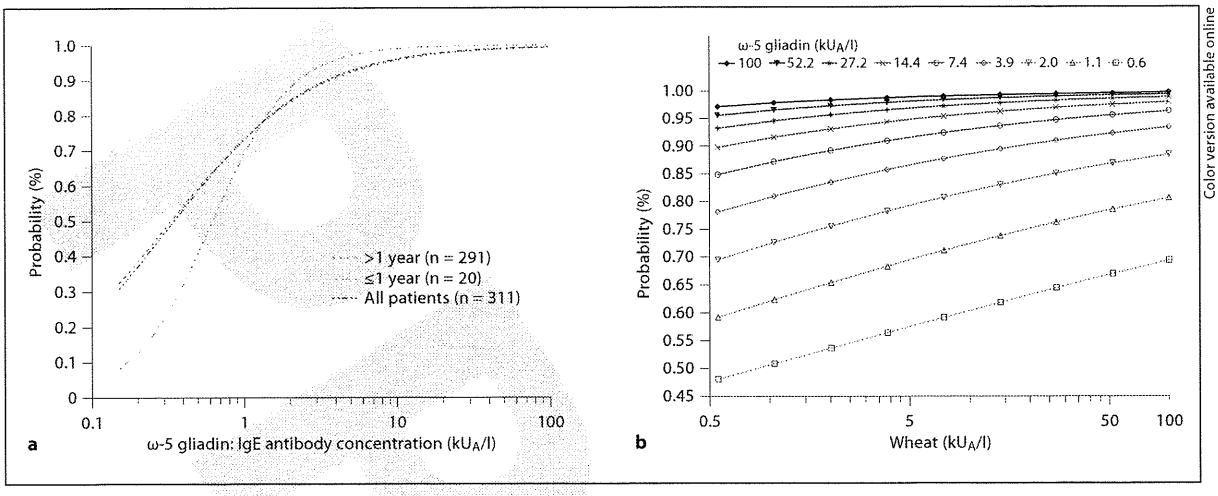

Fig. 4. Fitted predicted probability curves for the outcome of WA at a given IgE value for ω-5 gliadin for all children and for children ≤1 and >1 years of age (**a**) and for nine concentrations of ω-5 gliadin in relation to specific IgE to wheat (**b**).

els and the outcome of oral wheat challenges in a group of 57 German and 29 American children. One explanation could be the sample size, as our study contains 5 and 10 times more children, respectively. Another explanation could be that the inclusion criteria of the study population differed and the study participants had different phenotypes. With regard to the German group, almost half of the children (9 of 19) reacted to wheat challenge but were not sensitized to wheat. In our study, only 5 of 137 challenge-positive children were not sensitized to wheat. The German study population thus included more individuals with non-IgE-mediated WA with delayed symptoms than our study.

Further, no child had ω-5 gliadin levels >20 kU_A/l in their study compared to 11 individuals in our study. A third explanation as to why we arrived at a different conclusion could be the age factor. WA, which usually begins in early childhood, is outgrown in most cases by 3–5 years of age [12], whereas 35% show a persistent WA into adolescence [13]. The children in our study had a median age of 2.3 years and they were representative of typical pediatric cases of immediate allergy to wheat in our opinion.

We speculate that ω-5 gliadin may differ among populations in Asia and Europe due to dietary habits and the genetic background. Our study is however in agreement with the Finnish study, which demonstrated earlier that IgE to ω-5 gliadin is highly predictive of immediate allergy to ingested wheat in children [7]. Further studies are needed to clarify the impact of different races, food habits and genetic variations on immediate allergy to wheat.

Measuring IgE to ω-5 gliadin is useful in the diagnostic workup when investigating immediate-type WA in children and young adults. In Japan, both IgE to wheat and ω-5 gliadin are now routinely assessed when investigating these patients. Patients sensitized to ω-5 gliadin are not challenged with wheat as it is most likely to fail. Patients responsive to the test are asked to strictly avoid wheat. The serology of these patient is then followed prospectively in order to have an indication that tolerance had developed and wheat challenge should be performed. The other scenario is the patient sensitized to wheat but not to ω-5 gliadin during the initial investigation. A wheat challenge is performed as soon as possible in order to confirm or exclude a diagnosis of WA.

