes with measurement of IgE concentration

Keiichi Nakano^{a,b,*}, Junichi Sugita^a, Masanori Seimiya^b, Keiko Yasuda^a, Chiaki Watanabe^a, Hideki Goto^a, Takanori Teshima^a

^a Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan

^b Department of Medical Technology and Sciences, School of Health Sciences at Narita, International University of Health and Welfare, Chiba, Japan

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ABSTRACT

Background and aims: Patients with immunoglobulin G4 (IgG4)-related disease (IgG4-RD) have elevated immunoglobulin E (IgE) concentration compared to that in healthy individuals, which suggests the occurrence of IgE-mediated allergic reactions. We have previously shown that IgG4 and IgE form a complex in some patients with IgG4-RD. However, it is currently unknown whether and how the presence of the IgG4-IgE complex affects IgE concentration measurements by different assays.

Materials and methods: Twenty patients with confirmed presence or absence of IgG4-IgE complex were evaluated. We compared IgE concentrations measured by ST AIA-PACK IgE II (AIA-PACK), Elecsys IgE II Immunoassay (Elecsys), and Iatroace IgE (Iatroace) and evaluated to what extent the IgG4-IgE complex interfered with these measurements.

Results: In patients with the IgG4-IgE complex, IgE concentrations measured using Iatroace were significantly lower than those measured using Elecsys and tended to be lower than those measured using AIA-PACK. IgE concentrations determined by Iatroace were significantly different in patients with and without the IgG4-IgE complex, whereas no significant differences between these groups were detected when IgE concentrations were measured by AIA-PACK or Elecsys.

Conclusion: The formation of the IgG4-IgE complex underestimates measured IgE concentrations depending on the method used. Therefore, caution should be exercised when selecting a specific IgE assay for patients with IgG4-RD.

1. Introduction

Patients with immunoglobulin G4 (IgG4)-related disease (IgG4-RD) have elevated immunoglobulin E (IgE) concentrations compared to those in healthy individuals, which suggests that these patients present with IgE-mediated allergic reactions [1]. A prior study reported that the pathogenesis of IgG4-RD itself contributes to elevated IgE concentrations, even in the absence of atopy [2]. Therefore, IgE has been proposed to play a major role in IgG4-RD and be a useful marker of the therapeutic response and recurrence [1,3]. However, the clinical implications of elevated IgE concentrations in patients with IgG4-RD remain poorly understood.

Using immunoadsorption techniques, we have previously found that IgG4 and IgE form a complex in patients with IgG4-RD [4,5]. As the formation of the IgG4-IgE complex is a recent discovery, its clinical

significance has not yet been elucidated. Various assays have been developed to determine IgE concentrations. Because they are based on different principles (e.g., enzyme immunoassay, turbidimetric immunoassay, and latex agglutination immunoassay) and use different components (e.g., antibodies, substrates), they can yield discrepant results. We previously evaluated a patient with the IgG4-IgE complex and found discordant IgE concentrations determined by in two assays [4]. However, it is currently unknown whether and how the presence of the IgG4-IgE complex affects the accuracy of IgE concentration measurements by different assays.

In this study, we compared the performance of three assays measuring IgE concentration and evaluated to what extent the presence of IgG4-IgE complex interfered with these measurements.

Abbreviations: IgE, immunoglobulin E; IgG4, immunoglobulin G4; IgG4-RD, IgG4-related disease.

* Corresponding author at: Kita-12 Nishi-5, Kita-ku, Sapporo 060-8648, Japan.

E-mail address: nkeiichi@frontier.hokudai.ac.jp (K. Nakano).

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2. Materials and methods

2.1. Patients

The present study was conducted in accordance with the guidelines of the 1975 Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Hokkaido University Hospital (016-0351). All patients provided written informed consent. The presence or absence of the IgG4-IgE complex in the 20 included patients was verified in a previously published study [5]. Seventeen patients had IgG4-RD, whereas three had other diseases (those of final diagnoses were interstitial pneumonia, bronchial asthma, and food allergy). IgG and IgG4 concentrations were measured by the immunoturbidimetric assay using N-Assay TIA IgG-SH Nittobo (Nittobo, Tokyo, Japan) and the nephelometric assays using N Latex IgG4 (Siemens Healthcare Diagnostics, Eschborn, Germany). Clinical information and laboratory data of the subjects were obtained from the medical records.

