
Accuracy of skin prick tests in IgE-mediated adverse reactions to bovine proteins

Alessandro Fiocchi, MD*; Gabriel R. Bouygue, MSc*; Patrizia Restani, PhD†; Giorgio Bonvini, MD*; Rosario Startari, MD*; and Luigi Terracciano, MD*

Objectives: To review the recent literature on the diagnostic accuracy of skin prick tests (SPTs) in pediatric food allergy, focusing on adverse reactions to milk and beef. To present data about the test performance characteristics of beef extracts used in SPTs among children with atopic dermatitis (AD) reporting immediate hypersensitivity to beef.

Data Sources: MEDLINE search using the following algorithm ["skin prick test" AND "food allergy" OR allergen; 1997-2002; English; all children]. Prospective sensitivity study of SPTs in 34 patients.

Study Selection: Thirty-four children with AD (median age 2.29 years) were consecutively recruited between 1992 and 2000 because of immediate reactions to beef. On double-blind, placebo-controlled food challenges (entry criterion), 20 of the patients reacted to beef and 14 did not. Cut-off points for skin prick test wheal positivity was selected by receiver-operator characteristic analysis for fresh and commercial beef allergens. Sensitivity and specificity of skin tests and indices of reproducibility were calculated.

Results: In the literature, the positive predictive accuracies of skin prick tests vary between 69 and 100% and the negative predictive accuracies between 20 to 86% for cow's milk. In our series, SPTs with commercial beef extracts were highly diagnostic (100% sensitivity; 10% false positive rate) and SPTs with fresh beef were highly specific (100%), albeit with a false-positive rate of 21.42%.

Conclusions: From the literature, we conclude that the diagnostic accuracy of SPTs with milk should be reappraised in the workup of cow's milk allergy. Carrying out commercial and fresh food SPTs at the same time substantially reduces costs and diagnostic work. Oral provocation is necessary in 20.68% of children with AD who have immediate symptoms to beef. Greater allergen standardization and streamlining of the workup of cow's milk allergy are desirable future goals.

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INTRODUCTION

The properties of skin prick tests (SPTs) as indices of immunoglobulin (Ig)E-mediated food hypersensitivity are low specificity and low positive predictive value (PPV) in allergic pediatric populations.¹⁻³ Because diagnostic accuracy varies with the SPT method used and with the antigen characteristics, the positioning of SPTs in the diagnostic workup of adverse reactions to bovine proteins (ARBP) on the basis of test performance must be inferred by reviewing published caseloads and has never been investigated in a prospective study of children tested with various bovine allergens. In this study, we reviewed the clinical literature pertinent to infants and children diagnosed with cow's milk allergy (CMA) and beef allergy (BA) during workup with SPTs. We also present prospective data on SPTs in the workup of allergy to beef in a population of children with atopy.

Accuracy refers to concordance with a reference test, the double-blind, placebo-controlled food challenge (DBPCFC), in the case of food allergy. SPT sensitivity, specificity, and predictive values or performance characteristics are calcu-

lated against this standard and summarize data for the positioning of tests for screening or diagnostic purposes. In the literature, when the low specificity and PPV of SPTs have been mentioned, sources have not been clinical trial evidence, but reviews,^{4,5} book chapters,^{6,7} or reports more than 10 years old.⁸⁻¹² Further, the accuracy and the statistical nomenclature used to report the test performance characteristics of SPTs lack the consensus language that allergists have recently adopted.^{13,14} Although accepting these limitations, we note that a comparative study of three SPT interpretation methods against DBPCFC in the diagnosis of food allergy found that the negative predictive value (NPV) of SPTs with cow's milk is considered indicative of the screening capacity of the test,¹ and another study¹⁵ defined a negative SPT outcome as "predictive of clinical tolerance" in IgE-mediated CMA.

In this report, we review the test performance characteristics of SPTs with bovine allergens in the context of CMA and BA in infants and children. We also present original data about the accuracy of SPTs in the workup of BA.

SPT IN THE DIAGNOSIS OF CMA

Seven recent studies^{1,2,16-20} comparing the diagnostic properties of commercial and fresh food allergens (cSPT and ffSPT), the atopy patch test, and specific IgE determinations in a pediatric setting are summarized in Table 1. No single study reports all the statistical variables necessary for independent calculation of test utility and positioning. The mean

* Department of Child and Maternal Medicine, The Melloni Hospital, Milan, Italy.