In conclusion, the detection of IgE to ω-5 gliadin seems to be associated with ~~lack of~~ responsiveness to the challenge test and is particularly useful in infants with a suspicion of WA.

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Utility of the Peripheral Blood Basophil Histamine Release Test in the Diagnosis of Hen's Egg, Cow's Milk, and Wheat Allergy in Children

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Key Words
Food allergy · Histamine release · Hen's egg · Cow's milk · Wheat

Abstract
Background: The diagnosis of food allergy (FA) is made by oral food challenge tests (OFCs) that occasionally produce serious symptoms in patients; therefore, whether to perform OFCs should be carefully considered. The utility of the histamine release test (HRT) in the diagnosis of childhood FA has not been fully examined. ***Methods:*** Sixty-four subjects with suspected hen's egg allergy, cow's milk allergy (CMA), and wheat allergy (WA) were enrolled. The diagnosis of FA was made based on the outcomes of OFCs or a convincing history of symptoms after food ingestion within 6 months before or after sample collection. HRT was performed using an HRT Shionogi kit. The threshold of histamine release (HRT threshold), which was defined as the minimum concentration of food antigen to induce a 10% net histamine release, was analyzed in association with FA diagnosis. ***Results:*** Receiver operating characteristic analysis showed that the HRT threshold was useful in the diagnosis of heated egg allergy (HEA), raw egg allergy (REA), CMA, and WA. We were able to determine the cutoff value for the HRT threshold in relation to outcomes of OFCs. The cutoff value was 6 ng/ml of egg white antigen in HEA and REA ($p < 0.01$), 40 ng/ml of milk antigen in CMA ($p < 0.01$), and 500 ng/ml of wheat antigen in WA ($p < 0.05$). The efficiency was 70.3% for HEA, 78.0% for REA, 77.6% for CMA, and 70.7% for WA. ***Conclusions:*** We conclude that the HRT threshold measurement for egg white, milk, and wheat antigen is related to outcomes of OFCs and is useful in determining when OFCs should be performed.

Introduction

A definitive diagnosis of food allergy (FA) is generally made using oral food challenge tests (OFCs). Since OFCs occasionally induce severe reactions, whether to perform OFCs should be carefully considered. Antigen-specific IgE levels, a useful predictor of reaction symptoms in OFCs, have been widely used in clinical practice [1–4]. However, antigen-specific IgE is highly sensitive but less specific, and the development of more reliable tests for FA diagnosis is being pursued.

When a causative antigen is bound to specific IgE on the surface of basophil cell membranes, cross-linking of the high-affinity IgE receptor (FcεRI) occurs, inducing

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the release of histamine and the production of leukotrienes and cytokines [5, 6]. The expression of FcεRI on the surface of basophils correlates with serum IgE levels [7]. Moreover, it has been reported that basophils primed with IL-3 enhance histamine release in response to IgE-dependent activation [8]. In this way, basophils are influenced by biological conditions. Therefore, we hypothesized that examination of the histamine release test (HRT) is distinct from simply measuring antigen-specific IgE.

Recently, basophil activation tests have been reported to be useful in the diagnosis of IgE-dependent allergies and have been evaluated in association with a variety of different allergies [9–16]. More recently, Ocmant et al. [15] reported that corresponding performances of ovalbumin and peanut-induced basophil activation tests may be helpful in the diagnosis of egg and peanut allergies. Wanich et al. [17] reported that patients were able to tolerate heated cow's milk have suppressed basophil expression of milk-induced CD63.

On the other hand, the development of HRT in the 1960s [18] did not lead to its widespread use outside of research laboratories due to the large volume of blood required and the short testing window after blood sampling [19]. In recent years, a novel HRT method has been developed that is more convenient and requires a smaller volume of blood. This HRT is commercially available for diagnostic examinations in Japan [20].

