
The diagnostic accuracy of the atopy patch test in diagnosing hypersensitivity to cow's milk and hen's egg in unselected children with and without atopic dermatitis

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Background: Previous studies have suggested that the atopy patch test (APT) may make oral challenge superfluous in diagnosing children with food hypersensitivity.

Objective: To investigate the clinical relevance of APT in predicting hypersensitivity to cow's milk and hen's egg in 486 unselected children 3 years of age.

Method: The children were examined by APT, skin prick (SPT), histamine release (HR), and specific IgE followed by oral challenge when hypersensitivity to cow's milk or hen's egg was suspected.

Results: Food hypersensitivity confirmed by oral challenge was 1.6% to hen's egg and 0.6% to cow's milk. No hypersensitivity to cow's milk or hen's egg was predicted by APT alone.

Conclusion: APT could not predict food hypersensitivity not predicted by SPT, HR, or specific IgE. Thus, APT cannot be recommended in daily practice for the diagnosis of hypersensitivity to cow's milk and hen's egg in children 3 years of age. (J Am Acad Dermatol 2004;51:556-62.)

Previous studies have suggested that the atopy patch test (APT) may make oral challenge superfluous in children with atopic dermatitis (AD) and suspicion of food hypersensitivity (FHS).¹⁻⁴

Over the last three decades, oral challenge has become the gold standard in diagnosis of food hypersensitivity (FHS).^{5,6} However, oral challenge is time consuming, relatively expensive, and not always without a risk for the patient. An alternative to oral challenge with a high sensitivity and specificity would benefit daily practice in diagnosis of FHS.

Quantification of specific IgE to hen's egg and cow's milk has been suggested as an alternative to

oral challenge in diagnosis of FHS,⁷⁻⁹ but a recent study demonstrated no significant relationship between quantification of specific IgE (egg white) and the challenge threshold dose in egg allergic children with AD.¹⁰

Isolaure et al introduced APT in the diagnosis of delayed-onset reactions of FHS in infants with AD.¹¹ Further, recent studies suggested that the combination of APT together with quantification of specific IgE in serum could predict a clinical reaction to hen's egg and cow's milk with more than 95 % certainty, thus making oral challenge superfluous in children with AD.²

The objective of this investigation was to examine in unselected children the clinical relevance of APT in predicting hypersensitivity to cow's milk or hen's egg compared to the skin prick test (SPT), histamine release (HR) (REFLAB, Copenhagen, Denmark) and specific IgE (Magic Lite [ML], ALK-ALBELLÓ, Hørsholm, Denmark).

METHODS

Study population

The study was comprised of 495 children 3 years of age. The children were enrolled (the year of birth)

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in a cohort in the period from November 1998 to November 1999.¹² A questionnaire investigation was performed when the children turned 3 years of age with the main question concerning self-reported FHS. A clinical examination was made in all children focusing on possible food induced symptoms such as AD, asthma, rhinoconjunctivitis, gastrointestinal symptoms and urticaria.

The diagnostic procedures included APT, SPT, HR and specific IgE (ML) followed by oral challenge (European Academy of Allergology and Clinical Immunology [EAACI]) in children with possible hypersensitivity to cow's milk or hen's egg.⁶ Possible FHS was defined as self-reported FHS (questionnaire) or a positive outcome in at least one of the test procedures (SPT, APT, HR, and ML) without a clear-cut negative case history (not frequently eating the culprit food during the last year from the test date).

Approval for this study was obtained from the local ethics committee (#20010088) and written informed consent was obtained from the parents before enrollment in the study.

Scoring of atopic dermatitis

The lifetime prevalence of AD was recorded by the Danish questionnaire developed from the UK Party's questionnaire for AD.¹³⁻¹⁷ Moreover, the severity of AD was scored according to the SCORAD index.¹⁸

Skin prick test

SPT was performed with fresh cow's milk and hen's egg using the prick-prick technique.¹⁹ SPT was performed with a 1 mm lancet (ALK-ABELLÓ, Hørsholm, Denmark) at the volar surface of the forearm with histamine dihydrochloride (10 mg/ml) and diluent as positive and negative controls, respectively.²⁰ SPT was done in duplicate according to EAACI guidelines and wheals were read after 15 minutes.²¹ The mean value of duplicate tests was used. The wheal reactions were outlined with a marker and transferred to paper with transparent tape. The wheal size was measured using the formula: $(D+d)/2$, where D is the maximum diameter, and d its perpendicular diameter. A wheal size ≥ 3 mm was defined as a positive SPT.²¹

Histamine release from basophils

HR was performed with cow's milk and egg white using a glass-fiber-based histamine assay.²² A histamine release ≥ 10 ng/ml was considered as positive.

