

Specific serum IgE in the diagnosis of egg and milk allergy in adults

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Levels of specific serum IgE to cow's milk, whole hen's egg, egg white, and egg yolk were compared to the outcome of double-blind, placebo-controlled food challenge (DBPCFC) with fresh egg and or milk in 21 adults with a case history of immediate hypersensitivity to egg and or milk. Specific serum IgE was measured by four different commercially available tests and by an inhouse Maxisorp RAST using freshly prepared food extracts.

Sensitivities and negative predictive accuracies were generally high with egg white and milk, but low with egg yolk. Specificities and positive predictive accuracies were low for all allergens and tests. Changing the cutoff levels did not improve the ability of the tests to predict clinical allergy. Among commercially available test allergens, egg white gave the most consistent results in levels and class scores, and the highest degree of concordance with DBPCFC, whereas egg yolk and milk varied more. Applying freshly prepared food extracts in Maxisorp RAST did not improve diagnostic value. Measuring specific serum IgE levels in control subjects tolerant to egg milk showed that false positive reactions occurred frequently among patients with another food allergy and atopic dermatitis, whereas most tests were likely to be negative in pollen-allergic and nonallergic volunteers. In conclusion, specific IgE measurements with egg white and milk were useful for exclusion of symptomatic hypersensitivity to egg and milk in patients with a positive history, whereas DBPCFC is still mandatory in patients with positive history and positive test. Measuring egg-yolk-specific IgE or using freshly prepared food extracts for specific IgE measurements added no further diagnostic information. The rate of clinically insignificant positive test results seems to be influenced by the prevalence of other food allergies and or atopic dermatitis in the population under study.

**A. Norgaard, C. Bindlev-Jensen,
P. Stahl Skov, L. K. Poulsen**

Food Allergy Unit, National University Hospital,
Copenhagen, Denmark

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Astrid Norgaard, MD
Food Allergy Unit TTA 7523
Medical Department TTA
National University Hospital
20 Tansvej
DK-2200 Copenhagen N
Denmark

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Immediate hypersensitivity to milk or egg is the most common food allergy in children (10, 11, 15, 20, 23, 34) and is also frequently reported among food-allergic adults (29, 31). It is well established that sensitized individuals who ingest milk or egg may develop allergic reactions such as asthma rhinitis, urticaria, angioedema, vomiting diarrhea, and anaphylaxis (6, 10, 11, 15, 20, 23, 29, 31, 34). Since these immediate allergic symptoms are believed to be IgE-mediated, the measurement of specific serum IgE has long been applied as a diagnostic tool in food allergy. In earlier studies, a significant relation between a history suggestive of food allergy and an elevated specific IgE was demonstrated; on the basis of these findings, many patients were treated with extensive elimination diets (1, 26). Introduction of the double-blind, placebo-controlled food challenge (DBPCFC) as the standard method for diagnosing

clinical food hypersensitivity in patients older than 2-3 years revealed that only 30-60% of patients reporting immediate hypersensitivity in combination with a positive specific IgE test result or skin prick test (SPT) react during challenge (4, 5, 11, 12, 29, 33). In comparison of specific IgE tests to clinical reactivity substantiated by DBPCFC, as recommended by the American Academy of Allergy and Immunology (28), correspondingly low specificities and positive predictive accuracies were found (5, 29, 33, 35); furthermore, the sensitivity was shown to depend on the applied allergen (33, 35). Recently, several new specific IgE tests have been introduced, the above-mentioned documentation of which is still lacking (2). We have previously reported a lack of concordance between DBPCFC and RAST as well as SPT and basophil histamine release (HR) performed with commercially available milk and egg

extracts (29, 30). Using fresh foods in SPT and HR improved concordance with DBPCFC, and this suggests that optimized and standardized food extracts may improve the diagnostic value of specific IgE tests.

This study evaluated four different commercially available specific IgE tests, and investigated whether the use of fresh foods applied in inhouse Maxisorp RAST would improve allergen-specific IgE measurement as a diagnostic test of clinical egg and milk allergy in adults.

Material and methods

Patients

Sera drawn from 44 adults were investigated: 21 patients with a case history strongly indicating type I egg/milk allergy and 23 control subjects. Ten of the 21 patients reported egg allergy only and five reported both egg and milk allergy, whereas six reported milk allergy only. All patients underwent detailed questioning, SPT, and DBPCFC, as described elsewhere (29). Clinical egg allergy was confirmed by DBPCFC in 9/15 subjects, and clinical milk allergy was confirmed in 4/11.

Egg-tolerant control subjects reporting frequent intake of egg with no symptoms comprised three groups:

Group B ($n = 7$): patients suffering from another type I (IgE-positive) food allergy confirmed by DBPCFC: five patients allergic to codfish, soy, or wheat, and two milk-allergic patients (from the DBPCFC-positive, milk-allergic group). In addition to reporting frequent intake of egg with no obvious symptoms, these patients were tested by DBPCFC and found to be DBPCFC-negative.

Group C ($n = 9$): patients allergic to grass or birch pollen, as evidenced by seasonal rhinoconjunctivitis and positive specific IgE and SPT.

Group D ($n = 7$): nonallergic volunteers (laboratory personnel).