Various reports have described the utility of HRT in the diagnosis of FA [21–28]. Norgaard et al. [26] examined the utility of HRT in diagnosing hen's egg allergy and cow's milk allergy (CMA) in adults [28]. These reports suggested that improving the quality of allergen preparations may allow HRT to become a valuable method in FA diagnosis. Lau et al. [25] examined the utility of HRT in the diagnosis of hen's egg allergy in children and indicated that HRT was not effective in predicting outcomes of OFCs. However, in past reports, the utility of HRT in childhood FA diagnosis was not fully examined as many of the subjects were adults and the number of children included in the studies was low [21–28]. The objective of this study was to examine the utility of HRT in the diagnosis of FA in children.

Subjects and Methods

Subjects

Sixty-four subjects (50 males and 14 females; mean age 5.8 ± 3.7 years) with suspected FA, and who had received follow-up care at the Division of Pediatrics of Sagamihara National Hospital between July 2005 and October 2005, were recruited for this study. The diagnosis of FA was made using the results of OFCs or a convincing history of symptoms after food ingestion within 6 months before or after sample collection. Ethics approval was obtained through the Institutional Review Boards at Sagamihara National Hospital. Written informed consent was given by the child or by the child's parents prior to enrolment.

Grouping of Subjects Based on Acquisition of Food Tolerance

Of the subjects, those who were tested for HRT and antigen-specific IgE were grouped as follows: for egg allergy, subjects with heated egg allergy (HEA) who reacted after ingestion of heated eggs [HEA(+)] and those without HEA who did not develop symptoms after ingestion of heated eggs [HEA(–)], and subjects with raw-egg allergy who reacted after ingestion of raw eggs [REA(+)] and those without raw egg allergy (REA) who did not develop symptoms after ingestion of raw eggs [REA(–)]; for CMA, subjects with CMA [CMA(+)] who developed symptoms after drinking milk and those without CMA [CMA(–)] who did not develop symptoms after drinking milk; for wheat allergy (WA), subjects with WA [WA(+)] who developed symptoms after eating wheat and those without WA [WA(–)] who did not develop symptoms after eating wheat.

Oral Food Challenge Tests

OFCs were performed using an open challenge or a single-blind challenge [29]. The open challenge test was performed using heated egg (about 30 g) for HEA, yogurt (35 g) or cow's milk (200 ml) for CMA, and udon (made from wheat flour; 15–50 g) for WA. The single-blind challenge test employed freeze-dried powder (raw egg, cow's milk, and wheat) provided by QP Co., Ltd. Institute, Tokyo, Japan [30]. The initial dose was 1/16 of the amount of the total challenge dose, increasing gradually at 15-min intervals for a period of 1 h, and subjects were carefully monitored for the subsequent 24 h.

Histamine Release Test

The HRT was performed using an HRT Shionogi kit (Shionogi & Co., Ltd., Osaka, Japan) [20]. First, 2 ml of whole blood was drawn into plastic tubes containing EDTA2Na. Blood (20 μl) and anti-leukocyte antibody- (BA312) coated magnetic beads (100 μl) were added to each well of a 96-well microplate and incubated for 10 min at room temperature on a plate mixer. After incubation, a magnetic device was inserted in the 96-well microplate and the basophil-bead complexes were captured for 4 min on the magnetic device at room temperature. The basophil-bead complexes were transferred to another microplate, which was coated with streptavidin for enzyme-linked immunosorbent assay (ELISA) and contained 100 μl of anti-IgE; digitonin (200 μg/ml) for total histamine; HEPES-buffered saline with human serum albumin containing 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% glucose (HACMG) for spontaneous histamine release, and/or food antigen in the respective wells. The microplate was incubated at 37°C for 60 min, with the basophil-bead complexes remaining on the magnetic device. After incubation, the magnetic device was removed and histamine content was measured by ELISA. The following antigens were used: anti-IgE antibody [31], egg white, milk, and wheat (Greer Laboratories, Inc., Lenoir, N.C., USA). The antigens were diluted in HACMG to final concentrations of egg white at 0.5, 6, 70, 830, and 10,000 ng/ml; milk at 0.4, 4, 40,