Determination of specific IgE

Serum samples were analyzed by ML to cow's milk and egg white according to the manufacturer's

instructions.²³ Measurable specific IgE was defined as a positive test result (ML > 1.43 SU/ml).

Atopy patch test

APT was performed according to Niggemann et al³ (except for using an 8 mm cup instead of a 12 mm cup, and 20 μ L of food instead of 50 μ L of food). One drop (20 μ L) of fresh whole egg and fresh cow's milk (3.5 % fat) was placed on filter paper and applied to uninvolved skin of the back using an 8 mm aluminium cup on adhesive tape (Finn Chamber, Epitest Ltd., Finland).²⁴ Application site was checked after 20 minutes and scored finally after 72 hours. Immediate reactions (20 minutes) were defined as inconclusive and further testing stopped.

The outcome of APT was scored from + to +++³:

- (+) weak positive reaction: erythema and slight infiltration.
- (++) strong positive reaction: erythema, infiltration, papules.
- (+++ very strong reaction: erythema, infiltration, papules, vesicles.

A positive outcome of APT was defined as a score of $\geq +$ with infiltration as the major criteria.

Irritant or doubtful reactions, including sharply demarcated confluent erythema, or reactions confined to margins without infiltration were not regarded as positive.

Oral challenge

Adverse reaction to food was classified according to the revised nomenclature for allergy as food hypersensitivity (FHS) including both IgE and non-IgE mediated, and acute and delayed reactions.²⁵

Standardized open oral challenge was performed according to EAACI guidelines.⁶

The dose interval was 15 minutes. The outcome of oral challenge was divided into immediate and late reactions. Immediate reactions were defined as reactions within 2 hours after the last dose and late reactions in the interval from 2 hours to 24 hours after the last dose of the food was administered.

The children were examined for occurrence of late reactions by telephone and reported symptoms were verified/excluded by clinical examination.

Statistics

The sensitivity is the proportion of positive cases that are correctly classified by the diagnostic tests (SPT, HR, and CAP), while the specificity is the proportion of negative cases correctly classified by the diagnostic tests.²⁶

The positive predictive value (PPV) and the negative predictive value (NPV) are the likelihood