2.2. IgE concentration measurement

The following methods were used to measure IgE concentrations: ST AIA-PACK IgE II (AIA-PACK, TOSOH, Tokyo, Japan), based on the immunoassay using an AIA 1800 analyzer (TOSOH, Tokyo, Japan); Elecsys IgE II Immunoassay (Elecsys, Roche Diagnostics GmbH, Mannheim, Germany), based on the electrochemiluminescence immunoassay using a Cobas e801 analyzer (Roche Diagnostics, Basel, Switzerland); and Iatroace IgE (Iatroace, LSI Medicine, Tokyo, Japan), based on the latex immunoturbidimetry assay using a BM8030 analyzer (JEOL, Tokyo, Japan). The analytical measurement ranges were 3.0–4000.0 IU/mL, 0.2–2500.0 IU/mL, and 10.0–1700.0 IU/mL for AIA-PACK, Elecsys, and Iatroace, respectively, with the lowest range value representing the limit of quantitation. The three IgE measurements required approximately 30 min and were performed in the same order for all patients. All analyses were performed according to the manufacturers' instructions.

2.3. Immunoabsorption of IgG4 with an anti-IgG4 antibody conjugated affinity matrix

Immunoabsorption of IgG4 was performed to detect the IgG4-IgE complex using the CaptureSelect™ IgG4 (Hu) Affinity Matrix (Thermo Fisher Scientific, MA, USA), as described previously [4,5]. Briefly, 40 µL of serum were diluted to 1:9 with 100 mM sodium phosphate buffer (pH 7.4) and mixed with 100 µL of the affinity matrix. After overnight incubation, the matrices were washed with sodium phosphate buffer and eluted with 1 M glycine-HCl (pH 3.0). The IgE concentrations in the flow-through and bound fractions were measured by AIA-PACK.

The ratio of IgE bound to IgG4 to free IgE was calculated using the following formula:

$$\text{Ratio of IgG4-bound IgE to free IgE} =$$

$$\frac{\text{IgE concentration in bound fraction}}{\text{IgE concentration in flow-through fraction}}$$

Patients with an IgE ratio >1.0 were considered IgG4-IgE complex-positive and those with an IgE ratio ≤ 1.0 were considered IgG4-IgE complex-negative.

2.4. Statistical analyses

The Wilcoxon signed-rank test and Bonferroni *post hoc* correction were performed to assess differences in IgE concentrations detected by the three assays, as well as the distributions of IgE concentrations in the groups with and without the IgG4-IgE complex. Spearman correlation analysis was performed to evaluate the correlations between the ratio of IgG4-bound to free IgE (IgE ratio) and the ratio of IgE concentrations determined by individual assays (assay ratio). To obtain the latter ratios, the concentration measured by AIA-PACK was divided by that measured

by Iatroace, the concentration measured by Elecsys was divided by that measured by AIA-PACK, and the concentration measured by Elecsys was divided by that measured by Iatroace. All data were analyzed using JMP Version 16.0.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the study subjects

The baseline characteristics of the 20 patients are presented in Table 1, among which 5 were IgG4-IgE complex-positive and 15 were IgG4-IgE complex-negative. The groups that were positive for the IgG4-IgE complex comprised confirmed patients with IgG4-RD, whereas those that were negative for the IgG4-IgE complex comprised confirmed patients with IgG4-RD and suspected cases. The median age, IgG concentration, and IgG4 concentration across all patients were 69 years, 14.40 g/L and 2.10 g/L, respectively.

3.2. Comparison of IgE concentrations determined by the three assays

IgE concentrations determined by each of the three assays (AIA-PACK, Elecsys, and Iatroace) were plotted and compared. As shown in Fig. 1, there was a significant difference in IgE concentrations determined by Elecsys and Iatroace in the group with the IgG4-IgE complex. However, no significant differences in the concentrations determined by the three assays were observed in the IgG4-IgE complex-negative group or the total patient cohort.

3.3. Distribution of IgE concentrations in patients with and without the IgG4-IgE complex

As shown in Table 2, the median IgE concentration in the IgG4-IgE complex-positive and -negative groups, as measured using AIA-PACK, Elecsys, and Iatroace, were 226.1 IU/mL and 171.9 IU/mL, 556.6 IU/mL and 271.1 IU/mL, and 60.9 IU/mL and 177.4 IU/mL, respectively. Thus, IgE concentrations determined by Iatroace were significantly different between the two groups of patients, whereas no significant differences were detected when AIA-PACK or Elecsys were used (Fig. 2).