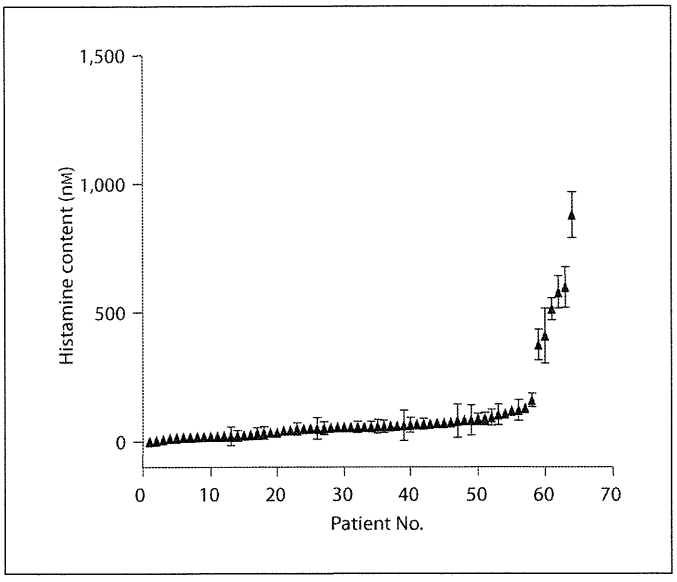

Fig. 1. Distribution of spontaneous histamine release (n = 64). Plots and bars represent means ± SD.

Table 1. Characteristics of subjects

Food antigen	Diagnosis of food allergy	Subjects, n	Average age, years	Antigen-specific IgE (Ua/ml)
Hen's egg (heated) (n = 64)	HEA(+)	38	4.9 ± 3.4	40.0 ± 36.7
	HEA(–)	26	7.1 ± 3.8**	13.9 ± 18.8*
Hen's egg (raw) (n = 50)	REA(+)	38	4.9 ± 3.4	40.0 ± 36.7
	REA(–)	12	6.6 ± 4.6	7.9 ± 14.3
Cow's milk (n = 49)	CMA(+)	27	4.7 ± 3.4	23.8 ± 32.9
	CMA(–)	22	8.2 ± 3.9*	4.6 ± 10.0*
Wheat (n = 41)	WA(+)	12	3.6 ± 3.0	16.9 ± 26.0
	WA(–)	29	7.1 ± 4.1*	7.3 ± 14.0

The diagnosis of food allergy was made based on the results of food challenge tests and a convincing history of food allergies within the past 6 months [HEA(+), REA(+), CMA(+), and WA(+)]. HEA(–), REA(–), CMA(–), and WA(–) denote no symptoms after ingesting a causative food. Mann-Whitney U test, * $p < 0.05$; ** $p < 0.01$.

400, and 4,000 ng/ml; anti-IgE antibody, and wheat at 0.5, 5, 50, 500, and 5,000 ng/ml. Antigen-induced histamine release was measured in duplicate and total histamine content and spontaneous histamine release were measured using 10 wells. HRT was performed in duplicate on the sampling day. Spontaneous histamine release was expressed as means ± standard deviation (SD). Antigen- (anti-IgE antibody, egg white, milk, and wheat) induced histamine release was calculated as: (antigen-induced histamine release – spontaneous histamine release)/total histamine content × 100 [24]. The maximum of percent histamine release (Max. of %HR) is defined as the peak value of antigen-induced histamine release [22] and the threshold of HRT (HRT threshold) was determined as the minimum concentration required to induce a 10% net histamine release.

Antigen-Specific IgE Antibody

Serum was obtained from all subjects on the HRT sampling day. Antigen-specific IgE antibodies to egg white, milk, and wheat were measured using ImmunoCAP (Phadia AB, Uppsala, Sweden). The assay's detection limit was 0.35 kUa/l. We divided the subjects into 2 group according to a cutoff value which was 95% of a positive predictive value of antigen specific IgE levels reported by Komata et al. [1] and Sampson [2].

Statistical Analysis

Results are expressed as means ± SD or means ± standard error (SEM). Comparisons between groups were performed using the Mann-Whitney U test and the χ^2 test. $p < 0.05$ was considered statistically significant. ROC curve analyses were performed to analyze antigen-specific IgE, Max. of %HR, and the HRT threshold. The cutoff value for the HRT threshold was based on the lowest statistically significant food antigen concentration, and sensitivity, specificity, and efficiency were calculated using the cutoff values. These data were calculated using Graph Pad Prism (version 5; GraphPad Software, Inc., Calif., USA).