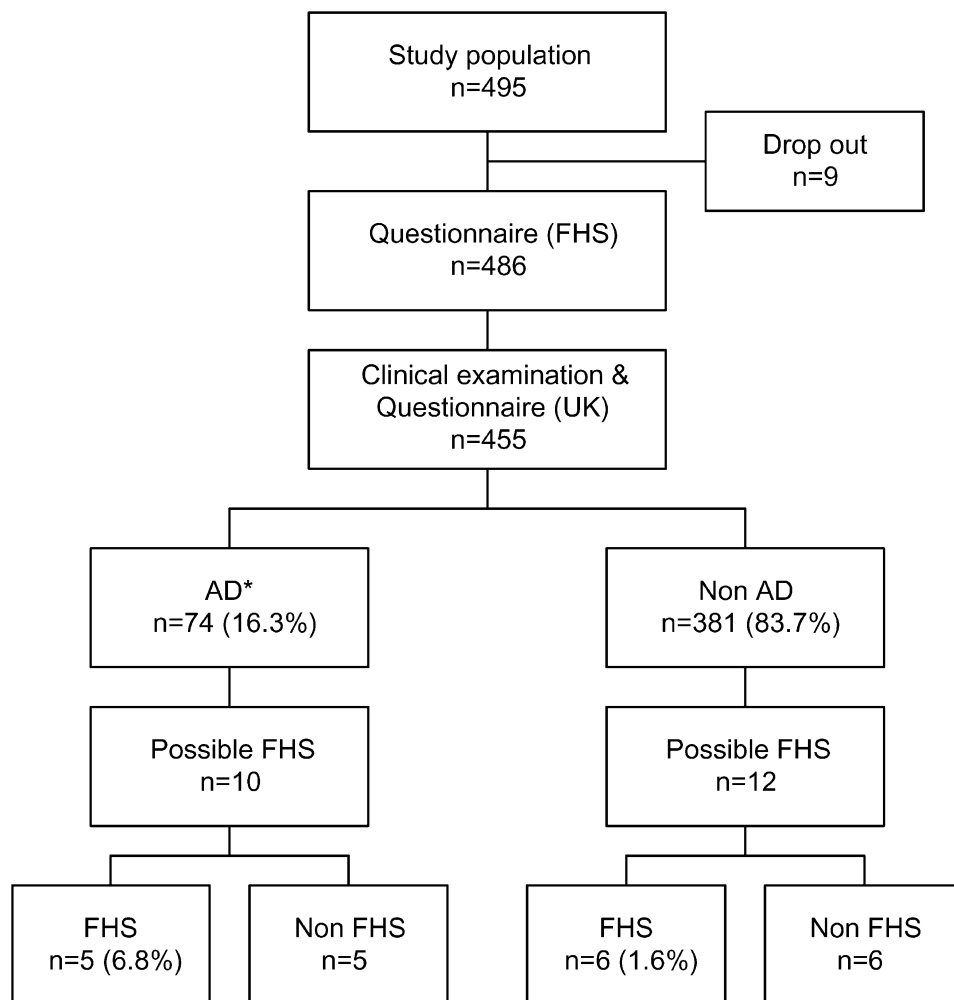


Fig 1. The prevalence of possible food hypersensitivity (FHS) in children with and without atopic dermatitis. *Positive SCORAD was found in 42 (9.2%) of 455 children, whereas 54 (11.9%) of 455 children fulfilled the UK criteria, in total 74 children.

that a positive and negative test result reflects the presence or absence of food hypersensitivity.

Data were double entered twice into a Microsoft Access Database, and statistical analyses were performed with Stata 8.1 (Stata corporation, Tex, USA).

RESULTS

In total, 495 children were enrolled in the study and 486 (98 %) completed the questionnaire about self-reported FHS. APT was performed in 396 children, SPT in 406, whereas a blood sample was obtained from 306 children and analyzed by HR (n = 305) and ML (n = 286).

In total, 455 children were examined for AD by questionnaire (UK) and clinical examination including SCORAD.^{13,18} Fig 1 shows the prevalence data of possible and confirmed FHS in children with and

without AD. In the group with AD, 5 (6.8%) of 74 were diagnosed FHS versus 6 (1.6%) of 381 children in the group without AD. However, the confirmed symptoms (FHS) were not an exacerbation of AD, but mostly urticaria (Table I). The lifetime prevalence of AD was estimated to 11.9 % (n = 54). SCORAD was positive in 9.2 % (n = 42) children with a mean score of 29 points. Seventy-four different children (16.3 %) fulfilled the criteria for AD by the Danish questionnaire and/or the SCORAD index. Eight children with AD had no APT because of parents' lack of time. However, none of these children reported possible hypersensitivity to cow's milk and hen's egg, and all were negative in SPT, HR, and specific IgE.

APT was performed in all children with possible hypersensitivity to cow's milk or hen's egg. Outcome of APT in the children with and without AD is shown in Fig 2, A and B.

Table I. The performance characteristics of *in vitro/in vivo* tests in the children 3 years of age with possible hypersensitivity to cow's milk and hen's egg

