Egg

Egg white is the most important source of allergens in egg and contains 23 different proteins (14). The most important allergens that have been identified and for which the clinician can test are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin/conalbumin (Gal d 3), and lysozyme (Gal d 4) (15).

Although ovomucoid comprises only 10% of the total egg white protein, it has been shown to be the dominant allergen. Ovomucoid has several unique characteristics, such as stability to heating and breaking down by proteinases. It also appears to be allergenic in minute quantities, and testing for ovomucoid has proven helpful in the prognosis and diagnosis of egg allergy.

High concentrations of ovomucoid-specific IgE are associated with persistent egg allergy (15).

When an individual allergic to egg starts to develop tolerance to hen's egg, he or she first becomes tolerant to heated egg and subsequently to raw egg, which has been described in several case reports (16).

Differences in IgE antibodies against ovomucoid were found in patients depending on the reactivity to raw and cooked egg (17). Low levels of IgE antibodies against ovomucoid were associated with tolerance to cooked egg. Furthermore, the quantification of ovomucoid antibodies can guide the clinician's decision on whether to perform a challenge. Ando et al. (17) show that a concentration of IgE antibodies against ovomucoid higher than *c.* 11 kUA/l (positive decision point) indicates a high risk of reacting to heated (as well as raw) egg. At the same time, a concentration below *c.* 1 kUA/l (negative decision point) means that there is a low risk of reaction to heated egg, although the patient may well react to raw egg.

Benhamou et al. (18) have found differences in egg-specific IgE levels for patients with severe, moderate, or absent reactions at challenge, highest for patients with severe reactions and decreasing with the severity of reaction. This kind of differences regarding levels for ovomucoid and severity in challenge are yet to be described.

Milk

The majority of patients allergic to milk are sensitized to several cow's milk proteins. However, the profile of the IgE response to these components varies greatly.

The most important allergens in milk are caseins (Bos d 8), beta-lactoglobulins (Bos d 5), and alpha-lactoglobulins (Bos d 4), although allergies to other minor proteins such as bovine serum albumins (Bos d 6) have also been reported (19).

There is now growing evidence that casein seems to be a major allergen component to test for in the treatment of a patient with cow's milk allergy.

Garcia-Ara and co-workers have been following children with cow's milk allergy. They have observed that casein is the protein that best discriminates between persistent and transient allergy (20). The same Spanish group has also been studying reactions to accidental exposure to milk in children with cow's milk allergy. They found that it was relatively common and that 15% of the group had severe reactions. The risk factors for such reactions include high levels of IgE against cow's milk and casein in combination with asthma.

Gern and Sampson have studied allergic reactions in patients with cow's milk allergy who eat so-called non-dairy products (21). They found that casein was often the cause of the reaction. Casein is used as an extender in sausages, soups, and stews.

D'Urbano et al. (22) showed that in patients with a positive food challenge to milk, casein (Bos d 8) was the milk allergen component against which they most frequently had IgE.

Wheat

Wheat allergy is common worldwide (8, 23) and is sometimes difficult to diagnose for the pediatric allergist. Part of the reason is that a positive result to wheat flour extract may not always correlate with clinical symptoms (24), which indicates that *in vitro* diagnosis of allergy to wheat may be improved by using wheat allergen components. There are a number of strong candidates among the wheat components currently undergoing clinical evaluation. They will most likely improve the management of patients allergic to wheat.

Wheat commonly cross-reacts with grass pollen, which causes a problem with overdiagnosis of wheat allergy. The typical situation is that the clinician performs a skin prick test for wheat or orders a wheat-specific IgE Ab measurement for a patient allergic to grass. Owing to cross-reaction, this test will most probably be positive. The clinician might interpret this as an indication of wheat allergy and incorrectly advise the patient to avoid wheat in the diet. Improved species-specific diagnostics for wheat are obviously needed.

To date, we can test for one major wheat component when investigating suspected hypersensitivity reactions to wheat in children and adults.

In children, IgE antibodies against omega-5 gliadin (Tri a 19) are associated with a risk of IgE-mediated reactions to wheat (25, 26). It has been suggested that the level of antibodies against omega-5 gliadin acts as a marker for clinical reactivity and an aid when deciding whether to perform a wheat challenge (26). IgE antibodies against Tri a 19 in adults are linked to a risk of exercise-induced reactions associated with the ingestion of wheat (27). LTP to wheat seems also to be a clinical-relevant component when investigating wheat hypersensitivity but is not yet available for clinical use.

Fish

Fish proteins are sometimes responsible for life-threatening IgE-mediated allergic reactions.

Fish parvalbumins from cod (*Gadus morhua*) Gad c 1 and carp (*Cyprinus carpio*) Cyp c 1 are both major fish parvalbumin proteins and representative markers for fish sensitization in general (5). The different content of parvalbumin in species like cod, whiff, or swordfish may explain tolerance to some

species (28). The extensive cross-reactivity between parvalbumins from different species means that Gad c 1 and Cyp c 1 are valuable tools in diagnosing patients with fish allergy. Both have remarkable stability, which may explain why sensitization can result because of ingestion even after cooking, via contact with and inhalation of vapor from cooking. Sensitization to a fish parvalbumin suggests caution in the administration of all fish species to reactive patients (29).

Soy

Soybean allergy in children is known to be mediated primarily by contact with allergen via the gastrointestinal tract, often in the form of soya-based milk substitute products, particularly in infants allergic to cow's milk. The primary sensitizers seem to be the most important soya proteins Gly m 5 and Gly m 6 (30, 31). Soy allergy may also be acquired following primary sensitization to birch pollen, owing to IgE cross-reactivity between the most important birch pollen allergen Bet v 1, and its homologous protein in soybean, Gly m 4 (32). To date, pollen-mediated soy allergy has been mainly described in adults. This type of soy allergy seems also to be a problem among the pediatric population, and there have recently been reported four children allergic to birch pollen who experienced severe allergic reactions following the ingestion of soy milk during the pollen season (33).

Gly m 4 has been shown to be a risk factor for severe OAS or systemic reactions to soya in patients allergic to birch pollen (32). Gly m 4 is also cross-reactive with Ara h 8, and in Europe, approximately two-thirds of patients allergic to soya are allergic to peanut. Targeted diagnostic testing with Gly m 4 is recommended in pollen-sensitized patients where allergy to soya is suspected, especially if the soya extract test result is negative. Some patients sensitized to Gly m 4 can show low or even negative IgE results with soya extract because of a low Gly m 4 content in the extract (33).