Results

Characteristics of the subjects are summarized in table 1. Thirty-six of the subjects (6.3%) presented with bronchial asthma and 51 (79.7%) with atopic dermatitis. There were 64 subjects with HEA [HEA(+) vs. HEA(–); 38 vs. 26], 50 with REA [REA(+) vs. REA(–); 38 vs. 12], 49 with CMA [CMA(+) vs. CMA(–); 27 vs. 22], and 41 with WA [WA(+) vs. WA(–); 12 vs. 29]. The number of subjects who had received OFCs was as follows: 37 for HEA, 25 for REA, 15 for CMA, and 9 for WA. The mean age of the subjects was significantly higher in HEA(–), REA(–), CMA(–), and WA(–) groups than in the respective (+) groups. The mean antigen-specific IgE level was significantly higher in HEA(+), REA(+), and CMA(+) groups than in the respective (–) groups; no significant differences were observed in WA.

Six of the 64 subjects showed elevated spontaneous histamine releases greater than 40%. The other 58 subjects showed relatively low spontaneous histamine releases, with a mean percent spontaneous histamine release of 9.6 ± 7.4% (fig. 1). The mean percent anti-IgE induced histamine release was 73.2 ± 18.8%. There was no subject

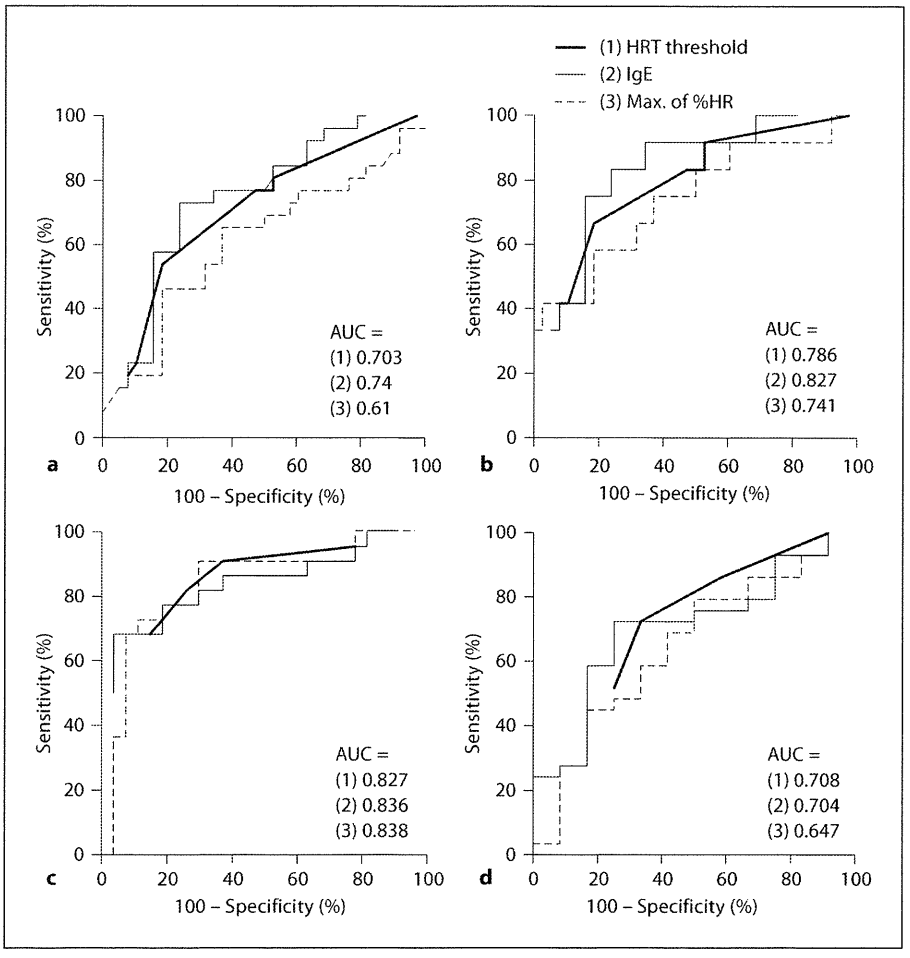

Fig. 2. ROC curves for HRT and antigen-specific IgE to egg white, cow's milk, and wheat. **a** HEA. **b** REA. **c** CMA. **d** WA.

whose anti-IgE induced histamine release was less than 10%.