Milk-tolerant control subjects reporting frequent intake of milk with no symptoms comprised three groups: **Group B ($n = 7$):** patients suffering from another type I (IgE-positive) food allergy confirmed by DBPCFC: five patients allergic to codfish, soy, or wheat, and two egg-allergic patients (from the DBPCFC-positive, egg-allergic group). Demographic characteristics of patients and control subjects are listed together with reported and verified symptoms in Table 1. Groups C and D were identical to the egg-tolerant control groups C and D.

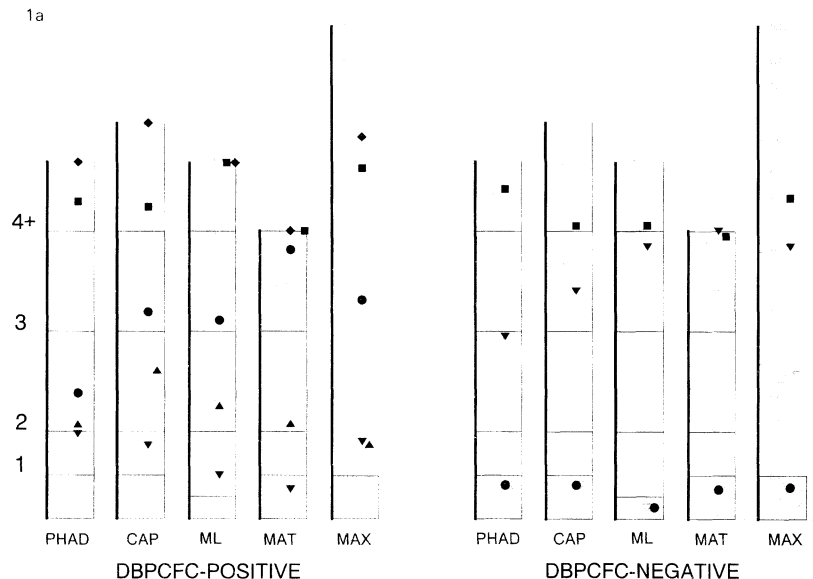
Allergenic material

Fresh cow's milk (Danish whole milk; pasteurized and homogenized, 3% fat and 3.5% protein) and hen's eggs bought locally were used for the challenges as well as in Maxisorp RAST. For Maxisorp RAST, the whole egg was mixed briefly, diluted 1 + 1 in PBS (sodium phosphate 0.1 M, sodium chloride 0.15 M, sodium azide 10 mg/ml, pH 7.4), and stirred slowly for 1 h. For egg-white extraction, an egg was separated carefully without mixing white and yolk. The egg white was treated as described above for whole egg. For egg yolk, an egg was separated carefully without mixing white and yolk. The yolk was rolled in moisture-absorbent tissue paper to absorb the adherent liquid white from the surface. The intact vitelline membrane was then pierced and liquid yolk was aspirated from the interior of the sac, thereby avoiding egg-white contamination. The yolk was extracted in PBS as described above. Freshly prepared crude extracts were diluted in PBS, as described below, immediately before their use in the Maxisorp RAST. The protein contents of the diluted extracts were measured spectrophotometrically using the Bio-Rad Protein Assay by comparison to oval-

Table 1. Demographic characteristics of patients and control subjects. Age and total IgE are given as median values with ranges in parentheses. Total IgE in kU/l, normal range 0–150. AD: atopic dermatitis (not related to food intake); RESP: respiratory symptoms; GI: gastrointestinal symptoms; CUT: cutaneous symptoms (see Material and methods/challenges). Control group B: for milk allergy, includes two of egg-allergic patients and five patients allergic to soy, wheat, or codfish. Control group B for egg allergy includes two of milk-allergic subjects and same five patients allergic to soy, wheat, or codfish

		Age (years)	Sex	Total IgE	AD	Symptoms on challenge		
						RESP	GI	CUT
Milk	DBPCFC-positive ($n=4$)	37 (29–46)	2 F/2 M	696 (285–738)	2	1	4	1
	DBPCFC-negative ($n=7$)	25 (18–30)	7 F	436 (5–12 522)	4	–	–	–
Egg	DBPCFC-positive ($n=9$)	26 (16–39)	8 F/1 M	738 (238–5219)	5	5	9	4
	DBPCFC-negative ($n=6$)	22 (20–26)	3 F/3 M	1787 (188–9551)	4	–	–	–
Control	B ($n=7$)	30 (19–47)	5 F/2 M	285 (52–5666)	4	–	–	–
	C ($n=9$)	36 (30–50)	3 F/6 M	50 (21–389)	1	–	–	–
	D ($n=7$)	32 (24–42)	6 F/1 M	14 (2–111)	–	–	–	–

Egg-white-specific IgE



bumin, grade III, or bovine serum albumin, grade III (Sigma Chemical Co, St Louis, MO, USA).