3.4. Effect of the IgG4-IgE complex presence on measured IgE concentrations

The ratio of IgG4-bound IgE to free IgE determined by immunoabsorption and the assay ratio of IgE concentrations in each method were plotted to assess whether the presence of the IgG4-IgE complex interferes with the accuracy of IgE concentration measurements. As shown in Fig. 3, significant correlations between the IgE ratio and AIA-PACK to Iatroace assay ratio ($r = 0.832$, $P < 0.001$), Elecsys to Iatroace assay ratio ($r = 0.862$, $P < 0.001$), and Elecsys to AIA-PACK assay ratio ($r = 0.810$, $P < 0.001$) were found. These correlations imply that the higher the amount of IgG4-bound IgE, the larger the discrepancy in the measurements afforded by an individual method.

Table 1
Patient characteristics.

	IgG4-IgE complex-positive	IgG4-IgE complex-negative	Total
N	5	15	20
Age, years	71 (70–80)	67 (63–74)	69 (63–75)
Sex, male/female	3/2	8/7	11/9
IgG, g/L	16.16 (14.82–21.18)	13.74 (11.45–15.19)	14.40 (11.87–16.15)
IgG4, g/L	5.15 (1.99–6.75)	1.81 (0.94–3.18)	2.10 (1.00–3.66)

Values are presented as the absolute number or median (interquartile range).

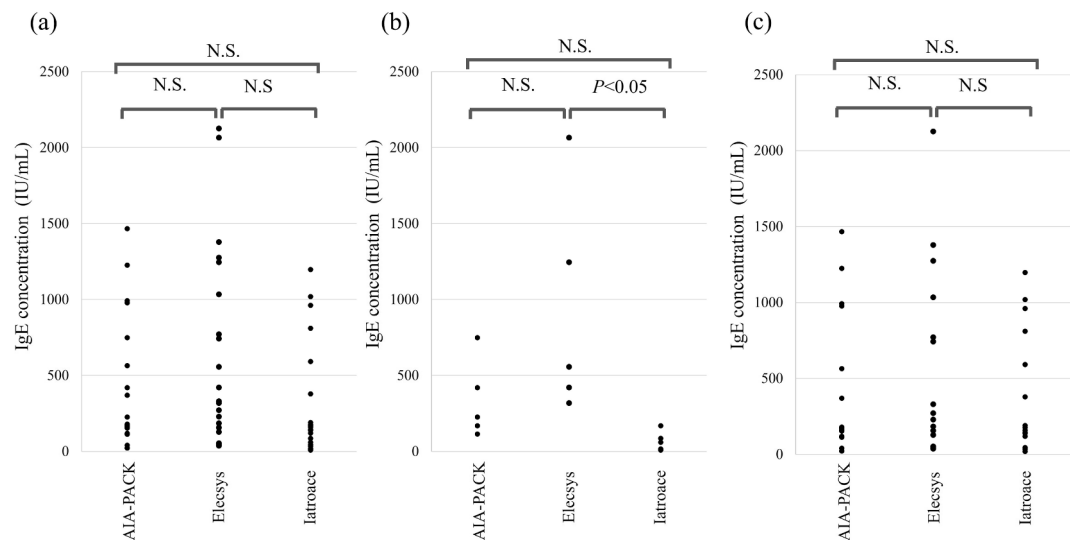


Fig. 1. Comparison of IgE concentrations measured using AIA-PACK, Elecsys IgE II, and Iatroace IgE assays in (a) all patients, (b) IgG4-IgE complex-positive patients, and (c) IgG4-IgE complex-negative patients. AIA-PACK, ST AIA-PACK IgE II; Elecsys, Elecsys IgE II; Iatroace, Iatroace IgE; N.S., not significant;

Table 2
IgE concentrations determined by different methods in patients with and without the IgG4-IgE complex.

	IgG4-IgE complex positive	IgG4-IgE complex negative	Total	P
AIA-PACK	226.1 (168.8–420.4)	171.9 (116.8–771.7)	175.9 (118.5–611.1)	0.76
Elecsys	556.6 (420.2–1245.0)	271.1 (142.0–902.7)	375.5 (177.5–1086.8)	0.21
Iatroace	60.9 (16.9–86.4)	177.4 (129.7–701.7)	152.2 (57.1–432.3)	0.03

Values are represent the median IgE concentration (interquartile range). *P*-value indicates the significance of differences in IgE concentrations between IgG4-IgE complex-positive and IgG4-IgE complex-negative groups.
AIA-PACK, ST AIA-PACK IgE II; Elecsys, Elecsys IgE II; Iatroace, Iatroace IgE.