† Laboratory of Toxicology, Department of Pharmacological Sciences, University of Milan, Milan, Italy.

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age of the patients ranged from 6.9 to 50 months, and the effect of this variable on test performance could not be evaluated. Having the investigator unaware of the food challenge test material was not a uniform requirement in these studies. Double-blind (two studies), open (two studies), either double-blind or open (one study), or single-blind (one study) challenges—and even a PPV >95% or, alternatively, an open or double-blind challenge¹⁹—have been used. The dose of challenge material, such as cow's milk, when reported, varied from 130 to 210 mL.

Different assumptions have also been made about interpreting the reference test. The times of evaluation for challenge positivity included immediate reactions only, both immediate and delayed reactions, and, in one case, no data on when symptoms developed.¹⁹ This information is relevant in the case of CMA. Immediate responses are expected to develop with SPTs, and cow's milk often induces delayed reactions in children with CMA. Thus, the discriminatory power of SPTs is even more relevant to their positioning in the diagnostic workup of CMA, because IgE-mediated sensitization is not present in all patients with positive responses to oral food challenge with cow's milk.²¹

In the studies reviewed, the set of indicators to determine clinical ARBP, crucially, also varies. In one study¹⁶ of children with CMA selected from a larger, prospectively recruited series, the full range of allergic symptoms is reported as sample prevalence. In another study,² an adverse reaction to cow's milk on days 1 and 2 after DBPCFC was used to define positivity, and reactions occurring after day 2 were considered if confirmed by diary and clinical evaluation.

SPT results are the primary indicator of sensitivity for the clinician to evaluate progression to IgE-mediated symptoms.

In their diagnostic use, one should be aware that variations in procedures determine different predictive utilities. Some uniformity has been achieved in conducting and interpreting SPTs, but basing clinical practice guidelines for SPT procedures on these studies is not yet possible (Table 2).

Wheal interpretation after 15 minutes has elapsed and the mean wheal diameter are main outcome measures in the protocol of the studies reviewed. Nevertheless, five research groups used commercial allergen extracts from different manufacturers, and what is meant by reagent standardization in the diagnostics industry relies on a weight by volume classification in the absence of known epitope characterization and even of the different allergenic proteins contained in the test materials.²² Many factors related to heat treatment and the methods of preparation of commercial allergen extracts may unpredictably affect their allergenicity,^{23,24} and thus the performance characteristics of cSPT. Because food antigens from different commercial sources can vary in sensitivity,¹¹ comparisons among different diagnostics are difficult. ffSPT is also difficult to define in the case of milk, and it has been variously interpreted as pasteurized cow's milk in two studies^{18,20} and as a cow's milk-based formula in another.¹⁶ A single study compared cSPT and ffSPT,²⁰ and whether a commercial or native allergen offers better discriminating power in diagnosing CMA is unknown. Isolated cow's milk proteins were used in one study, but their degree of purification was not specified.²⁰ Another study²¹ used bovine serum albumin (BSA), bovine β -lactoglobulin, and casein patch tests, but not in SPTs. Duplicate SPTs may have been necessary to improve reliability by reduction of interpersonal variations.²⁵ Evaluation of different sites for SPTs has not

Table 1. Overview of SPTs in the Recent Literature on CMA: Caseload Characteristics and Confirmation of Diagnosis*

Reference	CMA assessment (no.)	Mean age (range) (mo)	Symptoms	Challenge	Reaction time	Doses
Eigenmann & Sampson ¹	63 (35 challenge+, 28 challenge-)	4.6 yr†	AD	DBPCFC or convincing history	I	...
Sporik et al ²	310 (143 challenge+, 167 challenge-)	31† (1-192)	CMA	Open with PM	I & D (<1 wk)	<210 mL during challenge; <450 mL during open phase
Saarienen et al ¹⁶	239 (118 challenge+, 121 challenge-)	6.9 (6.3-7.5)	CMA	Open with CMF	I & D (<5 days)	<160 mL
Majamaa et al ¹⁷	143 (72 challenge+, 71 challenge-)	10.5 (SD 7.2)	AD, GI	DBPCFC or open with CMF	I & D (<7 days)	<186 mL
Roehr et al ¹⁸	71 (45 challenge+, 26 challenge-)	13† (2-134)	AD	DBPCFC with PM	I (<2 h), D (>48 h)	<143 mL
Eigenmann & Calza ¹⁹	13 (2 history+, 3 CAP >95% PPV, 6 challenge+, 2 challenge-)	30† (6-195)‡	AD	CAP >95% PPV or challenge (either open or DBPC) with dehydrated CM	NR	<10 g powder (130 mL reconstituted formula?)
Rancé et al ²⁰	40 (23 challenge+, 17 challenge-)	50 (2-240)	CMA	Labial—single-blind oral challenge	I	NR