Challenges

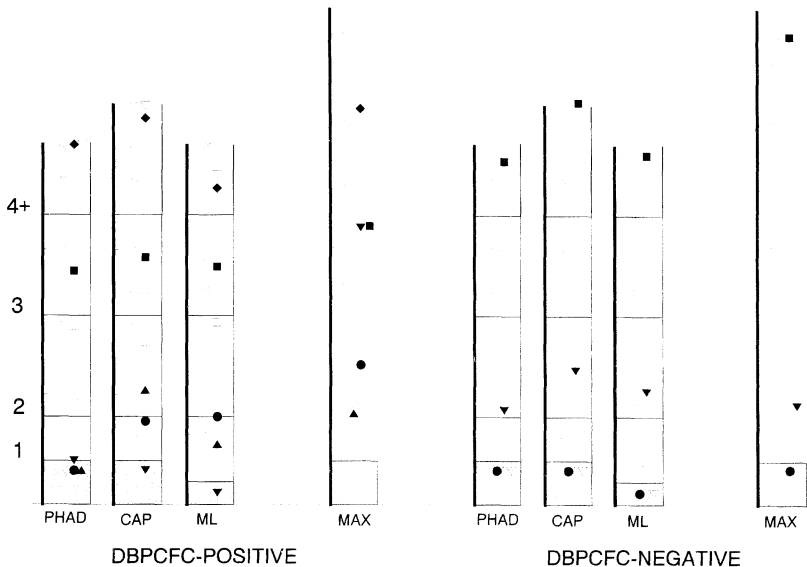
As described previously (29, 30), titrated DBPCFC consisted of liquid challenge doses using fresh cow's milk and whole hen's egg, starting at 5 mg in a vehicle of Vivonex elemental diet and fruit juice, and administered as 10-fold increasing doses at 30-min intervals until the appearance of objective symptoms or a maximum cumulated dose of 55 g of egg or 250 g of milk was reached. A challenge was considered positive if objective allergic symptoms signs occurred within 2 h after an active challenge, and no symptoms occurred after the corresponding placebo challenge. The following symptoms were observed: gastrointestinal: oropharyngeal itching and swelling, nausea vomiting, abdominal pain, diarrhea; respiratory: rhinitis with or without conjunctivitis, asthma; cutaneous: urticaria, angioedema. All reactions occurred within 40 min after challenge, and generally resolved within 1–3 h.

Commercially available tests for specific IgE

Serum was drawn prior to DBPCFC and stored at -20°C until analysis. Specific IgE to egg white, egg yolk, and milk was measured by four commercially available test kits: Phadebas RAST[®] and CAP RAST[®] (Pharmacia, Uppsala, Sweden); Magic Lite (ALK Laboratories, Horsholm, Denmark); Matrix[®] Food Aero (Abbott Laboratories, Abbott Park, IL, USA). The commercially available test kits were performed according to the manufacturers' instructions. In Fig. 1, each test has its own separate y-axis to display the different companies' units, and the class shifts have been aligned calibrated to compare visually the patients' class scores of the different tests. This was done because the specific IgE units cannot be compared numerically, as levels of allergen-specific IgE are measured by comparison with standard sera unique to each company laboratory. Moreover, the company laboratory assigns its own unit system to express the relative content of allergen-specific IgE in the samples. The companies categorize the test result in allergy classes ranging from 0

Egg-yolk-specific IgE

1b



to 4, 5, or 6, indicating increasing amount of specific IgE. For all four commercially available tests, the unit level of the shift between class 3 and 4 can be divided by 5 to obtain the class 2–3 shift, and by 25 to obtain the class 1–2 shift. For Phadebas, CAP, and Matrix it can further be divided by 50 to obtain the class 0–1 shift, while it must be divided by 70 for Magic Lite. All unit test results categorized by the companies as class 4, 5, or 6 were recorded as 4+ in Fig. 1.

Maxisorp RAST

Maxisorp RAST was performed essentially as described before (32). Briefly, to test tubes ("Maxisorp", NUNC, DK-4000 Roskilde, Denmark) was added 250 µl of the fresh food extracts appropriately diluted in PBS 7.4, and incubated overnight. After washing, serum samples were diluted (PBS plus 0.1% Tween-20), added to the test tubes, and incubated overnight. On the next day, the tubes were washed and ^{125}I -labeled anti-IgE (Pharmacia, Uppsala, Sweden) was added. After overnight incu-

bation, samples were washed and counted in a gamma-scintillation counter. Dose-response experiments showed optimal protein concentrations for the coating as follows: whole egg and egg yolk (approximate protein concentrations in parentheses): 1:2000 (65 µg/ml), egg white 1:20 000 (4 µg/ml), and cow's milk: 1:7000 (5 µg/ml). The test sera showed dilution curves parallel to the reference serum, and results were expressed as sorbent units (SU) relative to the reference serum (32). For whole egg, egg white, and egg yolk, the reference serum was that of a DBPCFC-positive patient. For milk, initial experiments were carried out with both a DBPCFC-positive serum and a DBPCFC-negative serum, showing very similar results (below interassay variation); thereafter, the DBPCFC-negative serum was used. With the reference serum in each allergen system, a maximum binding of specific IgE of 30–40% could be obtained, whereas a pool of normal human serum (RAST-negative sera from blood donors) and sera from grass- or birch-pollen-allergic patients bound 0.3–0.4% (signal-to-noise ratio 100). Preincubation of the egg/milk-positive reference serum