3.5. Correlation between IgE concentrations determined by different assays

Plots of correlation between outputs of different assays were overlaid with values for the IgG4-IgE complex-positive and -negative groups (Fig. 4). Regression curves that differed between IgG4-IgE complex-positive and -negative groups were identified. The slopes of the linear regression lines between AIA-PACK (x-axis) and Iatroace (y-axis), Elecsys (x-axis) and AIA-PACK (y-axis), and Elecsys (x-axis) and Iatroace (y-axis) in the groups with and without the IgG4-IgE complex were 0.228 vs 1.187, 0.351 vs 0.759, and 0.078 vs 1.497, respectively. Modified Bland–Altman plots of the agreement between the assays were overlaid for the IgG4-IgE complex-positive and -negative groups (Fig. 4). No proportional and fixed errors were identified, but differences in the distribution of values were observed, depending on the presence of the IgG4-IgE complex.

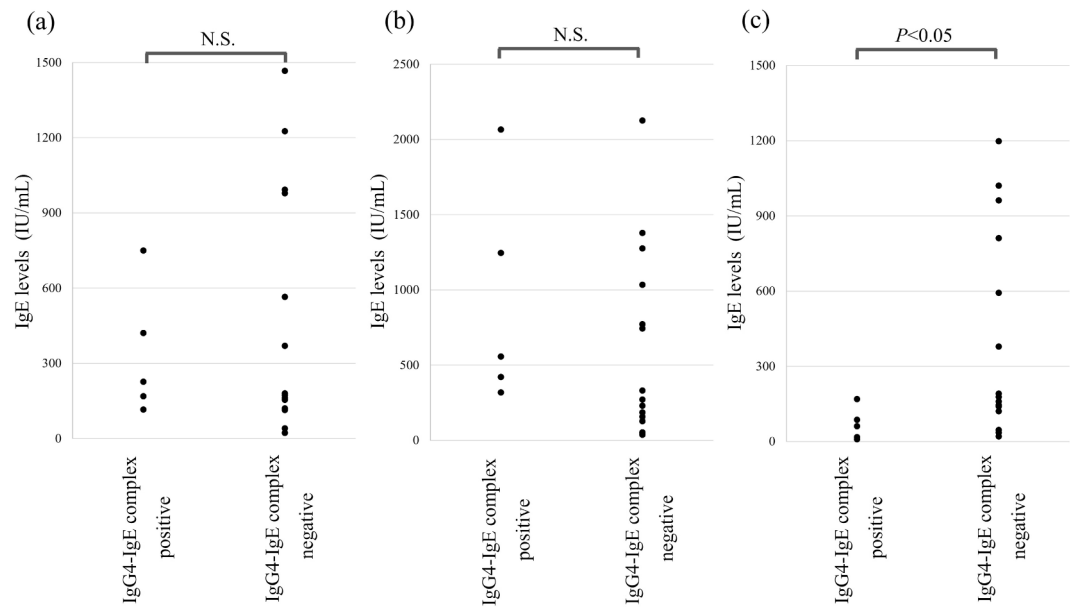


Fig. 2. Distribution of IgE concentrations in IgG4-IgE complex-positive and IgG4-IgE complex-negative patients. IgE concentrations were measured using (a) AIA-PACK, (b) Elecsys IgE II, and (c) Iatroace IgE. AIA-PACK, ST AIA-PACK IgE II; Elecsys, Elecsys IgE II; Iatroace, Iatroace IgE; N.S., not significant.