The results of ROC analysis for the HRT threshold, Max. of %HR, and antigen-specific IgE levels are shown in figure 2. By ROC analysis, the area under the curve (AUC) of milk was found to be highest with 0.827 for the HRT threshold and 0.838 for Max. of %HR, respectively. The AUCs of the HRT threshold in HEA, REA, and WA were higher than that of Max. of %HR, though that of the HRT threshold in CMA was similar to that of Max. of %HR. The AUCs of antigen-specific IgE levels (0.74, 0.827, and 0.836) were higher than that of HRT threshold in the subjects with HEA, REA, and CMA, respectively.

The correlation between the HRT threshold and antigen-specific IgE levels is shown in figure 3a–d. The cutoff value for the HRT threshold was 6 ng/ml of egg white antigen in HEA ($p < 0.01$); the sensitivity, specificity, and efficiency were 81.6, 53.8, and 70.3%, respectively. Similarly, the cutoff value for the HRT threshold was 6 ng/ml of egg white antigen in REA, 40 ng/ml of milk antigen in CMA ($p < 0.01$), and 500 ng/ml of wheat antigen in WA ($p < 0.05$). The sensitivity was 81.6, 74.0, and 66.7%; specificity was 66.7, 81.8, and 72.4%, and efficiency was 78.0, 77.6, and 70.7%, respectively.

As shown in table 2a–d, in the subjects with HEA, although levels lower than 25.5 Ua/ml for egg white-specific IgE were detected in 20/38 HEA(+) subjects, 14 (70%) of these subjects had an HRT threshold of less than 6 ng/ml of egg white antigen. On the other hand, in the subjects with HEA(–), levels lower than the cutoff value for antigen-specific IgE were detected in 21 subjects; 8 (38%) of these subjects had an HRT threshold of less than 6 ng/ml of egg white antigen. Similarly, in REA(+) and CMA(+) subjects the ratio of subjects who were below the cutoff value for the HRT threshold was significantly high.

