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Comparison of the Results of Skin Prick Tests, CAP System and ENEA System in the Diagnosis of Food Allergy

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The only accepted test to confirm an adverse reaction to food is the double-blind, placebo-controlled food challenge (DBPCFC). Once identified the food responsible for the reaction, the diagnosis of IgE-mediated food allergy is strongly supported by the demonstration either directly or indirectly (by skin tests) of the presence of specific IgE to that food [1].

It is well known that neither the cutaneous nor the serological method in use has a high diagnostic reliability in food allergy. This could be explained in part by insufficient standardization of food allergens and by the low sensitivity and/or specificity of the methods used [2–6].

The DBPCFC should not be applied in the case of history of severe systemic reactions to foods. Moreover, the DBPCFC may be avoidable for the diagnosis of the oral allergy syndrome when the clinical history is unequivocal. In this last case, the history can be confirmed by skin tests or by the presence of specific IgE and eventually by challenge tests.

The aim of our study is to evaluate the diagnostic reliability of a new, completely automated system, the ENEA system, for the detection of specific IgE antibodies to food allergens.

Outpatients referred to our clinic for the suspicion of food allergy were screened by skin prick test (SPT) and/or CAP system, and submitted to DBPCFC in order to differentiate patients with food allergy and patients without clinical food allergy. Twelve patients did not undergo the challenge because they had previous severe reactions and their history unequivocally indicated intolerance to the causative food.

Toward this end we have studied a population of 59 adults (36 female, 23 male) aged between 16 and 65 years (average 32). Based on unequivocal history and challenge results, the study population was divided into two groups: those with clinical food allergy (31 patients) and those without clinical food allergy (18 patients and 10 nonallergic controls). Control subjects had negative history of allergies to foods, to inhalants, to chemicals and to drugs. All the subjects under study were submitted to SPT, CAP system and ENEA system with the following foods: cow's milk, egg white, wheat, peanut, corn, pea, and soya.

DBPCFC

DBPCFC were performed in the fasting state. Increasing amounts of the lyophilized or powdered food or placebo (dextrose) were administered in opaque capsules. The suspected food and the placebo were administered on alternate days, starting with one capsule. The dose was then doubled every 30 min (or at different intervals, depending on the history) until the appearance of symptoms or a maximum cumulative dose of at least 15 g. In case of negative result of the DBPCFC with capsules, a second DBPCFC was performed in patients with suspected wheat intolerance, by masking wheat flour in minced meat in a sufficient portion to blind 100 g of wheat flour. In this case, the minced meat without wheat was administered as placebo. All negative challenges were confirmed by an open feeding.

SPTs

All subjects underwent SPTs with commercial food extracts 1:20 w/v (DHS, Slough, UK); a 1-mm tip standard lancet (Allergy Pricker, DHS) was used with the seven investigated food allergens. SPTs were done on the volar surface of the subject's forearm. To be considered positive, an SPT had to produce a wheal reaction graded at least 2+ (i.e., 50% of the one elicited by a solution of histamine hydrochloride 10 mg/ml). SPTs with a wheal mean diameter < 3 mm were considered negative.

CAP System

The CAP system (Pharmacia Diagnostic, Uppsala, Sweden) was performed according to the manufacturer's instructions.

ENEA System

The ENEA system (Bioallergy, Fiumicino, Italy) is a fully automated system, capable of performing an immunoenzymatic assay and the final photometric measurement of the resultant color change [7]. To the inner walls of the reactive chambers (ACE = allergy chamber enzymatic) are coupled the purified allergens. IgE in the serum sample forms a specific complex with the insolubilized allergens, which is subsequently reacted with monospecific anti-IgE antibody conjugated to urease enzyme. Bound enzyme, in a final incubation with urea substrate, causes a rise in pH and a yellow to purple change in the color of the Bromocresol Purple indicator. The intensity of the color is directly proportional to the concentration of spe-

Table 1. Absolute values and classes of specific IgE antibodies, detected by CAP and ENEA

CAP		ENEA	
kU/l	class	U/ml	class
<0.35	0	<0.35	0
0.36-0.7	1	0.36-0.7	1
0.71-3.5	2	0.71-3.5	2
3.51-17.5	3	3.51-17.5	3
17.51-50	4	>17.5	4
51-100	5		
>100	6		

Table 2. Statistic indices

TP (true positive): positive test in patients with food allergy
FP (false positive): positive test without food allergy
TN (true negative): negative test without food allergy
FN (false negative): negative test with food allergy
Sensitivity: $TP/TP + FN$
Specificity: $TN/TN + FP$
Efficiency: $TP + TN/TP + TN + FP + FN$
P = Prevalence: patients with food allergy/total patients tested
PPV (positive predictive value): $TP \times P/TP \times P + FP(1 - P)$
NPV (negative predictive value): $TN(1 - P)/TN(1 - P) + FN \times P$

cific IgE in the serum. The level of specific IgE is determined by automatic interpolation of the net change in optical density from stored standard curves corresponding to the group to which the test allergen belongs, e.g. foods, as reported in table 1.