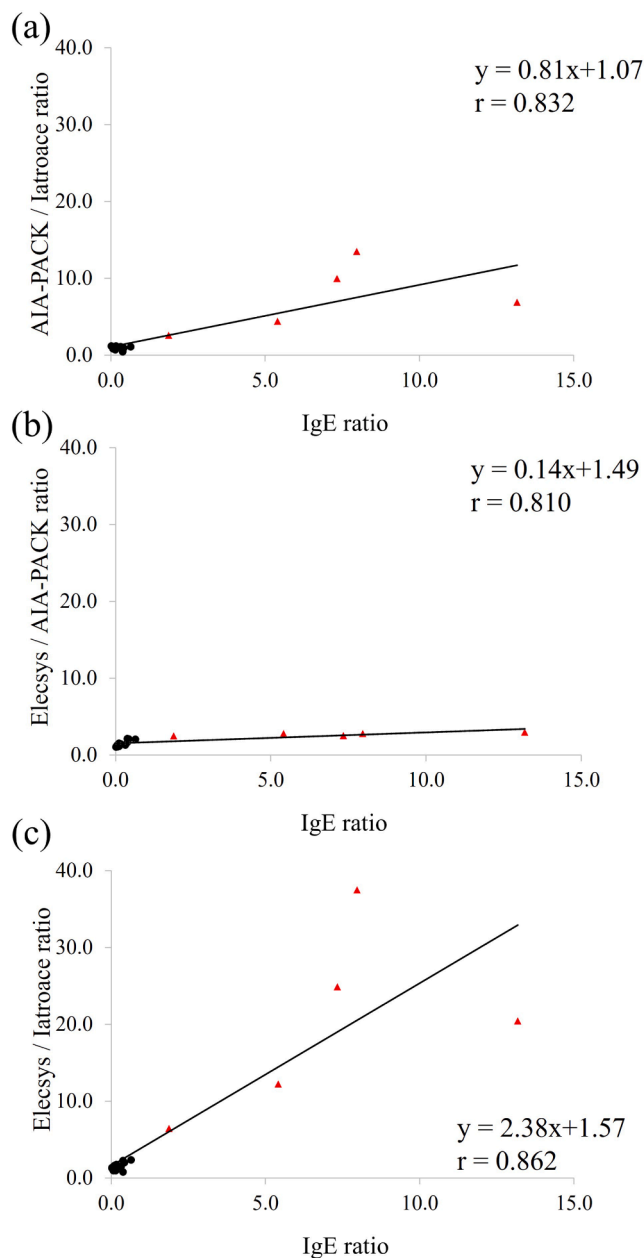


Fig. 3. Two-dimensional plots of the relationships between ratios of IgE concentrations determined by (a) AIA-PACK and Iatroace, (b) Elecsys and AIA-PACK, and (c) Elecsys and Iatroace (y-axis) and the ratio of IgG4-bound IgE to free IgE (x-axis). Values determined in patients with and without the IgG4-IgE complex are illustrated by red triangles and black circles, respectively. AIA-PACK, ST AIA-PACK IgE II; Elecsys, Elecsys IgE II; Iatroace, Iatroace IgE. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Our study found a discrepancy in IgE concentrations determined by different assays in patients that was dependent on the presence of the IgG4-IgE complex. Among patients with the IgG4-IgE complex, IgE concentrations measured using Iatroace were significantly lower than those measured using Elecsys and tended to be lower than those measured using AIA-PACK. In contrast, in patients without the IgG4-IgE complex, IgE concentrations measured using Iatroace did not differ from those determined by other assays (Fig. 1). In addition, IgE concentrations measured using Iatroace in IgG4-IgE complex-positive and

-negative groups were significantly different (Fig. 2). These results indicated that the IgG4-IgE complex may interfere with the measurement of IgE concentration using Iatroace, although our evaluation of the three assays cannot predict how the performance of other existing IgE detection assays may be affected.

IgE concentrations measured using Iatroace were significantly lower in the IgG4-IgE complex-positive group compared to those in the IgG4-IgE complex-negative group. In contrast, significant inter-group differences were not observed when IgE concentrations were measured using AIA-PACK or Elecsys (Fig. 2). These results demonstrate that the IgG4-IgE complex interferes with the method of IgE concentration measurement used in Iatroace. Positive interference caused by autoantibodies [6,7] and heterophilic antibodies [8–10] has been reported for immunoassay measurements. Other studies have also reported that autoantibodies can cause negative interference [11,12]. A similar effect has been observed with rheumatoid factor [13]. The results of the present study suggest that the IgG4-IgE complex negatively influences the ability of at least one method to detect IgE concentration accurately. IgE concentrations were measured in each assay using a specific monoclonal antibody against IgE. It is possible that the IgG4-IgE complex may mask some IgE epitopes, so the specific antibody used in the Iatroace assay may not adequately detect it.