Milk-specific IgE

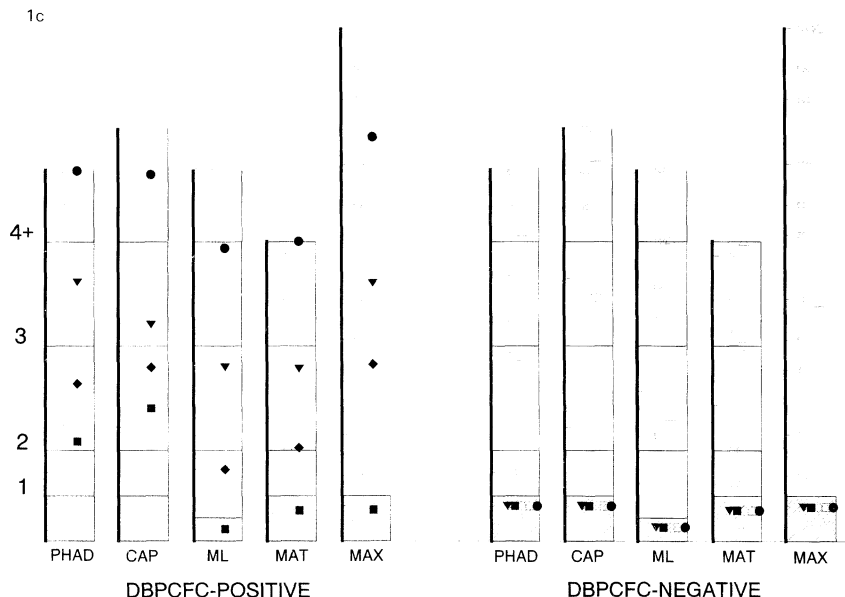


Fig. 1a-c. Serum levels of allergen-specific IgE in DBPCFC-positive and DBPCFC-negative patients as measured by four commercially available test kits and Maxisorp RAST (MAX) in SU/ml (see Material and methods). PHAD: Phadebas RAST[®] in PRU/ml; CAP: Pharmacia CAP system[®] in kU/l; ML: Magic Lite[®] in SU/ml; MAT: Matrix Food AeroTM in IU/ml. Separate logarithmic y-axis is displayed for each test system, and class 3, 4, 2, 3, and, where possible, class 1, 2 shift has been aligned to compare visually class scores in different tests. Axis break denotes upper detection limits, whereas hatched area denotes lower cutoff. In both patient groups, each symbol refers to one particular patient.

with the allergen extracts used for coating showed a dose-related inhibition, reaching a maximum of 90–99% at 0.1–1 mg/ml in the four allergen systems. Preincubation of the serum with an inhalant allergen (10^2 – 10^5 SQ grass or birch pollen, Aquagen[®], ALK Laboratories, DK-2970 Hørsholm, Denmark) to which the patient was also sensitized produced no inhibition. In Maxisorp RAST using grass birch as coating allergen, the serum showed a strongly positive reaction for the relevant pollen allergen, which could be inhibited by the coating allergen, but not by the fresh milk/egg extracts (to which the patient was also sensitized). The Maxisorp RAST using fresh food allergen extracts was thus considered of sufficient specificity. When three sera were run in duplicate on three different days, new food extracts being prepared each time, the intra-assay mean coefficient of variation (CV) was 5–7%. The interassay CV

varied between 20% (whole egg) and 29% (white). Calculation of the CV on the percent binding of radioactivity gave similar results.

Statistics

Diagnostic performance was described as the ability of a test to predict the outcome of DBPCFC in patients selected by a history indicating type I allergy. The performance parameters listed below thus cannot be transferred to an unselected population. Sensitivity (SE) was calculated by dividing the number of positive DBPCFCs correctly identified by the test by the total number of positive DBPCFCs, and the specificity (SP) was calculated by dividing the number of negative DBPCFCs correctly identified by the test by the total number of negative challenges. Positive predictive accuracy (PPA) was cal-

culated by dividing the number of positive DBPCFCs correctly identified by the test by the total number of positive test results, and negative predictive accuracy (NPA) was calculated by dividing the number of negative DBPCFCs correctly identified by the test by the total number of negative test results. Levels of specific IgE in DBPCFC-positive and -negative patients were compared with the Mann-Whitney U nonparametric test, defining a P value of 0.05 as statistically significant. Spearman's correlation coefficient was used to test the nonparametric association between continuous variables (double negative results were omitted from calculations). The SPSS statistical software was used for the calculations.

Results

Fig. 1 shows the test results in units and classes that each patient obtained in the different tests.

Egg-white-specific IgE

Levels of egg-white-specific IgE in sera from the 15 patients suspected of egg allergy (nine DBPCFC-positive) measured by the five different tests are shown in Fig. 1a. Egg-white-specific IgE was positive in all nine DBPCFC-positive patients in Phadebas, CAP, Magic Lite, and Maxisorp, and was positive in 8/9 patients in Matrix. In DBPCFC-negative patients, Phadebas, CAP, and Magic Lite detected egg-white-specific IgE in 4/6 patients, whereas Matrix and Maxisorp detected IgE in 5/6. Diagnostic performance parameters (SE, SP, PPA, and NPA) of the tests in the patients with a positive case history are listed in Table 2 together with the performance parameters when control subjects were included. SE and NPA were high in all tests, accompanied by a low SP and PPA. Thus, DBPCFC-positive patients could not be distinguished by the type of answer (negative/positive), but a negative test result with Phadebas, CAP, Magic Lite or Maxisorp excluded a positive challenge in patients with a positive case history. Due to the considerable overlap of the levels of specific IgE between the DBPCFC-positive and DBPCFC-negative group observed in all tests (3/6 DBPCFC-negative patients being scored as class 2 or more), no improvement in diagnostic performance could be obtained in any test by raising the cutoff. The groups also could not be distinguished by the level of specific IgE (difference between group levels not significant). Correlation between the units measured by the different egg-white-specific tests was high, r_s being between 0.77 and 0.93, $P < 0.0001$ (listed in Fig. 2). However, the tests did not score the patients uniformly in allergy classes. Fig. 2 summarizes the pattern of deviation in class score per al-