No*	Atopic dermatitis [‡]		Food [~]	Symptoms		Threshold [∞]	APT [∞]	SPT	IgE	HR
	SCORAD	UK		reported	confirmed [□]					
138	0	÷	Egg	U, T	U	4305	0	9	58	52
248	38	÷	Egg	U	U	805	0	11.5	174	- ↑
526	0	÷	Egg	U, C, V	U	305	+	6.5	0	27
540	0	÷	Egg	U	U	305	+	3.5	24	44
729	0	÷	Egg	U	U	305	•	15	0	134
767	30	÷	Egg	E, R	U, C	1805	•	8	51.5	24
1436	0	+	Egg	U	U	4305	•	7	0	0
1490	0	+	Egg	U, E	U	4305	0	1.5	8	0
164	0	÷	Egg	Sus	Negative	-	0	3	0	0
485	0	NI	Egg	U, E	Negative	-	0	0	17.8	0
1448	47	+	Egg	U	Negative	-	0	0	0	0
1452	0	÷	Egg	Sus	Negative	-	0	0	0	35
1479	30	÷	Egg	Sus	Negative	-	+	5	13	0
1385	54	+	Egg	U	Negative	-	-⇒	0	0	34
100	0	+	Milk	As, E, U	U	31500	0	10	15.7 [⇐]	24
239	0	÷	Milk	V, T	V	210800	IR	5	7	17
535	0	NI	Milk	V, D	V, D	135000	0	0	0	0
138	0	÷	Milk	E	Negative	-	0	0	3	41
493	0	NI	Milk	D	Negative	-	0	0	0	0
519	0	÷	Milk	Ap, Ob	Negative	-	0	0	0	0
1436	0	+	Milk	U	Negative	-	0	0	5	0
1490	0	+	Milk	U, E	Negative	-	0	1	3	0

Ap, abdominal pain; APT, atopy patch test; As, asthma; C, conjunctivitis; Co, cough; D, diarrhea; Di, dizziness; E, eczema; HR, histamine release (ng/ml); IgE, specific IgE concentration measured by magic lite (SU/ml); IR, irritant or doubtful reactions including redness with no infiltration; NI, non-responders to the questionnaire; Ob, obstipation; R, rhinitis; SPT, skin prick test (mm); Sus, suspicion to food hypersensitivity, but unable to direct the suspicion toward a specific food; T, tiredness; U, urticaria; V, vomiting.

*ID number of the children used in the study.

[‡]Examined by the SCORAD index and the UK diagnostic criteria (+ fulfilled the criteria and ÷ for children not fulfilling the criteria for atopic dermatitis)

[~]Suspected food

[□]Confirmed symptoms during oral challenge

[∞]Challenge threshold dose in mg (whole hen's egg and fresh cow's milk [3.5 % fat])

[∞]Scored from + to +++

•Immediate reaction (urticaria) after 20 minutes

[†]Not enough serum for histamine release

[⇐]Measured by CAP (Pharmacia & Upjohn, Uppsala, Sweden)

[⇒]APT not performed (parents' lack of time)

Oral challenge was performed in 22 children with possible hypersensitivity to cow's milk or hen's egg (Table I). Possible hypersensitivity was 2.9 % to hen's egg and 1.6 % to cow's milk with 1.6 % and 0.6 % confirmed by oral challenge, respectively. The confirmed reactions were all immediate reactions and no hypersensitivity to cow's milk or hen's egg was predicted by APT alone (Table I), and no late reactions were reported at all. In three of the eight egg-allergic children, an immediate reaction (20 minutes) was elicited by APT, and further testing stopped.

Fourteen children with AD suspected food-induced eczema, but were unable to direct the suspicion towards a specific food. However, the children were negative in APT, SPT, HR, and ML to cow's milk

and hen's egg. Table II lists the performance characteristics of the children with a positive outcome of APT, but without possible hypersensitivity to cow's milk or hen's egg.

The performance characteristics of the different diagnostic tests are listed in Table III and Table IV. SPT and HR showed the best diagnostic ability correctly classifying the children as hypersensitive to cow's milk or hen's egg.