Furred animals

Cross-reactions also occur between our most common domestic animals, such as cats, dogs, and horses. This is partly new knowledge and might explain why so many of our patients allergic to furred animals are often sensitized to more than one species. In the German MAS (Multicentre Allergy Study) cohort, Matricardi and co-workers identified 56 children sensitized to cat at the age of 10. Fifty-seven per cent of them reported having concomitant allergic sensitization to dog. Forty-one children were sensitized to dog, and 73% were also sensitized to cat (34). Liccardi and co-workers identified 35 adults sensitized to horse, of whom 23 were reported to have concomitant allergic sensitization to dog and 25 to cat (35). Baatenburg de Jong et al. have recently shown that among a group of 776 polysensitized children, 87% were sensitized to dog and 74% were sensitized to cat (36). These studies indicate either a strong comorbidity between furry animal allergies or prevalent cross-reactions or a combination of both. Challenge with animal dander is theoretically possible and would reveal the actual reactions to each dander. However, this is not a method commonly used today because of the risk of severe reactions. These conditions can be now studied through allergen component testing.

Fel d 1 is the most important allergen component in cat (1), which indicates primary sensitization. Up to 90% of patients allergic to cat have IgE antibodies against Fel d 1. This allergen component can be used as a specific marker, which indicates that immunotherapy treatment of cat is probably of clinical value. Among individuals allergic to cat, Grönlund and co-workers found higher levels of IgE against Fel d 1 in children with asthma compared with children with allergic rhinoconjunctivitis (37). This indicates that Fel d 1 could be used as a marker for an increased risk of lower respiratory disease among cat-sensitized individuals. Other cat components available for testing are Fel d 2 and Fel d 4.

IgE against cat serum albumin Fel d 2 is likely to cross-react with most other mammalian albumins, such as dog Can f 3, horse Ecu c 3, pig Sus s PSA (pig serum albumin), and cow Bos d 6. It can also cause reactions following the ingestion of pork (the cat-pork syndrome); about 15–40% of patients allergic to cat have IgE against Fel d 2 (1).

The picture for primary sensitization to dog is more complex.

To date, Can f 1, Can f 2, and Can f 5 have been found to be specific allergen components that indicate primary sensitization; *c.* 50–90%, 20–33%, and 70% of patients allergic to dog have IgE antibodies against Can f 1, 2, and 5, respectively (1). A completely new finding is the recent identification of Can f 4 as another species-specific allergen component for dog (38). This complexity might explain why dog allergy can, in some cases, present clinical difficulties. It is quite common for a patient allergic to dog to tell the clinician that he/she can tolerate some dogs but reacts to contact with others. Future research will clarify whether the composition of the dog allergen components differs between various breeds of dog, which would explain the clinical picture.

Equ c 1, a lipocalin, is considered the major allergen in horse dander. New data have been presented but not yet published that Equ c 1 cross-reacts with Fel d 4, which belongs to the same protein family. This new knowledge and insight means that we may be overdiagnosing horse allergy at present. It may be that patients are only sensitized to cat, but our interpretation may be that they are also allergic to horse, and vice versa. We now have the tools to understand the underlying mechanisms behind sensitization in more detail. Consequently, we should be more careful in advising on avoiding animal dander before we know the primary sensitizer.

Allergen components on microarrays

The term 'microarray or biochip' refers to the distribution of small amounts of biomolecules on a surface in a regular, compact pattern. In contrast to conventional diagnosis, microarrays allow us to investigate IgE reactivity against a large number of different allergenic components with a single, rapid test.

The amount of patient serum required is far smaller than in conventional immunoassay. In fact, as little as 20 μl is enough to determine IgEs against hundreds of individual allergens, while conventional tests require 50 μl for each allergen tested.

This facilitates the use of the technique in pediatric patients, because such a minute amount of serum can be obtained from a simple blood sample.

The first experimental microarray system for allergy diagnosis was reported in 2000 (39). Later on, microarrays were developed with a growing number of recombinant and purified molecules. The ISAC prototype commercialized by VBC Genomics/Phadia was the first protein microarray applied to the detection of sIgE (40). There are a number of studies (41–43) validating microarray technology using homework or commercially available technology (ImmunoCAP® ISAC).

ISAC is an IgE antibody assay specifically designed to help clinicians identify the presence and quantify the amount of cross-reactive IgE antibodies among the different food and pollen allergen groups that are known to share extensive homology (44). The microarray generates a fluorescent image, which is analyzed by special software that calculates the IgE results semiquantitatively for each allergenic component. IgE concentration is measured in arbitrary units termed ISAC Standardized Units (ISUs), and these values are divided into four classes (negative, low, intermediate, and high).

Interpreting 112 allergen component test results per patient is challenging for the clinician. Soon, microarray technology will be combined with PC-based intelligent support for interpretation. Clinical trials have been performed, and preliminary data indicate that an interpretation tool helps the practicing allergy specialist assimilate and interpret the vast amount of IgE antibody data from the chip-based microarray assay. This should make the issue of food cross-reactivity more manageable for the practicing clinician.

Could molecular allergology replace the food challenge?

The measurement of allergen components has the potential to reduce the number of food challenge. The reason why food challenge not yet can be replaced is that not all allergic sources in the various foods have yet been completely characterized and evaluated. From a health economic perspective, the health service would save money and reduce the risks if allergen components were used instead of food challenges. We therefore need a method even safer and better than the challenges we use today. The double-blind placebo-controlled food challenge (DBPCFC) has long been the standard in the diagnosis of food allergy as a benchmark test from which to judge the diagnostic characteristics of the clinical history, skin prick test, and IgE antibody serology. The drawback is that open challenge can give false-positive results ranging from 20% to 71% (44). However, positive placebo reactions that may occur during the DBPCFC can be as high as 35%. False-negative open challenges occur in 1–3% of cases.

Furthermore, the problem today is that too few patients are offered or are prepared to undergo a challenge, owing to scarce resources and the risk of severe reactions. Especially, nut allergies are difficult to food challenge, and as a consequence, few patients with suspected nut or peanut allergies undergo challenges. There is therefore a great need for improved diagnosis, in which the testing of allergen components can be very helpful. Zijlstra and co-workers have performed food challenges on this group, finding that 58% of the individuals had unnecessarily avoided hazelnuts and 33% peanuts (45).

D'Urbano et al. (22) have investigated children with suspected cow's milk allergy, comparing milk allergen components with milk challenges. The results indicate that serial testing of IgE against milk and microarray ImmunoCAP® ISAC have a clinical performance very close to that of the food challenge. Using this two-step approach, the clinician would have detected that 27 of 29 children should have a milk-free diet. Using only IgE against cow's milk at the pediatric clinic in primary health care would have eliminated the need for a challenge in about 27% of the patients.

This sequential use of the two tests would have led to a 50% reduction in the number of challenges.

Even more remarkable is that this reduced the number of positive challenges to five compared with the previous 17. These data are very promising with regard to reducing risks for children with allergies.

According to D'Urbano et al., serial use of the two tests could be considered from the point of view of clinical application, based on the opportunity that:

1 Pediatricians in outpatient care or general practitioners in primary health care identify patients with a high probability of allergy, based on case history and detection of IgE against cow's milk. The children with high probability of milk allergy are referred to secondary care.