Table 2. Diagnostic performance parameters of specific IgE tests. PHAD: Phadebas RAST; CAP: Pharmacia CAP; ML: Magic Lite; MAT: MATRIX Food Aero; MAX: Maxisorp RAST; SE: sensitivity; SP: specificity; PPA: positive predictive accuracy; NPA: negative predictive accuracy (see text: Calculations and statistics); subscript_{DBPCFC}: parameter calculated in both case-history-positive patients and in all three groups of control subjects. B, C, and D (see Material and methods)

Antigen	Parameter	PHAD	CAP	ML	MAT	MAX
Egg white	SE	1.00	1.00	1.00	0.89	1.00
	SP	0.33	0.33	0.33	0.17	0.17
	SP _{DBPCFC}	0.79	0.79	0.72	0.79	0.72
	PPA	0.69	0.69	0.69	0.61	0.64
	PPA _{DBPCFC}	0.60	0.60	0.53	0.57	0.53
	NPA	1.00	1.00	1.00	0.50	1.00
	NPA _{DBPCFC}	1.00	1.00	1.00	0.96	1.00
Egg yolk	SE	0.67	0.89	0.89	–	0.89
	SP	0.50	0.50	0.50	–	0.17
	SP _{DBPCFC}	0.86	0.83	0.76	–	0.79
	PPA	0.67	0.73	0.73	–	0.62
	PPA _{DBPCFC}	0.60	0.62	0.53	–	0.57
	NPA	0.50	0.75	0.75	–	0.50
	NPA _{DBPCFC}	0.89	0.96	0.96	–	0.96
Milk	SE	1.00	1.00	0.75	0.75	0.75
	SP	0.71	0.71	0.71	0.71	0.71
	SP _{DBPCFC}	0.90	0.87	0.70	0.90	0.90
	PPA	0.67	0.67	0.60	0.60	0.60
	PPA _{DBPCFC}	0.57	0.50	0.25	0.50	0.50
	NPA	1.00	1.00	0.83	0.83	0.83
	NPA _{DBPCFC}	1.00	1.00	0.95	0.96	0.96

lergen and test. CAP and Magic Lite totally agreed in the class scores, and a good agreement was also observed between Phadebas vs. CAP and Magic Lite, respectively. Matrix scored about 50% of the patients as different, and, as the only test, scored 7–21% at two classes' distance from the score given by the three other tests.

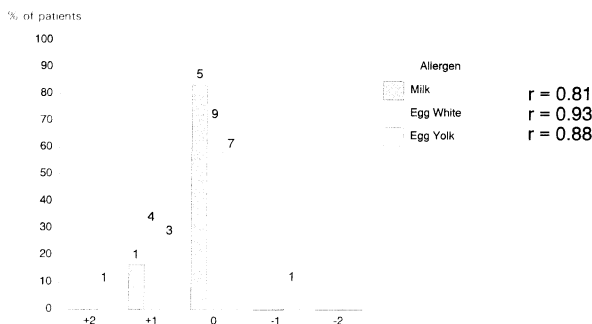
Egg-yolk-specific IgE

Egg-yolk-specific IgE levels in the same patients are shown in Fig. 1b. Phadebas detected egg-yolk-specific IgE in 6/9 patients, and CAP, Magic Lite, and Maxisorp detected egg-yolk-specific IgE in 8/9 patients reacting to whole egg in DBPCFC. In DBPCFC-negative patients, Phadebas, CAP, and Magic Lite in agreement detected specific IgE in 3/6 patients, while Maxisorp detected specific IgE in 5/6. SE and NPA were lower than for egg white, and still with a low SP and PPA (Table 2). Identification of DBPCFC-positive or -negative patients from the type of answer (positive/negative) was impossible due to these low performance parameters. Combining the white- and yolk-specific test results as *both* white and yolk positive (PPA 0.64–0.72, NPA 0.50–1.00) or as *white or yolk* positive (PPA 0.64–0.79, NPA 1.00) added no further diagnostic information.

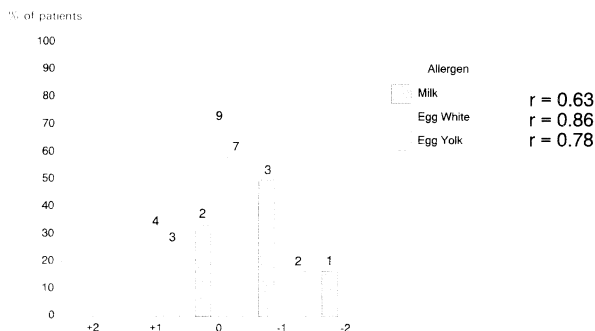
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Difference in class scores