* AD, atopic dermatitis; CM, cow's milk; CMA, cow's milk allergy; CMF, liquid cow's milk formula; D, delayed; GI, gastrointestinal symptoms; I, immediate; NR, not reported; PM, pasteurized milk; SD, standard deviation.

† Median age.

‡ Age given is for all food-allergic children; age of children with CMA is not reported.

Table 2. SPT Methods in the Recent Literature on CMA*

Reference	cSPT	ffSPT	Purified CM proteins	Duplicate tests	Localization	Device	Reading (min)	Wheal recording	Cutoff point (mm)†
Eigenmann & Sampson ¹	G	...	...	No	Back, VA	Wyeth bifurcated needle	15	Diameter Surface	5 29‡
Sporik et al ²	DHS	...	...	No	Back, VA	Lancet	15–20	Diameter	8§
Saarinén et al ¹⁶	...	CMF	...	No	VA	Lancet	15	Diameter	3 6 8
Majamaa et al ¹⁷	ALK	...	...	No	VA	ALK lancet	15	Diameter	3
Roehr et al ¹⁸	...	PM	...	No	VA	ALK lancet	15	Diameter	3
Eigenmann & Calza ¹⁹	A	...	...	No	Back, VA	Lancet	15	Diameter	3
Rancé et al ²⁰	S	PM	Casein, lactalbumin	No	VA	DHS lancet	15	Diameter	3

* A, Allergopharma (Reinbek, Germany); ALK, Allergologisk Laboratorium A/S (Hørsholm, Denmark); CM, cow's milk; CMF, liquid cow's milk formula; DHS, Dome/Hollister-Stier (Spokane, WA); G, Greer Laboratories, Inc (Lenoir, NC); PM, pasteurized milk; S, Stallergènes (Antony, France); VA, volar area of the forearm.

† Wheal diameter.

‡ Area in mm².

§ 100% specificity point.

been considered, and how localization may influence the reaction remains unknown.

All but one report¹ relied on the original allergy pricker method,²⁶ and lancets were used in 6 of the 7 studies. The test detection rates are outlined in Table 3. The NPV of a cSPT in some of these studies was poor;² even if cSPT with cow's milk extracts has been used with good results to screen for CMA in children with atopic dermatitis.¹⁵ In contrast, a strongly positive SPT with cow's milk (and with use of a >8-mm wheal diameter cutoff point) is associated with an adverse reaction on open challenge and supports the good diagnostic value of cSPT in this series.² The 74% sensitivity and 79% specificity found using a 3-mm wheal diameter cutoff (after receiver operating characteristic [ROC] curve analysis) might reflect the fact that non-IgE-mediated CMA is far from rare in children younger than 2 years. Another study,¹ which used an allergen extract made by Greer Laboratories (Lenoir, NC), suggested that using the mean wheal diameter, as opposed to the mean area, identifies most pa-

tients with a >5-mm wheal diameter cutoff point; 89% sensitivity and 68% specificity were found (78% PPV and 83% NPV).

Most studies have established a cutoff for cSPT and ffSPT with cow's milk formula at a 3-mm wheal diameter; however, with inclusion of immediate and delayed reactions in the analysis, the concomitant use of SPTs, CAP, atopy patch tests,¹⁸ and serum eosinophil cationic protein¹⁶ improves the detection rate of CMA, probably because of the variety of the immune responses involved. The sensitivity of cSPT with cow's milk is particularly low when eczema and gastrointestinal symptoms are taken into account, but the specificity remains high, particularly among children younger than 2 years.¹⁷ This same study suggested that the diagnosis of CMA can be made on the basis of a positive skin test result and a favorable response to a diagnostic elimination diet.