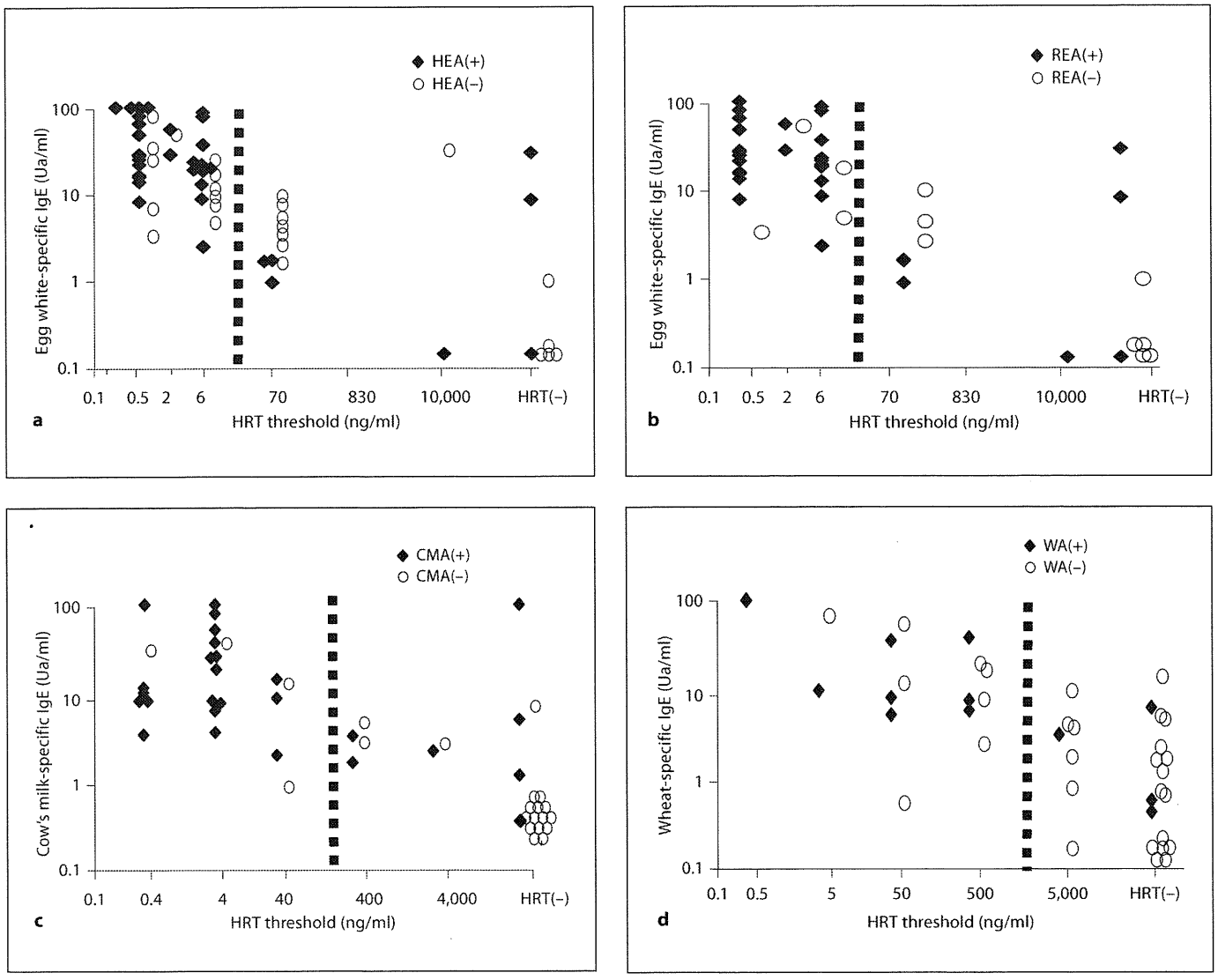

Fig. 3. Correlation between antigen-specific IgE and the HRT threshold. The diagnosis of FA was made using the results of an FCT and a convincing history of food allergies within the past 6 months [HEA(+), REA(+), CMA(+), and WA(+)]. HEA(–), REA(–), CMA(–), and WA(–) denote no symptoms after ingesting a causative food. The threshold of HRT was determined as the minimum concentration of antigen to induce a 10% net histamine release. HRT(–) means that the net percent histamine release by the maximum concentration of antigen is less than 10%. **a** HEA (n = 64). **b** REA (n = 50). **c** CMA (n = 49). **d** WA (n = 41).

Discussion

We examined the utility of HRT in the diagnosis of hen's egg allergy, CMA, and WA in children. The results of our study suggest that the HRT threshold for egg white, milk, and wheat antigen is related to the outcome of OFC after ingestion of a causative food and is useful in determining when OFC should be performed.

Six of 64 subjects (9.4%) showed high spontaneous histamine releases of 40% or more. Sampson et al. [32] reported that FA patients with atopic dermatitis who had eliminated the offending food allergen from their diet had a significantly lower rate of histamine release. It was suggested that mononuclear cells were being continuously stimulated by the offending food antigen, producing a histamine-releasing factor that results in basophil histamine release. As a result, basophils from these patients

were found to have high rates of spontaneous histamine release. In this study, 5 of 6 subjects with high spontaneous histamine release had concomitant atopic dermatitis. However, their atopic dermatitis was well controlled at the time of HRT measurement. All 6 subjects eliminated the offending food. Our cases did not agree with these reports and we plan to pursue this discrepancy further in future studies.

To evaluate the diagnostic utility of HRT in the diagnosis of HEA, REA, CMA, and WA, ROC analysis for each test was performed. In subjects with HEA, REA, and WA, the AUC of the HRT threshold was higher than that of Max. of %HR. Kleine-Tebbe et al. [24] also reported that birch pollen-allergic patients with oral allergy syndrome had lower thresholds of birch pollen-induced histamine release than did patients without oral allergy syndrome. From these results, it was thought that the HRT threshold was more useful than the Max. of %HR for the outcome prediction of OFCs.