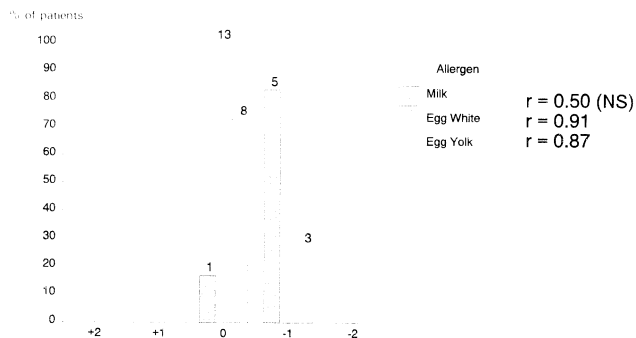
PHA
vs
CAP



PHA
vs
ML



CAP
vs
ML



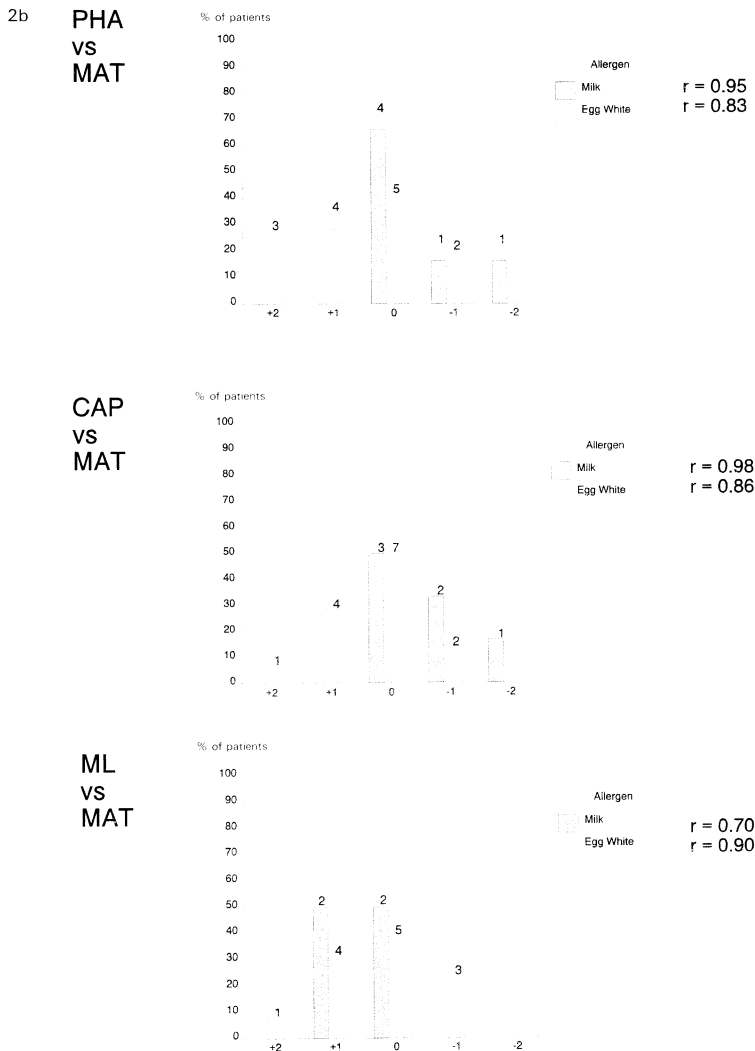


Fig. 2a–b. X-axis: difference in class scores when subtracting Phadebas (PHA) from CAP, Phadebas from Magic Lite (ML), CAP from Magic Lite, Phadebas from Matrix (MAT), CAP from Matrix, and Magic Lite from Matrix, respectively. Each bar filling represents different allergens, as indicated right. Height of bars indicates percentage of test-positive subjects scored uniformly, or one or two classes higher or lower, respectively, when going from one test to another. Numbers above bars indicate the actual number of subjects. Spearman's rank correlation coefficient for correlation between units is indicated on right for each allergen. Double negative test results were omitted from rank calculations. P values for coefficient are < 0.0001 , unless indicated otherwise. NS: not significant, $P > 0.05$.

Table 3 Number of positive specific IgE test results in three different groups of control subjects: B = allergic to another food; C = pollen allergic; D = nonallergic. See Material and methods: patients. Class scores: positive results were either class 1 or 2. Maxisorp RAST was not scored in classes

Egg white	Phadebas	CAP	Magic Lite	Matrix	Maxisorp
B (n = 7)	2	2	2	1	2
C (n = 9)	0	0	2	0	1
D (n = 7)	0	0	0	0	0
Egg yolk					
B (n = 7)	1	2	2		0
C (n = 9)	0	0	2		1
D (n = 7)	0	0	0		0
Milk					
B (n = 7)	1	2	4	1	1
C (n = 9)	0	0	1	0	0
D (n = 7)	0	0	2	0	0

The level of yolk-specific IgE was lower (difference not significant) than the level of egg-white-specific IgE in 13/15 patients (one DBPCFC-positive and one DBPCFC-negative patient excepted), and there was no significant difference between DBPCFC-negative and -positive patients. The three yolk-specific IgE tests correlated less well than egg-white-specific tests, r_s ranging from 0.53 ($P < 0.05$) to 0.88 ($P < 0.0001$), and the class score agreed in 55–75% of the patients, somewhat less than for egg white.

Milk-specific IgE

Milk-specific IgE levels are shown in Fig 1c. Phadebas and CAP in agreement identified the four DBPCFC-positive patients, whereas Magic Lite, Matrix, and Maxisorp identified only 3/4 patients, the three tests missing the same patient. In DBPCFC-negative patients, Maxisorp was positive in 3/7, and the other tests in 2/7. In Phadebas and CAP, SE and NPA were 1.00, thus predicting a negative DBPCFC in 5/7 patients, whereas PPA was almost equally low in all tests. No improvement could be obtained by raising the cutoff. The differences between the levels of specific IgE were not statistically significant in any of the tests. The correlation between the milk-specific tests varied between $r_s = 0.50$ (not significant) and $r_s = 0.98$; $P < 0.0001$ (Fig. 2).