2 The referred patients are screened with the microarray to assess whether a food challenge should be carried out in secondary- or tertiary-level health care.

Clinical advantages of microarrays

Allergic patients with a complex symptomatology, such as severe eczema, unstable asthma, and chronic urticaria, are especially suitable for the investigation of IgE reactivity using microarrays. The number of molecular allergens gives comprehensive and detailed information about the patient's sensitization profile.

To illustrate the clinical advantages of microarray, we show two pediatric cases from a birth cohort with high risk of developing allergies, and blood samples were taken at the ages of 6 and 18 months and at 6 and 18 yrs (46, 47). In this study, sera from these four sampling occasions were analyzed retrospectively with a component-resolved *in vitro* diagnosis technique using the ImmunoCAP® ISAC microarray assay.

The IgE antibody assay results were compared with each patient's clinical history. Two cases with severe allergic and asthmatic disease are described below to demonstrate the value of component-resolved diagnostics.

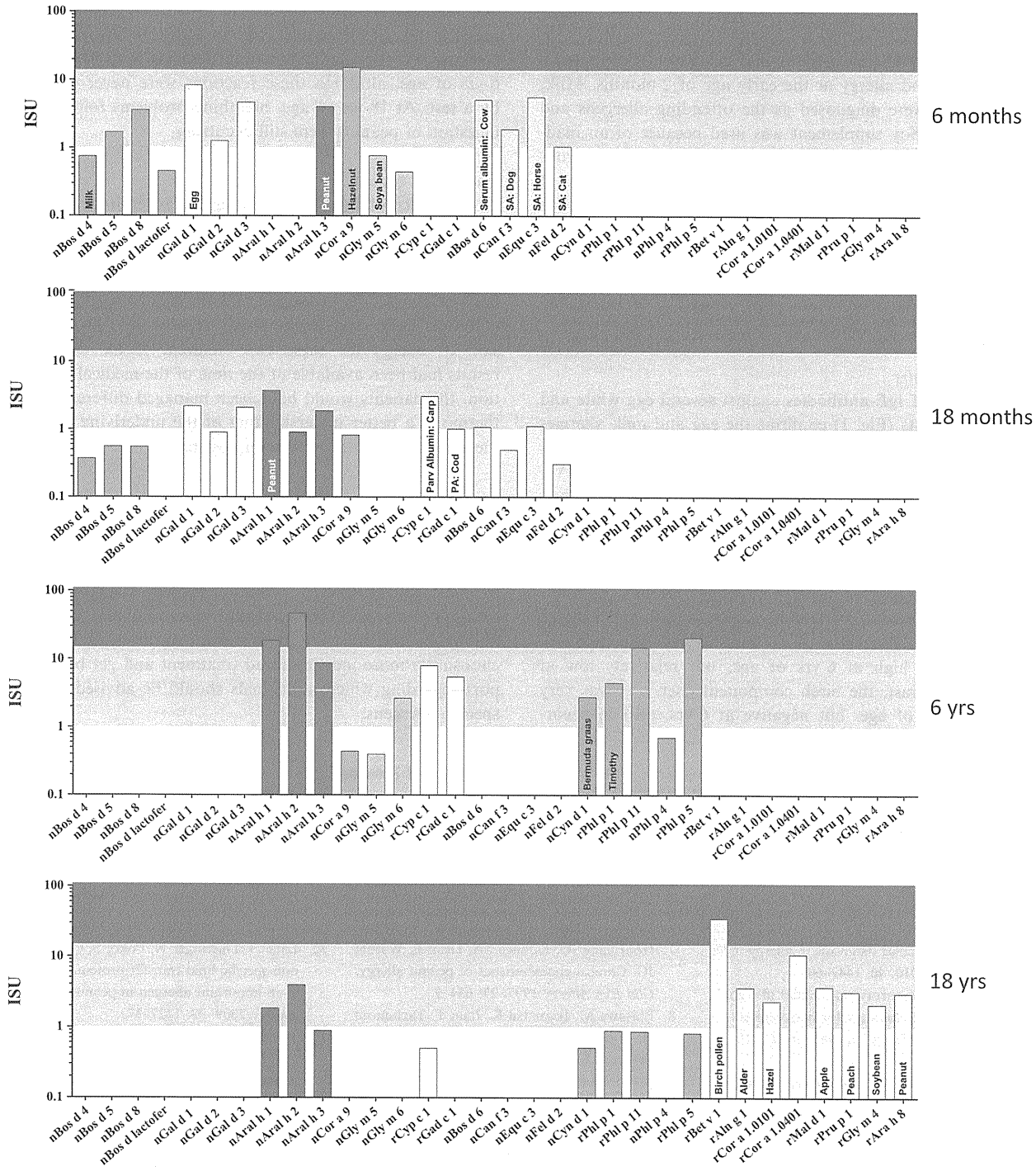

Figure 1 Test results from case number 1. The presence of IgE antibodies against several egg white and milk components confirms the egg and milk allergy in early childhood. In parallel, there were also positive values at an early age for hazelnut (Cor a 9), peanut (Ara h 1, Ara h 2, Ara h 3) and soya (Gly m 5, Gly m 6). The values for fish (Cyp c 1 and Gad c 1) peaked at 6 years of age, in accordance with the medical history. The value of the timothy pollen component (Phl p 5) was high at 6 years of age, but relatively low at 18 years. In contrast, the birch component (Bet v 1) was very high at 18 years of age, but negative at 6 years, also in accordance with the clinical history.

Case 1

A boy with two allergic parents had developed severe atopic eczema and food allergy at the early age of 2 months. Milk, egg, and fish were diagnosed as the offending allergens and were avoided. Soy supplement was used because of an inadequate supply of breast milk. He also had wheezing problems at an early age and was diagnosed with asthma at 6 months of age. Allergic rhinitis was diagnosed at the age of 2, and he was sensitized to pollen and furred animals. The eczema disappeared at the age of 10, and his severe asthma became mild at 15 yrs. At 18 yrs, he had allergic rhinitis and mild asthma and was sensitized to birch and timothy.

Component results

The presence of IgE antibodies against several egg white and milk components (Fig. 1) confirms the egg and milk allergies in early childhood. In parallel, there were also positive values at an early age for hazelnut (Cor a 9), peanut (Ara h 1, Ara h 2, and Ara h 3), and soy (Gly m 5 and Gly m 6). The boy subsequently experienced breathing problems when eating nuts and peanuts. Retrospectively, he was most likely allergic to soy because the soy supplement caused colic and the eczema did not fully improve. The values for fish (Cyp c 1 and Gad c 1) peaked at 6 yrs of age, in accordance with the medical history. The value of the timothy pollen component (Phl p 5) was high at 6 yrs of age, but relatively low at 18 yrs. In contrast, the birch component (Bet v 1) was very high at 18 yrs of age, but negative at 6 yrs, also in accordance with the clinical history.