The IgE ratio and assay ratio were plotted to verify whether the lower IgE concentrations measured using Iatroace, compared to those determined by AIA-PACK and Elecsys, were caused by the IgG4-IgE complex. As shown in Fig. 3, the IgE ratio was significantly positively correlated with the assay ratio. Moreover, a higher IgE ratio indicates a greater fraction of IgG4-bound IgE (i.e., more IgG4-IgE complex), and the higher y-axis ratio indicates a greater difference between assays. Thus, these results suggested that the higher the IgE ratio, the larger the discrepancy between assays. In other words, these results show that the presence of the IgG4-IgE complex correlates with the discrepancy in IgE concentrations determined by different assays. Although this relationship implies an interference, it does not prove it, as none of these three assays can be considered the “gold standard” that is free from interference.

Several methods for measuring IgE concentration have been developed, including immunoadsorption [14], enzyme immunoassays [15], chemiluminescent-enzyme immunoassay [16], and Quantum-Dot-Based Immunochromatographic Assay [17]. As shown in Fig. 4, there were significant correlations of the measured IgE concentrations between the three assays used by us. However, regression lines for the IgG4-IgE complex-positive and -negative groups had different slopes. Modified Bland–Altman plots demonstrated different distributions of IgE concentrations in patients with and without the IgG4-IgE complex (Fig. 4). The IgG4-IgE complex positive group exceeded the agreement limits calculated for the negative group. These results indicated the presence of considerable differences between the IgE measurements. In other words, these results reflect interference of IgE measurements due to the IgG4-IgE complex. From this evaluation of the three assays, it is not clear how the other assays may be affected.

Most patients with IgG4-RD show elevated IgE concentration independently of atopic symptoms [2]. Notably, although no association was found between the IgG4-IgE and IgG4 or IgE concentrations, the IgG4-IgE complex was found in patients with IgG4-RD [5]. Whether the IgG4-IgE complex has a biological function or is associated with the pathogenesis of IgG4-RD is unclear. Thus, it remains unknown whether IgE bound to IgG4 should be measured.

Delayed clearance from blood of several autoantibody-bound proteins, such as macro-prolactin, macro-prolactin [7], macro-AST [18], and macro-troponin [19], has been reported. Elevated concentrations of such proteins often should be interpreted with caution, because they do not necessarily reflect the true pathophysiology of the patient. To date, there have been only few reports on the IgG4-IgE complex, and none described the parameters of its clearance. It is possible that the IgG4-IgE complex has a different, likely slower clearance rate than free IgE, so diagnostic results in patients with IgG4-RD require more careful