Test results in control subjects

The number of positive test results in egg milk-tolerant controls is listed in Table 3. In group B (allergic to another food), 1–2/7 subjects had positive test results in almost all allergens and tests. In group C (pollen-allergic subjects), all tests gave negative results except Magic Lite (egg white and yolk, positive in 2/9 patients, and milk, positive in 1/9), and Maxisorp (egg white and yolk, positive in 1/9).

Group C (nonallergic subjects) had negative test results in all five white-specific tests and in the four yolk-specific tests, whereas Magic Lite with milk was positive in 2/7.

Discussion

Few studies have determined the predictive indices of measurement of specific IgE in food-allergic adults as compared to DBPCFC (4, 5, 13, 17, 29). We compared the levels of specific serum IgE to cow's milk, whole egg, egg white, and egg yolk measured by various methods with the outcome of DBPCFC in adults strongly suspected of egg and/or milk allergy. DBPCFC was considered the standard in diagnosing IgE-mediated food allergy, and only immediate reactions were studied. Other allergic mechanisms possibly influencing the outcome of DBPCFC were not considered in this study. The study included a small number of patients selected by case histories of moderate to severe clinical food allergy. Therefore, the predictive indices (especially SP/PPA) thus obtained therefore cannot be transferred to a general population, but show the ability of the tests to identify clinical disease in a "model group" of clinically food-allergic adults. We consider the SE NPA obtained in these adults important parameters because a test must at least "have the diagnostic capacity to reveal clinical allergy" (16) in moderate to severe clinical disease, before being applied in epidemiologic diagnostic studies comprising individuals with different degrees of clinical disease, and because it is not known whether diagnostic performance parameters obtained in pediatric populations can be transferred to adults. Several factors can be expected to influence the value of specific IgE measurements: the applied allergen, the quality of the method and the allergen extract, and the clinical setting.

Allergens

Egg white. Studies investigating predictive indices of egg-white specific IgE in children (Phadebas RAST compared to DBPCFC or controlled challenges in small children) have shown PPA from 0.40 to 0.71, and NPA from 0.82 to 0.99, rendering them useful for exclusion of symptomatic hypersensitivity (1, 10, 31, 33). In agreement with these studies, Phadebas RAST, CAP, Magic Lite, and Maxisorp RAST showed a maximal NPA of 1.00 in case-history-positive patients, and, among these, Phadebas RAST and CAP showed the highest PPA in both case-history-positive patients and overall PPA (including all controls). Egg white was the extract showing the highest degree of units correlation between the tests, probably because it is a stable, well-characterized

extract. However, a low SP was generally observed, perhaps indicating a degree of unspecific binding.

Egg yolk. CAP showed the highest observed, although not maximal, NPA of 0.75 in combination with the highest PPA in both case-history-positive patients and overall. The egg yolk extract generally showed the lowest degree of units correlation and a high degree of diversity among class scores between the tests. The egg white has been regarded as the source of allergens in the avian egg (1, 9, 21, 25–27), but recent studies have established the existence of specific yolk allergens (3, 36). In this study, DBPCFC was performed with fresh whole egg, and none of the case histories indicated exclusive reaction to yolk or white. Still, the lower SE obtained by the yolk-specific IgE tests might be caused by the fact that the patient is clinically sensitive only to egg white. A whole-egg-specific IgE test was not available, but would be preferable, since this represents the allergen mixture most commonly ingested by the patient. When this is not available, and there is no suspicion of separate sensitization, an egg-white-specific IgE test should be preferred, since the yolk-specific tests alone or combined with the white-specific tests added no further diagnostic information.

Milk. Studies investigating the predictive indices of milk-specific IgE in children (Phadebas RAST compared to DBPCFC or controlled challenges in small children) have shown PPAs from 0.27 to 0.81 (1, 7, 10, 33, 35), and NPAs from 0.50 to 0.94. In contrast to the high NPAs generally seen with egg white, NPAs observed in some studies are lower, rendering milk-specific IgE less useful for exclusion of symptomatic hypersensitivity. Phadebas RAST and CAP showed maximal NPA of 1.00 in case-history-positive patients, making them excellent for excluding symptomatic hypersensitivity to milk. They had a similar PPA in case-history-positive patients, although Phadebas RAST has a slightly higher overall PPA.

In Magic Lite, Matrix, and Maxisorp RAST, there was a considerable variation in diagnostic performance parameters, correlation between units, and class score from test to test, indicating variation in the different milk extracts used, and perhaps that one or more components were absent or present in a too low concentration. In Magic Lite, an unexpectedly high number of false positive test results (30%) in the control subjects was observed compared with 4–9% in other tests. Taken together with the missed DBPCFC-positive patient, this suggests that simply changing the cutoff will not improve the diagnostic performance of the test, but that the allergen extract composition should be investigated.