Case 2

A boy with severe atopic eczema and food allergy (vomiting), which started at the age of 3 months, was studied. Egg, fish, and birch pollen were positive in skin prick tests and diagnosed as the offending allergens. In addition, OAS-like symptoms in response to peanut and shellfish were reported at 6 yrs of age, although these reactions were never confirmed by a test. At 18 yrs of age, breathing problems following the ingestion of peanuts were still occurring.

Component results

The presence of IgE antibodies against several egg, fish, and birch pollen components confirms the diagnosed allergies. Furthermore, IgE antibodies against peanut (Ara h 1, 2, and 3) and shellfish (tropomyosin) components are registered before the reactions to peanut and shellfish are reported.

The allergen component results clearly show the progression of allergy for these two children. If the component results had been available at the time of the medical examination, the patients would have been managed differently. Furthermore, a better understanding of the underlying causes of the symptoms would have been possible.

Conclusions

The use of allergen components will pave the way for a more individual approach when we investigate and care for our patients with suspected allergic diseases. Using molecular allergology, we can now already better diagnose, prognose, and grade the allergic diseases. We can also get help with choosing a more individualized treatment and get better support regarding which individuals should be advised to avoid specific allergens.

Conflict of interest

Magnus Borres is medical director at Phadia AB. Philippe Eigenmann has received speakers honoraria from Phadia and ALK.

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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,

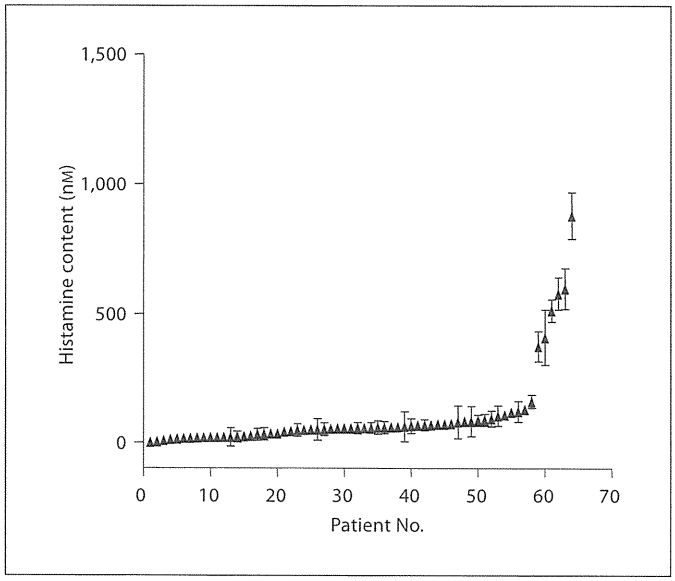

Fig. 1. Distribution of spontaneous histamine release (n = 64). Plots and bars represent means ± SD.

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

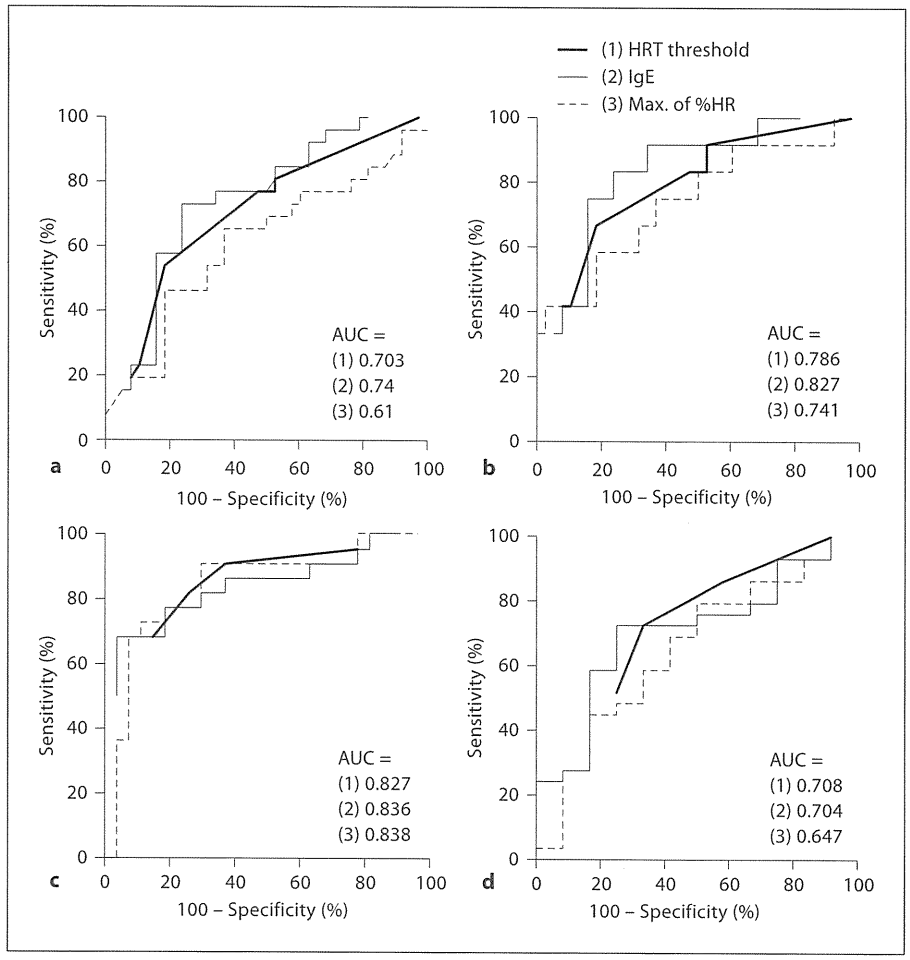

Fig. 2. ROC curves for HRT and antigen-specific IgE to egg white, cow's milk, and wheat. **a** HEA. **b** REA. **c** CMA. **d** WA.

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.