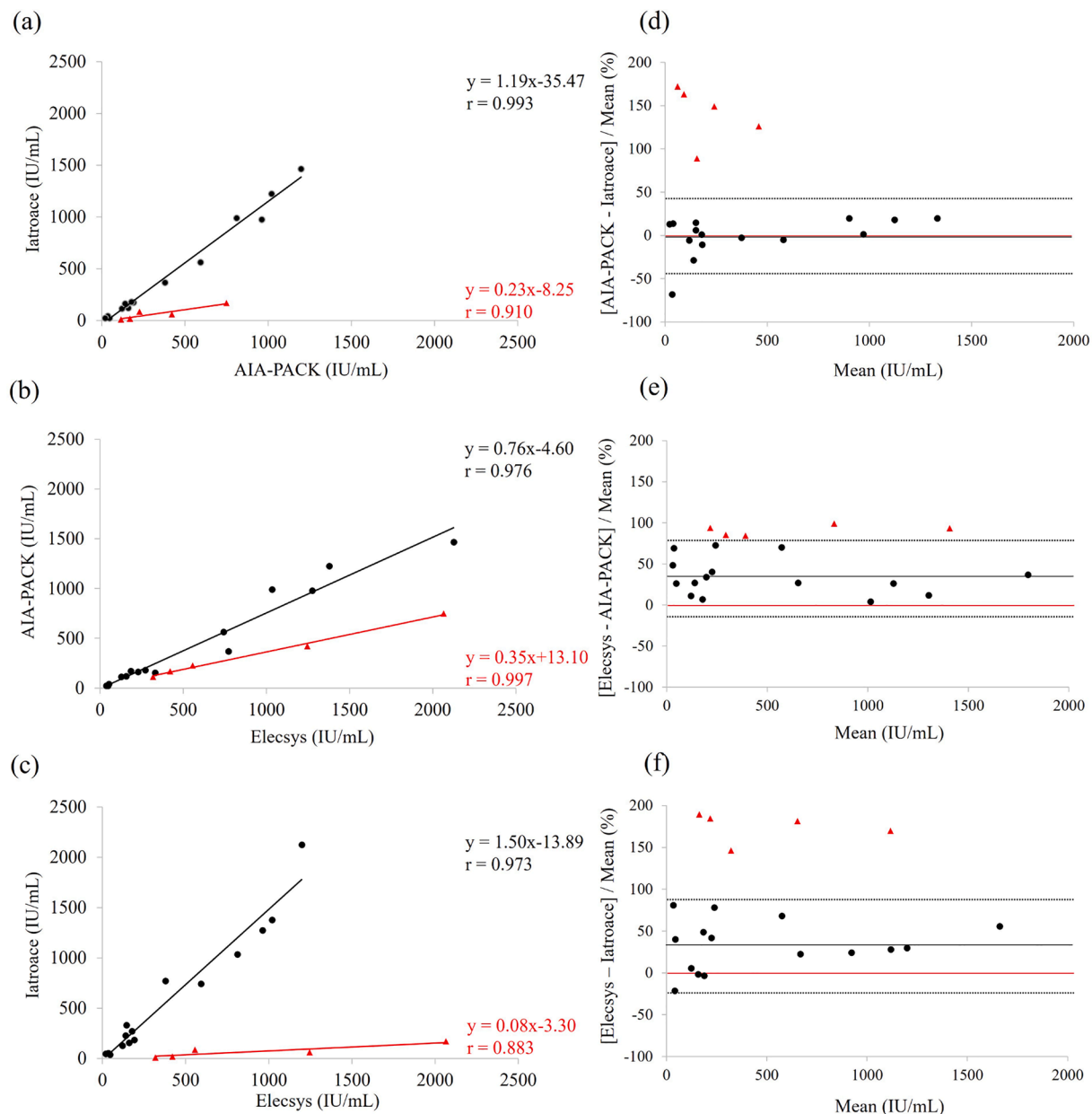


Fig. 4. Two-dimensional plots and modified Bland–Altman plots of IgE concentrations determined by the following pairs of methods: (a, d) AIA-PACK and Iatroace, (b, e) Elecsys and AIA-PACK, and (c, f) Elecsys and Iatroace. (d–f) The solid red line indicates no bias, the solid black line shows the mean difference, and the dotted lines indicate the limits of agreement (mean difference $\pm 1.96 \times$ standard deviation of the difference), respectively. Values determined in patients with and without the IgG4-IgE complex are illustrated as red triangles and black circles, respectively. AIA-PACK, ST AIA-PACK IgE II; Elecsys, Elecsys IgE II; Iatroace, Iatroace IgE. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interpretation.

However, the limitation of our study is that the sample size was too small to sufficiently evaluate the role of the IgG4-IgE complex interference in the measurement of IgE by different assays. We recognize that this limitation increases the risk of errors. In particular, although the interference by the IgG4-IgE complex was clearly observed when Iatroace was used, the limited sample size may have precluded accurate detection of such interference in the case of the other two assays. Therefore, a further study, enrolling a larger number of patients and including detailed data on patients, may be needed to assess the discrepancy between assays.

Recently, it has been reported that most patients with IgG4-RD experience allergic reactions induced by IgE and that determining IgE concentration may facilitate the diagnosis of IgG4-RD and predict its recurrence [1,3]. Our previous study identified the presence of the IgG4-

IgE complex in 21.2 % of patients with IgG4-RD and demonstrated the clinical significance of measuring IgE concentration in this condition [5]. This study is the first to reveal that interference by the IgG4-IgE complex results in an underestimation of IgE concentration, depending on the method used. Therefore, caution should be exercised when assessing IgE concentration in patients, especially those with the IgG4-IgE complex.

5. Conclusions

We showed that the presence of the IgG4-IgE complex in some patients with IgG4-RD interferes with the measurement of IgE concentration and contributes to a discrepancy in detected IgE concentrations between three different assays.

CRediT authorship contribution statement

Keiichi Nakano: Conceptualization, Methodology, Writing – original draft. **Junichi Sugita:** Conceptualization, Writing – original draft. **Masanori Seimiya:** Conceptualization, Data curation. **Keiko Yasuda:** Resources, Methodology. **Chiaki Watanabe:** Resources, Project administration. **Hideki Goto:** Writing – review & editing. **Takanori Teshima:** Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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