Methods

Phadebas RAST showed the best diagnostic performance except with egg yolk, which had a low sensitivity. If we assume that all DBPCFC-positive patients were sensitive to egg yolk, and that that extract was the same as the one used in CAP, this may be due to the coupling procedure. CAP tended to score the patients a little higher in all allergens than did Phadebas RAST (Fig. 2); correspondingly, CAP added a few more false positive results in the control group. Magic Lite scored 1–2 pollen-allergic control subjects positive with all three food allergens; with milk, false positive results were observed in all control groups, rendering Magic Lite with milk less useful as a diagnostic test. Matrix was not maximally sensitive in any of the two available allergens; taken together with the lowest observed frequency of false positive results in the control subjects, this suggests that the allergen concentration or the cutoff might be increased.

We previously reported an improvement in the diagnostic performance of SPT and HR when commercially available (not standardized) extracts were replaced with freshly prepared extracts of milk and egg (30). No improvement was seen with these extracts in the inhouse RAST, a finding confirmed by another study with fresh codfish (18). This might be caused by the allergen content in the fresh foods, by interfering factors such as lipids, or by a different degree of coupling of the single allergens in the mixture. However, due to the small number of patients, only major degrees of improvement may be detected.

Interpretation of the allergen-specific IgE test result

The use of the allergen-specific IgE tests in relation to IgE-mediated food allergy may be applied to three different clinical settings: for verification of a positive case history of food allergy, for longitudinal monitoring of the level of specific IgE, and for screening a certain population for food allergy.

Verification of a positive case history. When there is a clear case history indicating egg or milk allergy, a negative test result could exclude clinical allergy and thus replace DBPCFC due to maximal NPA, whereas a positive test result must be followed by DBPCFC due to the low PPA. In the population consisting of case-history-positive patients, only the test PPA was 0.60.

Longitudinal monitoring of the level of specific IgE. The level of specific IgE has been found to correlate with the clinical course in milk-allergic children (22, 24). Giving the result in units is necessary for this kind of survey, whereas no diagnostic information regard-

ing the outcome of DBPCFC in the adult population under study was obtained from the level of specific IgE, and scoring the result as positive or negative was equally informative for this purpose. The allergy class score has been found to be of value in children with atopic dermatitis (33), whereas this was not the case in the adult population under study.

Screening a certain population for food allergy. A negative test result could be used to exclude clinical egg or milk hypersensitivity due to a high NPA. However, the PPA was considerably lower. According to Bayes' theorem, the PPA varies with the prevalence of the disease. This means that by extrapolation of the PPA of 0.60 found in this population with a disease prevalence of 0.47 to a population with a prevalence of, for example, 10 or 1%, the PPA will be greatly reduced, since the absolute number of false positive test results affects the PPA heavily (8). However, correcting for the prevalence in this way demands that the SP derived from the study population be considered a constant when extrapolating to the larger, unselected population. In this study population, a low SP was observed due to a high number of subjects with clinically irrelevant positive test results (other food allergies/atopic dermatitis). When pollen-allergic and nonatopic control groups were added to the calculation, an increase in SP was observed. Few reports are available on the prevalence of specific IgE against milk and egg in nonatopic individuals. In a study of 262 consecutive blood donors, the prevalence of milk- or gliadin-specific IgE $\geq$ class 2 was 2.3%, i.e., an SP of approximately 0.98 (14). Wüthrich et al. showed an SP of milk RAST $\geq$ class 2 of 0.99 in 60 atopic and 40 nonatopic control subjects (37), and Hattevig et al. found a RAST class $\geq$ 1 to milk in 2% and to egg in none of 59 nonatopic children (19). Our data showing a low false positive rate among nonatopic subjects confirm these results and suggest that if the test is applied in an unselected population, or a population attending an outpatient allergy clinic, a much higher specificity can be expected due to another composition of the population, resulting in a virtually unchanged PPA. This is confirmed by the study of Onorato et al. comprising 300 consecutive patients referred to an asthma clinic, where an SP of RAST with egg of 0.98 and a PPA of 0.46 (prevalence of positive DBPCFC to egg 4%) were found when screening for food allergy by positive skin prick test, RAST, or case history, and establishing clinical diagnosis by DBPCFC (31).

By case history, one may view a population attending an outpatient clinic as divided into four different categories: 1) patients with positive case history indicating egg/milk allergy, 2) an intermediate group with a vague history perhaps because of per-

sistent symptoms (with or without atopic dermatitis) and/or other known food allergy, 3) patients with other allergic diseases and no case history, and 4) nonatopic individuals.

If one chooses to test an unselected population, the prevalence of food allergy will be low, but the SP will be relatively high due to a low number of false positives. If one chooses to test, for example, groups 1 and 2 mentioned above, the SP will be much lower, but the prevalence will be higher. Thus, when one moves from selected groups with high prevalences to larger and more unselected groups, the rise in SP tends to counterbalance the drop in prevalence affecting the PPA.

In conclusion, specific IgE measurements with egg white and milk were useful for exclusion of symptomatic hypersensitivity to egg and milk in patients with a positive history (NPA=1.00), whereas DBPCFC is still mandatory in patients with positive history and positive test. Measuring egg-yolk-specific IgE or using freshly prepared food extracts for specific IgE measurements added no further diagnostic information. The rate of clinically insignificant positive test results was influenced by the prevalence of other food allergies and/or atopic dermatitis, whereas few false positive results were observed among pollen-allergic and nonatopic subjects. Changing the cutoff did not improve the diagnostic performance of the test in this small group of patients, but a larger sample size is needed to confirm this conclusion. If one applies a sensitive method containing the clinically relevant allergens, a PPA of 0.40–0.60 can generally be expected.

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