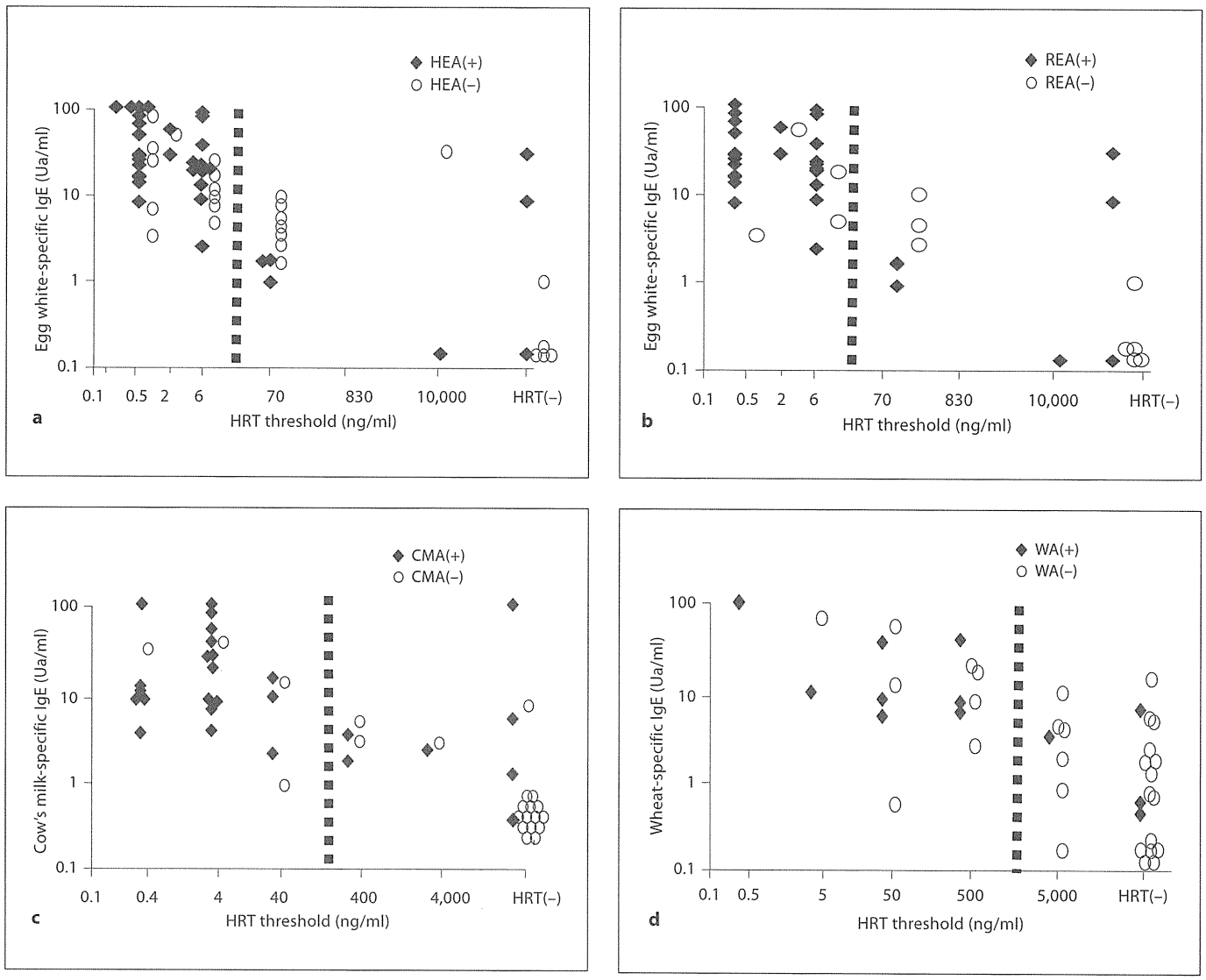

Fig. 3. Correlation between antigen-specific IgE and the HRT threshold. The diagnosis of FA was made using the results of an FCT and a convincing history of food allergies within the past 6 months [HEA(+), REA(+), CMA(+), and WA(+)]. HEA(–), REA(–), CMA(–), and WA(–) denote no symptoms after ingesting 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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REVIEW ARTICLE

Japanese Guideline for Food Allergy

Atsuo Urisu[1], Motohiro Ebisawa[2], Tokuko Mukoyama[3], Akihiro Morikawa[4] and Naomi Kondo[5]

ABSTRACT

Food allergy is defined as "a phenomenon in which adverse reactions (symptoms in skin, mucosal, digestive, respiratory systems, and anaphylactic reactions) are caused in living body through immunological mechanisms after intake of causative food."

Various symptoms of food allergy occur in many organs. Food allergy falls into four general clinical types; 1) neonatal and infantile gastrointestinal allergy, 2) infantile atopic dermatitis associated with food allergy, 3) immediate symptoms (urticaria, anaphylaxis, etc.), and 4) food-dependent exercise-induced anaphylaxis and oral allergy syndrome (i.e., specific forms of immediate-type food allergy).

Therapy for food allergy includes treatments of and prophylactic measures against hypersensitivity like anaphylaxis. A fundamental prophylactic measure is the elimination diet. However, elimination diets should be conducted only if they are inevitable because they places a burden on patients. For this purpose, it is highly important that causative foods are accurately identified. Many means to determine the causative foods are available, including history taking, skin prick test, antigen specific IgE antibodies in blood, basophil histamine release test, elimination diet test, oral food challenge test, etc. Of these, the oral food challenge test is the most reliable. However, it should be conducted under the supervision of experienced physicians because it may cause adverse reactions such as anaphylaxis.

KEY WORDS

elimination diet, food allergy, IgE-mediated type, non-IgE-mediated type, oral food challenge test

1. DEFINITION OF FOOD ALLERGY

The Japanese Pediatric Guideline for Food Allergy 2005,[1,2] published in 2005, defines food allergy as "a phenomenon in which adverse reactions (symptoms in skin, mucosal, digestive, respiratory systems, and anaphylactic reactions) are caused in living body through immunological mechanisms after intake of causative food."

2. EPIDEMIOLOGY OF FOOD ALLERGY

2.1. PREVALENCE OF IMMEDIATE-TYPE FOOD ALLERGY

Food allergy is common among infants aged 0-1 years and decreases with aging, which indicates that tolerance develops with aging. The estimated prevalence in Japan is 5-10% among infants and 1-2% among schoolchildren. The prevalence of food allergy, reported from various countries, is shown in Table 1.

2.2. CAUSATIVE FOODS

Eggs, dairy products, wheat, buckwheat, shrimp and peanuts are the common causative foods of immediate-type food allergy, indicated by the national surveys of food allergy during 1998-1999, conducted by the Review Committee on the Countermeasure for the Food Allergy of the Ministry of Health and Welfare (Fig. 1). As shown in Figure 2, patients aged less than 1 year of age account for 29.3%, and those aged $\leq$8 years account for 80.1%. The number of patients decreases with aging. Patients aged $\geq$20 years account for 9.2%. This is not a small number. Eggs, dairy products and wheat are 3 major allergens among those aged $\leq$6 years, while shrimp, fish, and

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Table 1 Prevalence of food allergy reported from various countries