The ENEA system was performed according to the manufacturer's instructions. Despite the different distribution of IgE levels according to classes (7 for CAP, 5 for ENEAS; see table 1), the test has been considered positive in both systems at class 2 or higher. There were minimal differences in the results if the tests were considered positive at class 1 or higher.

Statistics

On the basis of unequivocal history and challenge results, we calculated: true positives (positive test results in patients with food allergy); false positives (positive test results in patients without food allergy); true negatives (negative test results in patients with food allergy), and false negatives (negative test results in patients with food allergy) [8] by CAP, ENEA and SPTs. Table 2 summarizes statistic indices.

Table 3. Results of the CAP system, ENEA system and SPT

Food	Pts, n	P	Test	Se, %	Sp, %	Eff, %	PPV	NPV
Egg white	17	0.41	CAP	100	100	100	100	100
			ENEA	100	100	100	100	100
			SPT	83	100	93	100	94
Milk	15	0.2	CAP	100	91	93	43	100
			ENEA	100	100	100	100	100
			SPT	66	91	86	33.3	98
Wheat	34	0.15	CAP	100	41	50	49	100
			ENEA	40	86	80	81	98
			SPT	60	83	79	95	99
Peanut	37	0.3	CAP	100	38	57	23	100
			ENEA	37	73	62	20	86
			SPT	82	80	81	43	96
Corn	33	0.125	CAP	100	44	51	34	100
			ENEA	25	86	79	34	98
			SPT	75	68	70	45	99
Pea	24	0.125	CAP	100	57	62	45	100
			ENEA	66	76	75	54	99
			SPT	100	66	71	58	100
Soya	24	0.125	CAP	100	62	66	51	100
			ENEA	66	86	84	87	99
			SPT	100	62	62	20	98
Overall			CAP	100	55	64		
			ENEA	60	84.5	80		
			SPT	70	79	75		

Pts = Patients; P = prevalence; Se = sensitivity; Sp = specificity; Eff = efficiency; PPV = positive predictive value; NPV = negative predictive value.

Results

Table 3 shows the results obtained with the CAP system, ENEA system, and SPT. For each tested food is listed the number of patients submitted to the test, the prevalence of the disease, and the data relating to sensitivity, specificity, efficiency, positive, and negative predictive values. There are different test results with different foods.

Discussion

The CAP system has a higher sensitivity than ENEA, with conversely a lower specificity. The SPT yields values intermediate to those of the two *in vitro* methods. It is important to underline that false positive results (that is a low specificity) do not imply the presence of irrelevant specific IgE antibodies, but rather the absence of clinical manifestations.

In general, the negative predictive value (NPV) is very good with all three tests, particularly with the CAP system. This result is in agreement with data from the literature [4, 5, 9] and indicates the lowest probability that a patient with a negative test to a food could develop clinical manifestations to that food. The positive predictive value (PPV) also agrees with the literature [4, 5, 9] and is rather low in all three tests and with all food allergens that we have tested, with the exception of egg white. This indicates that the positive result of a test can be evaluated only in reference to the clinical state of the patient; hence the importance of the food challenge to confirm the diagnosis.

The preliminary data of our study indicates that in case of allergens of animal origin, such as milk and egg white, the reliability of the test results is higher than in the case of foods of vegetable source. In fact it has been shown that test results are reliable when purified allergens of animal source, like Gad 1, are used [10].

The low PPV of tests with vegetable foods probably results from poor standardization of food extracts and from the known cross-reactivity between foods and pollens and among various foods, most of all, foods of vegetable source. For example, it has been shown that *in vitro* cross-reactivity among legumes did not correlate with clinical hypersensitivity [11]. Similarly, false positive results of specific IgE to wheat probably arise from cross-reactivity between wheat flour and grass pollen allergens [12].

In conclusion, these preliminary results confirm the opinion that the crucial factor in the direct or indirect measurement of specific IgE is the availability of standardized allergens. It is therefore essential to identify the major allergens of individual foods. In this way the efficiency of the tests in the diagnosis of food allergy will be improved. At present, the evaluation of the reliability of diagnostic tests with available food allergens should be made with caution and cannot provide absolute data.

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