DISCUSSION

This study demonstrates the difficulties in using APT as a diagnostic tool in the diagnosis of FHS in children 3 years of age. APT could not predict

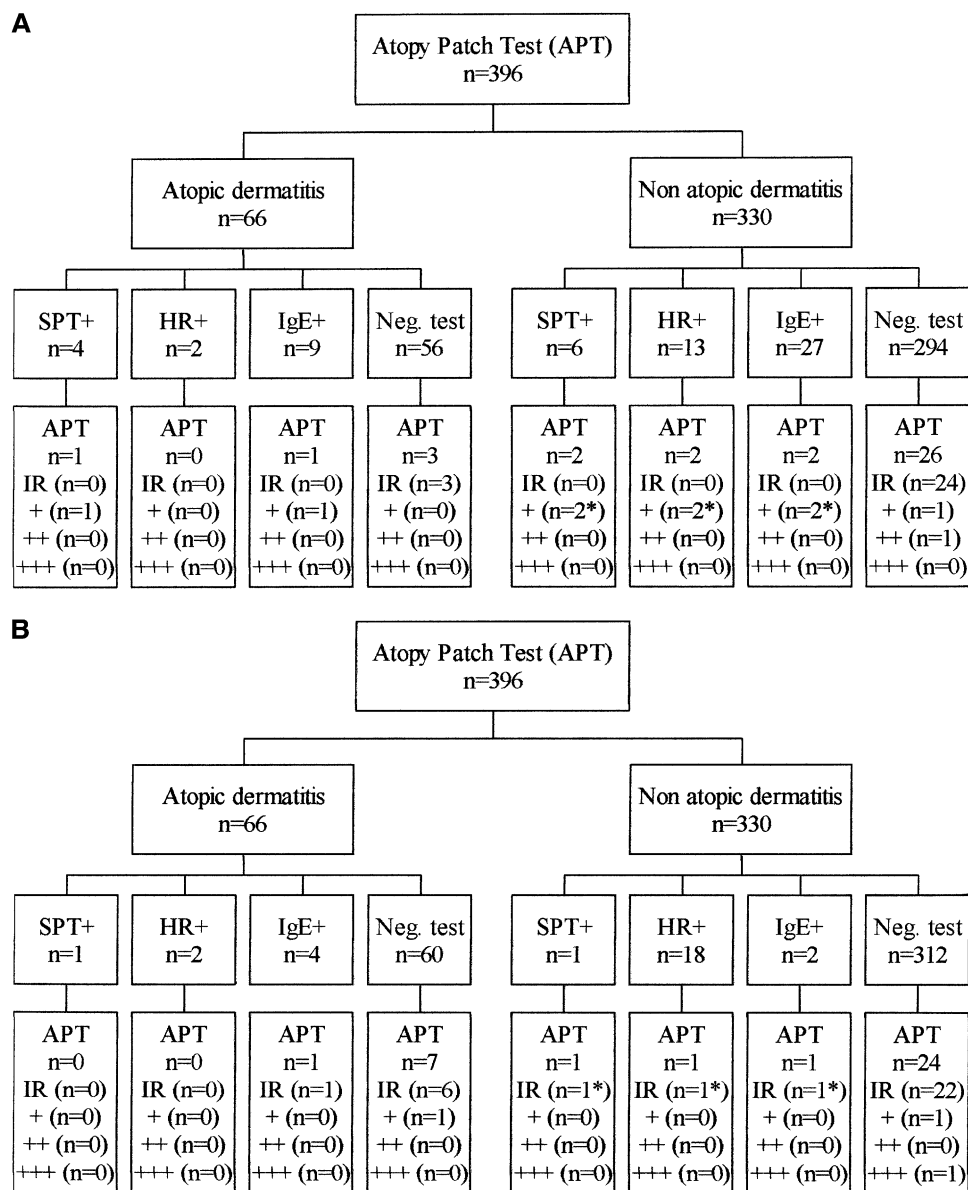


Fig 2. A, Hen's egg; outcome of the APT in children with and without atopic dermatitis. **B**, Cow's milk; outcome of the APT in children with and without atopic dermatitis. *Same two children. *APT*, Children with a positive atopy patch test to the respective food scored from +, ++, +++; *HR+*, positive histamine release; *IgE+*, positive specific IgE; *IR*, irritant or doubtful reactions including redness with no infiltration; *Neg. test*, negative result of SPT, HR, and IgE to the respective food; *SPT+*, positive skin prick.

hypersensitivity to hen's egg or cow's milk not identified by SPT, HR, or ML.