Year	Reporter	Country	Subject	Number of subjects	Methods	Diagnosis	Prevalence	Journal
1994	Young E	UK	All ages	7,500 households	Interview + DBPCFC	Food intolerance	1.4-1.8%	Lancet
1994	Jansenn JJ	Netherlands	Adults	1,483 persons	Questionnaire + DBPCFC	Food allergy, food intolerance	0.8-2.4%	J Allergy Clin Immunol
1999	Kristjansson I	Sweden, Iceland	Children (aged 18 months)	652 persons	Questionnaire + DBPCFC	Food allergy	2.00%	Scand J Prim Health Care
2001	Kanny G	France	All ages	33,110 persons	Questionnaire (two-step survey)	Food allergy	3.52%	J Allergy Clin
2004	Zuberbier T	Germany	All ages	4,093 persons	Questionnaire + DBPCFC	Food allergy	3.60%	Allergy
2005	Imai	Japan	School children	8,035,306 persons	Questionnaire	Food allergy	1.30%	J Jpn Pediatr Soc
2005	Rance F	France	School children	2,716 persons	Questionnaire	Food allergy	4.70%	Clin Exp Allergy
2005	Pereira B	UK	School children (aged 11 years)	757 persons	Questionnaire + Open challenge test	Food allergy	2.30%	J Allergy Clin Immunol
			School children (aged 15 years)	775 persons	Questionnaire + DBPCFC	Food allergy	2.30%	
2005	Osterballe M	Denmark	3 years old	486 persons	Questionnaire + Food challenge test	Food allergy	2.30%	Pediatric Allergy Immunol
			Aged ≥3 years	301 persons	Questionnaire + Food challenge test	Food allergy	1.00%	
			Adults	936 persons	Questionnaire + Food challenge test	Food allergy	3.20%	
2005	Penard-Morand C	France	School children (aged 9-11 years)	6,672 persons	Questionnaire	Food allergy	2.10%	Allergy
2006	Venter C	UK	1-year-old children	969 persons	Questionnaire + Open challenge test	Food allergy	5.50%	J Allergy Clin Immunol
					Questionnaire + DBPCFC	Food allergy	2.20%	
2006	Venter C	UK	6-year-old children	798 persons	Questionnaire + Open challenge test	Food allergy	2.50%	Pediatric Allergy Immunol
					Questionnaire + DBPCFC	Food allergy	1.60%	

fruits are common among those aged >6 years (Table 2).

3. PATHOLOGY, SYMPTOMS AND CLINICAL TYPES OF FOOD ALLERGY

3.1. PATHOLOGY OF FOOD ALLERGY

IgE is often involved in food allergies (IgE-mediated food allergy).[3] In some patients, symptoms develop via immunological mechanisms not involving IgE (non-IgE-mediated food allergy).[4] Both IgE-mediated and non-IgE-mediated reactions may be involved in the development of food allergies (mixed type food allergy).

Food provides essential nutrients for humans. The antigenicity of foods is reduced when they are digested into low-molecular substances. However, even in adults with mature digestive functions, the antigenicity remains to some extent after foods are absorbed into the living body. Orally ingested foods are foreign substances (non-self). If antigenicity remains, they should be immunologically eliminated, but are not eliminated. Healthy individuals have mechanisms for preventing allergic reactions to foreign food antigens, including a physicochemical barrier during food digestion and absorption in the digestive tract and an immunological barrier to reduce the antigenicity of foods absorbed in the digestive tract. The former includes digestion into low-molecular substances by digestive enzymes (e.g., pepsin) and denaturation by gastric acid. The latter includes the inhibition of

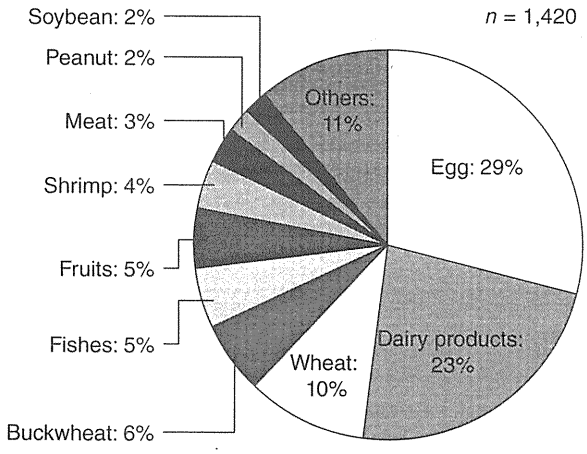

Fig. 1 Causative foods of immediate-type food allergy (national surveys by the Ministry of Health and Welfare during 1998-1999).

absorption of food antigens via secretory IgA and the establishment of oral immunotolerance to suppress allergic reactions to food antigens ingested from the digestive tract.[5]

In patients with food allergy, oral immunotolerance, which is normally established against orally ingested food antigens, may not be established or may be compromised after establishment. However, it is unknown why oral immunotolerance is not established in patients with food allergy.

Food allergy is common in infants because physical, biochemical and immunological barriers are underdeveloped during infancy.

3.2. SYMPTOMS OF FOOD ALLERGY

Symptoms of food allergy include skin, digestive, nasal, ocular, respiratory and systemic symptoms (Table 3).

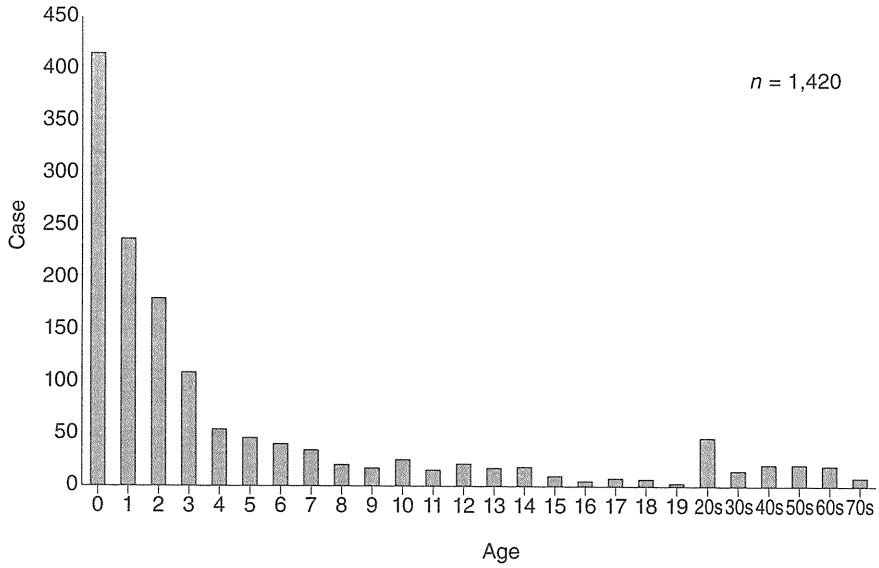

Fig. 2 Age distribution of immediate-type food allergy (national surveys by the Ministry of Health and Welfare during 1998-1999).