In the only study that compared cSPT and ffSPT in ARBP,²⁰ the specificity and PPV were higher with ffSPT than with cSPT. Cow's milk was the only food to yield a higher

Table 3. Accuracy of SPT Methods in the Recent Literature on CMA

Reference	Sensitivity (%)	Specificity (%)	Positive predictive accuracy (%)	Negative predictive accuracy (%)
Eigenmann & Sampson ¹	94	46	69	86
Sporik et al ²	74	79	...	...
Saarinén et al ¹⁶				
3 mm*	61	76	71	67
6 mm*	37	93	83	60
8 mm*	19	98	92	55
Majamaa et al ¹⁷	14	98	91	51
Roehr et al ¹⁸	78	69	81	64
Rancé et al ²⁰				
cSPT	73	50	91	20
ffSPT	66	100	100	28

* Wheal diameter cutoff point.

false-negative rate with the fresh allergen and a higher false-positive rate with the commercial allergen. Skin test sensitivity was higher for all food allergens when native materials were used rather than kit tray allergen extracts. Standardization of allergen extracts, however, awaits epitope characterization of the most clinically relevant immunogens to be practicable.²² The use of purified cow's milk and beef proteins as test materials should be investigated in clinical trials, despite problems with the target allergen extract purity, concentration, and characterization.

Overall, SPTs with cow's milk preparations have low diagnostic value, but their use in screening self-reports of ARBP is justified by their specificity. The use of 100% specificity decision points has been proposed in selected, high-risk populations (as defined by history) but, on the strength of caseload findings, widespread use necessitates further clinical and statistical evaluation. A need persists for standardization of diagnostic materials in the workup of CMA, pending immunochemical characterization of the antigenic determinants of bovine proteins. The use of purified cow's milk proteins remains unexplored in SPTs outside research settings.

SPT IN THE DIAGNOSIS OF BA

In comparison to CMA, the literature on BA has a notable lack of definition of (and consensus about) the role of SPTs in the diagnostic workup of this less frequent, albeit by no means rare, condition.²⁷ Accurate reporting of sample statistics or characteristics has also eluded researchers who did not focus on ARBP but specifically on CMA.²⁸ Nonetheless, allergists can draw from their experience with CMA when having to make an informed decision with a patient reporting an immediate reaction to beef and to address the following questions: 1) What are the performance characteristics of SPTs with beef, and does the nature of the beef proteins in the allergen extract affect these? 2) Should both fresh and commercial allergen extracts be used? 3) If so, which allergen should be ordered first? 4) Organizing a DBPCFC is a time- and labor-intensive activity^{7,29} for patients and for health facilities. 5) Can using SPTs (primary investigation of IgE-mediated sensitivity in a clinical setting) reduce the need to mobilize such resources and decrease the costs for my patient?

To address the problems linked to the diagnostic accuracy of SPTs with beef from a clinical standpoint, we prospectively recruited young patients who came to our allergy care unit between 1992 and 2000 for evaluation of symptoms occurring immediately after the ingestion of beef.

CURRENT CASES

Study Subjects

Thirty-four children (age range, 1.00 to 4.41 years; median, 2.26) with atopic dermatitis and IgE-mediated sensitization to foods (at least one of the following allergens: wheat, egg, cod, peanut, tomato, and cow's milk) were enrolled in this study when they reported immediate symptoms attributed to consumption of beef.

All patients underwent DBPCFC with beef in accordance with a previously described procedure.³⁰ Twenty DBPCFC-positive patients (age range, 1.00 to 4.41 years; median, 2.29) and 14 patients testing negative on DBPCFC (age range, 1.25 to 3.66 years; median, 2.16) underwent SPTs with use of a commercial beef extract and fresh beef.

SPTs

A Dome/Hollister-Stier lancet with a 1-mm tip was used in both SPTs. A Bayer Pharmaceutical Allergy Products (Spokane, WA) extract consisting of lyophilized skeletal muscle tissue, defatted in acetone and ethyl ether and extracted into 50% glycerol at 1 mg/mL concentration (D. Constable, Bayer Pharmaceutical Allergy Products, personal communication, 1998), was used for cSPTs. The physicochemical properties of extracts were evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.³¹ Separations of reagents from Greer Laboratories and Bayer Pharmaceutical Allergy Products were similarly evaluated for comparison of protein profiles (Fig 1). SPTs with the commercial beef extract were performed by percutaneous lancing through a drop of the extract and wiping with a tissue immediately afterward. Raw, unfrozen skeletal muscle from locally obtained beef was prepared by grinding and centrifugation in our laboratory after ffSPTs were ordered. The lancet tip was dipped in the fresh material and immediately drawn across the volar aspect of the arm to prick the skin.¹⁰ Histamine phosphate (10 mg/mL) in 50% glycosaline and glycosaline alone were used as positive and negative controls, respectively. Wheal diameters were read through a clear plastic caliper disk scaled in tenths of millimeters under 4× magnification and were interpreted when the wheal margin was included within a complete caliper circle to the nearest millimeter.²