Lau et al. [25] examined the utility of HRT in the diagnosis of HEA in patients 3.5 months to 12 years of age. They reported that HRT was not an effective predictor of the outcome of OFCs in childhood egg allergy because the sensitivity of HRT was 64.7%, the specificity was 40.0%, and the efficiency was 55.6%. However, the results of our study showed that the HRT threshold for egg white in subjects with HEA was 81.6% for sensitivity, 53.8% for specificity, and 70.3% for efficiency and that of REA was 81.6% for sensitivity, 66.7% for specificity, and 78.0% for efficiency. The efficiency of our data was higher than that in the report of Lau et al. [25]. It has been reported that differences in HRT sensitivity may be due to several factors, such as the quality of allergen extracts [28]. Lau et al. [25] employed a different basophil-stimulating antigen, i.e. ovalbumin, while we used raw egg white antigen. Furthermore, they decided that a percentage of histamine release of 30% or more was the HRT cutoff value. The results of our study suggest that the HRT threshold is more effective than the Max. of %HR for predicting the outcome of OFCs. These might be the reasons that the results of Lau et al. [25] differed from ours.

It has been reported that the antigen-specific IgE levels could predict the results of OFC after ingesting a causative food; cutoff values were set to a positive predictive value of 95% or greater [1, 2, 33]. In the patients with HEA and REA, the AUCs of antigen-specific IgE levels were higher than that of the HRT threshold. However, about 50% of subjects appeared to have symptoms caused by ingesting a causative antigen when antigen-specific IgE levels were below the cutoff value.

Table 2. Diagnosis of FA and its relationship to egg-, milk-, and wheat-specific IgE and the HRT threshold

a Heated egg

HEA(+)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤6	17 (94%)	14 (70%)	31 (82%)
	>6	1 (6%)	6 (30%)	7 (28%)
Total		18	20	38
HEA(–)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤6	4 (80%)	8 (38%)	12 (46%)
	>6	1 (10%)	13 (62%)	14 (54%)
Total		5	21	26

b Raw egg

REA(+)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤6	17 (94%)	14 (70%)	31 (82%)
	>6	1 (6%)	6 (30%)	7 (28%)
Total		18	20	38
REA(–)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤6	1 (100%)	3 (27%)	4 (33%)
	>6	0	8 (73%)	8 (67%)
Total		1	11	12

c Cow's milk

CMA(+)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤40	4 (80%)	16 (73%)	20 (74%)
	>40	1 (10%)	6 (27%)	7 (26%)
Total		5	22	27
CMA(–)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤40	0	4 (18%)	4 (18%)
	>40	0	18 (82%)	18 (82%)
Total		0	22	22

d Wheat

WA(+)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤500	3 (100%)	5 (44%)	8 (67%)
	>500	0	4 (56%)	4 (33%)
Total		3	9	12
WA(–)		>IgE	≤IgE	Total
HRT threshold, ng/ml	≤500	2 (100%)	6 (22%)	8 (28%)
	>500	0	21 (78%)	21 (72%)
Total		2	27	29

The cutoff value was decided based on previous reports. >IgE = Greater than the cutoff value of antigen-specific IgE levels; <IgE = lower than the cutoff value of antigen-specific IgE levels.

On the other hand, as shown in figure 3a–d, the HRT threshold differed in each case and was widely distributed, from low to high concentrations, in the subjects in which antigen-specific IgE levels were similar. When the HRT threshold was below the cutoff value, the ratio of subjects [HEA (+), REA (+), and CMA (+)] was significantly greater even if antigen-specific IgE levels were below the cutoff value.

In conclusion, the HRT thresholds for egg white, milk, and wheat antigen are related to the outcome of OFCs after ingesting a causative food and are useful in determining when OFCs should be performed, even if antigen-specific IgE levels are lower than the cutoff value. Therefore, measuring egg white-, milk-, and wheat-induced HRT could decrease the number of necessary OFCs and be useful in determining when OFCs should be performed.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the contents of this article.

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