Table 2 Causative foods of immediate-type food allergy by age

	0 year (n = 416)	1 year (n = 237)	2-3 years (n = 289)	4-6 years (n = 140)	7-19 years (n = 207)	>20 years (n = 131)
No. 1	Egg	Egg	Egg	Egg	Buckwheat	Seafood
	47.4%	30.4%	30.8%	25.0%	14.0%	16.0%
No. 2	Dairy products	Dairy products	Dairy products	Dairy products	Shrimp	Shrimp
	30.8%	27.8%	24.2%	24.3%	13.0%	14.5%
No. 3	Wheat	Wheat	Wheat	Wheat	Wheat	Buckwheat
	9.6%	8.4%	12.1%	8.6%	10.6%	12.2%
Total	87.8%	66.6%	67.1%	57.9%	37.6%	42.7%

Table 3 Symptoms of food allergy by organ

Organ	Symptoms
Digestive system	Oral discomfort, lip swelling, abdominal pain, nausea and vomiting, diarrhea
Respiratory system	Sneezing, rhinorrhea, nasal congestion, coughing, wheezing, dyspnea, chest tightness, laryngopharyngeal edema
Eyes	Conjunctival hyperemia and edema, blepharedema, and lacrimation
Skin	Erythema, urticaria, angioedema, itch, burning sensation, blister, eczema
Nervous system	Headache
Urinary system	Hematuria, proteinuria, nocturnal enuresis
Systemic	Anaphylaxis

3.2.1. Skin Symptoms: Skin Symptoms Are Most Common in Food Allergy

(1) Urticaria and angioedema: Acute urticaria and angioedema are common. Rash often occurs within several minutes after ingestion, accompanied by itch.

(2) Atopic dermatitis: Atopic dermatitis is not caused by a single factor. There are various exacerbation factors. Many papers have been published regarding the involvement of food allergies. Reports of its incidence vary widely, depending on the methods used to select subjects (e.g., selection based on severity, history, specific IgE antibodies, or skin test results), methods used for the oral challenge test (open food challenge, double-blind, placebo-controlled food challenge (DBPCFC), and test timing, i.e., before or after the remission of skin symptoms).

3.2.2. Digestive Symptoms

(1) Immediate-type gastrointestinal allergy: Nausea, vomiting, abdominal pain, colic and diarrhea occur during food ingestion or at about 2 h after food ingestion. These are often accompanied by skin and airway symptoms. Some infants present with intermittent vomiting and poor weight gain. Most affected infants ($\geq$95%) are positive for specific IgE antibodies against causative foods and in a skin test.

(2) Oral allergy syndrome (OAS)[6]: OAS is caused by contact urticaria in the oral mucosa. IgE antibodies are involved. Itch, redness, tingling, swelling, etc., often occur in the mouth, lips, and throat mostly within 15 min after ingestion. Some patients present with systemic symptoms, such as throat constriction, generalized urticaria, cough, wheezing, dyspnea, and anaphylactic shock. These may be caused by food antigens absorbed from the oral mucosa and distributed throughout the body. OAS occurs in infants, schoolchildren, and adults. Common causative foods are fruits (kiwi, banana, melon, peach, pineapple, apple, etc.) and vegetables. OAS is often complicated by pollinosis. OAS complicated by pollinosis is called pollen-associated food allergy syndrome or pollen-food allergy syndrome (PFS). Reportedly, in Hokkaido (Japan), 16% of patients with birch pollinosis develop OAS due to fruits, such as apple.

(3) Eosinophilic gastroenteritis: Eosinophilic gastroenteritis is a rare disease with eosinophil infiltration in the intestinal mucosa from the esophagus to the rectum. Abdominal pain, nausea and diarrhea occur. Eosinophilic gastroenteritis is accompanied by malabsorption, protein leakage and iron deficiency anemia caused by intestinal hemorrhage. While an infiltration of eosinophils is usually localized to the mucous membrane, it may spread to submucosa or muscle layer, being complicated by eosinophilic ascites. Food allergy is involved in 25-50% of these cases.

(4) Neonatal and infantile gastrointestinal allergy: In Europe and America, several disease types have been reported, which mainly present with digestive symptoms and occur among newborns and infants, and in which IgE is not involved.[7,8] Many Japanese patients also fall into these categories regarding their symptoms and test results. However, some patients do not fall into any of these disease types. Thus, the Guideline Committee for Food Allergy in the Japanese Society of Pediatric Allergy and Clinical Immunology bracket together these food allergies, which mainly present with digestive symptoms and occur among newborns and infants, into "neonatal and infantile gastrointestinal allergy." Many patients are negative for IgE antibodies and are positive for an allergen-specific lymphocyte stimulation test (ALST). Thus, this disease may be mainly caused by the hyperreactivity of cellular immunity.

About 70% of patients develop symptoms during the newborn period, while some do at several months after birth. Half of neonatal patients develop symptoms until 7 days after birth. Symptoms may develop after the first milk ingestion on the day of birth. Common symptoms are vomiting, bloody stool, diarrhea, and abdominal fullness. Other symptoms include shock, dehydration, sluggishness, hypothermia, acidosis, and methemoglobinemia. Of note, some patients present with fever and positive CRP. Differential diagnosis of these patients from those with severe infections, such as bacterial enteritis, is difficult. Some patients develop neonatal transient eosinophilic colitis, which causes bloody stool immediately after birth (before nursing). This disease may occurs in utero.[9]

The most common causative food is cow's milk.

Table 4 Classification of food allergy

Clinical type		Age of onset	Common causative foods	Tolerance acquisition (remission)	Possibility of anaphylactic shock	Mechanism of food allergy
Neonatal and infantile gastrointestinal allergy		Neonatal and infantile period	Cow's milk (powdered milk for infants), soybean, rice	(+)	(±)	Mainly non IgE-mediated type
Infantile atopic dermatitis associated with food allergy†		Infancy	Egg, cow's milk, wheat, soybean, etc.	(+) in many cases	(+)	Mainly IgE-mediated type
Immediate-type (urticaria, anaphylaxis, etc.)		Infancy-adulthood	Infants-young children: egg, cow's milk, wheat, buckwheat, fishes, etc. School children-adults: crustacean shellfish, fish, wheat, fruits, buckwheat, peanut, etc.	Egg, cow's milk, wheat, soybean, etc.(+) Others (±)	(++)	IgE-mediated type
Specific type	Food-dependent exercise-induced anaphylaxis (FEIAn/FDEIA)	School age-adulthood	Wheat, shrimp, squid, etc.	(±)	(+++)	IgE-mediated type
	Oral allergy syndrome (OAS)	Infancy-adulthood	Fruits, vegetables, etc.	(±)	(+)	IgE-mediated type

† Some cases are complicated by digestive symptoms, such as chronic diarrhea, and hypoproteinemia. Foods are not involved in all cases of infantile atopic dermatitis.

Modified from Food Allergy Management Guideline 2008.

Others include soybean milk and rice. Some cases were fed by mother's milk or hydrolyzed whey formula.

Diagnosis is made based on i) development of digestive symptoms after causative food ingestion, ii) improvement and disappearance of symptoms by eliminating causative foods (positive elimination test), and iii) positive food challenge test.