Previous studies have demonstrated that up to 40% of selected children with AD have FHS with cutaneous reactions occurring in 3/4 of the positive oral food challenges.²⁷⁻²⁹ In this study with unselected children with and without AD, these findings could not be reproduced since no late reactions were verified by clinical examination followed up to 24 hours after the last test dose administered. **Fig 1**

illustrates that FHS seems more common in children with AD (6.8%) in relation to children without AD (1.6%). In spite of this discrepancy between the prevalence of FHS in these groups, it is important to emphasize that the symptoms elicited in food hypersensitive children were not an exacerbation of AD, but mostly immediate urticaria. Furthermore, even in the children with a positive SCORAD at the time of testing, no isolated late phase reactions were seen, although the SCORAD index as a mean demonstrated

Table II. Performance characteristics of children 3 years of age with a positive APT without possible hypersensitivity to cow's milk or hen's egg

No*	Food	APT	Atopic dermatitis†		Asthma	SPT	HR	IgE
			SCORAD	UK				
260	Hen's egg	+	0	+	0	0	0	0
830	Hen's egg	+	0	÷	0	0	0	0
630	Cow's milk	+	0	+	0	0	0	0
720	Cow's milk	+	0	÷	0	0	0	0
267	Cow's milk	+++	0	÷	1‡	0	0	0

APT, Atopy patch test; HR, histamine release; IgE, specific IgE with a score grade from + to +++; SPT, skin prick test.

*ID number of the children used in the study.

†Examined by the SCORAD index and the UK diagnostic criteria (+ fulfilled the criteria and ÷ for children not fulfilling the criteria for atopic dermatitis).

‡Asthma under infections.

Table III. Hen's egg: The diagnostic value (%) of the atopy patch test, skin prick test, histamine release, and specific IgE compared to the outcome of oral challenge and case history of possible hypersensitivity to hen's egg

	APT	SPT	HR	IgE
Sensitivity	40	88	71	75
Specificity	99	99	96	89
PPV	39	59	22	10
NPV	99	99	99	99

APT, Atopy patch test; HR, histamine release; IgE, specific IgE; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.

Table IV. Cow's milk: The diagnostic value (%) of atopy patch test, skin prick test, histamine release, and specific IgE compared to the outcome of the oral challenge test and case history to possible hypersensitivity to cow's milk

	APT	SPT	HR	IgE
Sensitivity	0	67	67	50
Specificity	99	100	94	98
PPV	0	45	6	14
NPV	99	99	99	99

APT, Atopy patch test; HR, histamine release; IgE, specific IgE; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.

moderate severe eczema. These findings are in contrast to the data by Roehr et al who found that the combination of APT and quantification of specific IgE could predict clinical reactivity with more than 95 % certainty with no differences between immediate and late reactors, thus making oral challenge superfluous in children with AD.² PPV was 100 % when the specific IgE (cow's milk or egg-white) concentration exceeded 17.5 KU_A/L and 89 % when using APT alone.² However, the study population (Roehr et al) consisted of selected children with AD compared to unselected children in this study.² In this study, PPV was 59 % (SPT) and 39 % (APT) indicating that APT may only be valid in highly selected children with AD. However, the differences in the cup size and the amount of food placed on the filter paper could explain the different results.

A cohort study by Saarinen et al³⁰ examined 6209 unselected infants from birth for development of cow's milk allergy by APT, SPT, IgE, and oral challenge. Saarinen et al did not find any single test or combinations useful in predicting cow's milk

hypersensitivity compared to oral challenge. However, APT procedures were different from this study (we used the procedure described by Niggeman et al³); occlusion time 48 hours and reading 48 hours after removal of the Finn chamber versus 72 hours in this study, marked erythema was defined as a positive outcome versus infiltration and erythema (this study) and cow's milk powder versus fresh cow's milk (this study) was used in APT.

Bygum et al³¹ investigated the clinical interpretation and reproducibility of APT in 48 selected young adult patients with and without AD using standard inhalation allergens and fresh cow's milk. In agreement with this study, Bygum et al could not find any clinical relevance of a positive APT to fresh cow's milk.

In conclusion, APT cannot predict hypersensitivity to hen's egg and cow's milk not identified by SPT, HR, or ML in unselected children 3 years of age with and without AD. Thus, APT cannot be recommended in the diagnosis of hypersensitivity to hen's egg or cow's milk in children 3 years of age in daily practice.

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