To treat gastrointestinal allergy caused by cow's milk in an early stage, therapeutically effective products, such as amino-acid-based formula and extensively hydrolyzed formula, are preferably used.

The prognosis is relatively favorable. About 70% of patients acquire tolerance at 1 year of age, and about 90% acquire tolerance by their second birthday.

3.2.3. Respiratory Symptoms

Upper respiratory tract symptoms include symptoms of allergic rhinitis, such as nasal discharge, nasal congestion, and sneezing. Lower respiratory tract symptoms include symptoms of airway narrowing (wheezing) and laryngeal edema.

The Heiner syndrome is characterized by pulmonary hemosiderosis caused by milk,[10] Heiner syndrome a rare disease, which causes hemoptysis due to alveolar hemorrhage and features chronic cough, dyspnea, wheezing, fever, and bloody sputum, resulting in iron deficiency anemia. Precipitating antibodies against cow's milk proteins are detected in the sera of affected infants.

3.2.4. Ocular Symptoms

Symptoms of allergic conjunctivitis, such as conjunctival hyperemia and edema, blepharedema, and lacrimation, may occur.

3.2.5. Systemic Symptoms

(1) Anaphylaxis: Severe allergic symptoms occurring in multiple organs are called anaphylaxis. The most severe symptoms result in shock accompanied by decreased blood pressure and impaired consciousness. Causative agents of anaphylaxis, besides foods, include medicines, blood transfusion, bee, and latex. Food allergy is the most common cause. Food-induced anaphylaxis is an immediate reaction, in which IgE antibodies are involved. While symptoms usually occur within several minutes after ingestion, they occasionally occur 30 min or later. Symptoms may occur either in monophasic or biphasic. In Europe and America, causative foods of anaphylaxis include peanuts, nuts and seeds, seafood, eggs, and cow's milk. In Japan, they include eggs, cow's milk, seafood, shellfish, buckwheat, and peanuts in this order.

(2) Food-dependent exercise-induced anaphylaxis (FEIAn or FDEIAn): FEIAn is induced by exercise after food ingestion (mostly within 2 h after ingestion), but does not occur after either food ingestion or exercise alone. Nonsteroidal antiinflammatory drugs, such as aspirin, are an exacerbation factor. FEIAn occurs in an IgE-mediated manner.

The prevalence of FEIAn in schoolchildren and students is 0.0085%, i.e., one incidence per 12,000 per-

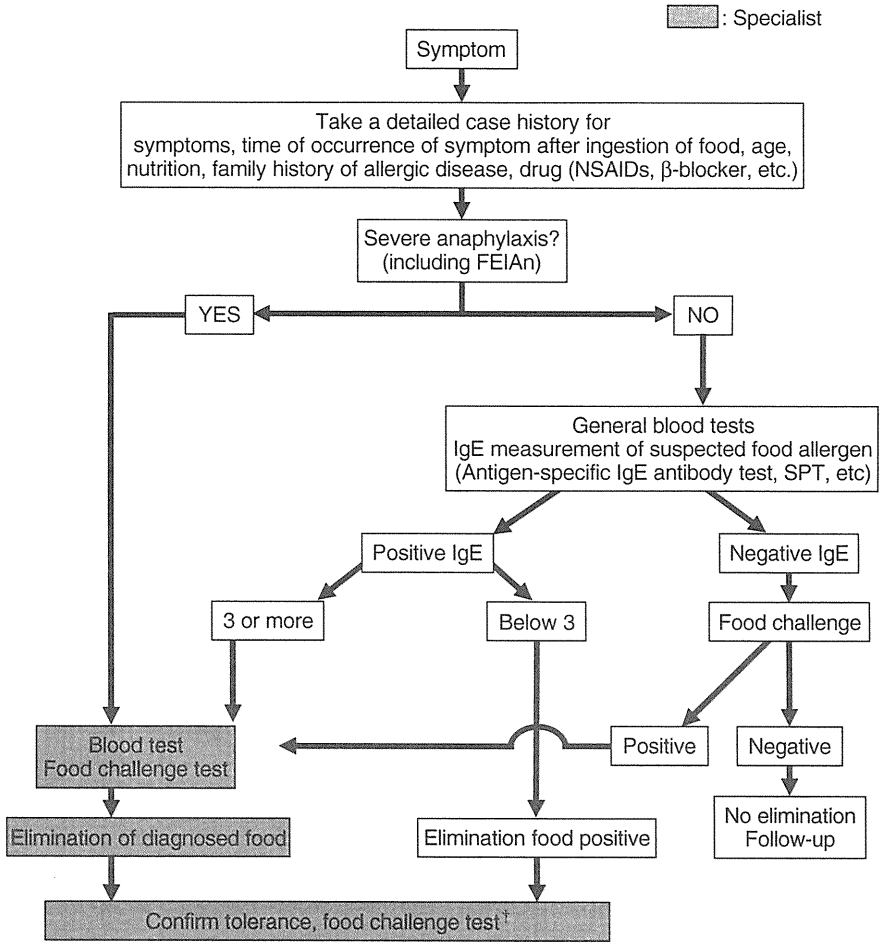

Fig. 3 Procedure for Diagnosis of Food Allergy (for "Immediate Type Reaction"). NSAIDs, non-steroidal antiinflammatory drugs; FEIAn, food-dependent exercise-induced anaphylaxis; SPT, skin prick test.
†Generally, patients who demonstrate immediate type reaction in later childhood are less likely to acquire tolerance.
Adapted from reference 12.

sons. FEIAn is most common among junior high school students, and is more common in males than in females (male-female ratio, 4 : 1). Common causative foods are shellfish (55%) and wheat products (45%).[11]

Definitive diagnosis can be made by presuming the causative foods through history taking, allergy testing, and checking hypersensitivity in a provocation test with food challenge followed by exercise loading. Few patients have a positive provocative test. In patients with negative results, consider administering aspirin before the food challenge.

3.3. CLINICAL TYPES OF FOOD ALLERGY

Four representative clinical types of food allergy are shown in Table 4, a revision to "Food Allergy Management Guideline 2008".[12]

"Neonatal digestive symptoms" in the Food Allergy Management Guideline 2008 was altered to "neonatal and infantile gastrointestinal allergy" after approval by the Guideline Committee for Food Allergy in the Japanese Society of Pediatric Allergy and Clinical Immunology.

Atopic dermatitis during infancy is often associated with food allergy, of which symptoms become immediate type and is usually resolved with aging. This type atopic dermatitis is called "infantile atopic dermatitis associated with food allergy." Common causative foods are eggs, cow's milk, wheat, and soybeans.

The food allergy which promptly develop after ingestion of causative food are "immediate-type food allergy which is common in young children to adulthood." The causative foods are buckwheat, peanuts, fish, curastacean shellfish, and fruits. Tolerance ac-