Statistical Analysis

Hypotheses of mutually independent diagnostic tests were evaluated by analyzing the number of disagreements (McNemar test) and by calculating the κ statistic for reliability. Sensitivity analysis was performed to calculate sensitivity and specificity by using a 2-by-2 array. Wheal diameter data were submitted to ROC curve analysis on dedicated software of our design. The best discriminatory thresholds for cSPT and ffSPT were set as a wheal diameter ≥ 3.01 mm.

Ethics

Institutional review was sought and granted for the study objectives and protocols.

RESULTS

Eighteen children with a positive cSPT to beef responded positively to beef during DBPCFC, and 11 ffSPT-negative patients responded negatively to provocation tests (Table 4). On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the protein lanes of the Bayer extract appeared similar to those of fresh beef, although the BSA content of this allergen extract was high. Myosin, tropomyosin and actin featured prominently on the lane corresponding to the Greer Labora-

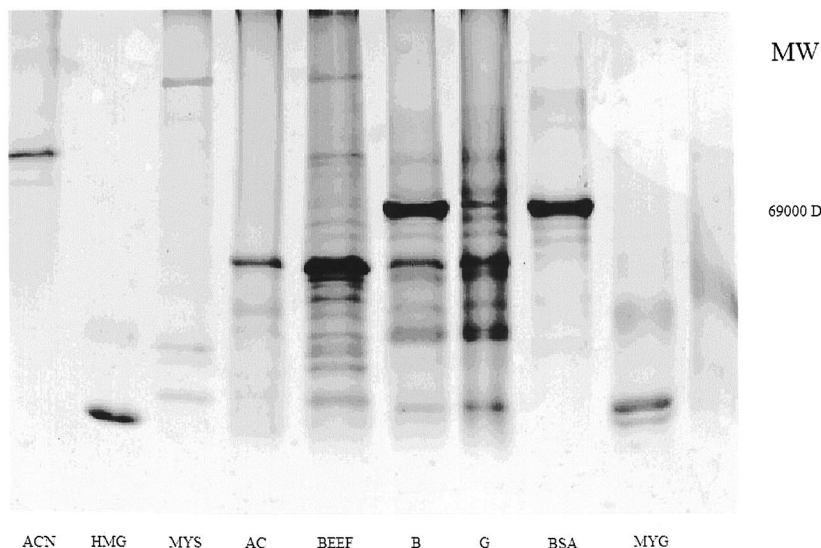


Figure 1. Immunoblots of beef protein fractions and commercial beef extracts. AC, actin; CAN, actinidin; B, Bayer commercial extract; G, Greer commercial extract; HMG, hemoglobin; MW, molecular weight; MYG, myoglobin; MYS, myosin.

ACN=actinidin HMG = haemoglobin MYS = myosin AC = actin B = Bayer's commercial extract
G = Greer commercial extract BSA = bovine serum albumin MYG = myoglobin

Table 4. Results of Sensitivity Analysis of Skin Prick Tests With Use of Commercial and Fresh Beef Allergens*

Factor	cSPT	ffSPT
No. of tests	34	34
DBPCFC with beef (no. of patients)		
Positive results	20	
Negative results	14	
SPT (no. of patients)		
Positive results	18	23
Negative results	16	11
Observed agreement (%)	94.11	91.17
Observed disagreement (%)	5.88	8.82
Sensitivity (%)	90	100
Specificity (%)	100	78.57
False-positive rate (%)	0	21.42
False-negative rate (%)	10	0

* cSPT = skin prick test with commercial extract; DBPCFC = double-blind, placebo-controlled food challenge; ffSPT = skin prick test with fresh food allergens.

tories extract. A 3.01-mm mark presented the best discriminating cutoff point in the study population on analysis of the cSPT and ffSPT ROC curves (sensitivity 90% and specificity 100% with cSPT; sensitivity 100% and specificity 78.57% with ffSPT). The results of the accuracy analysis are shown in Table 4. Although commercial and fresh test foods were statistically comparable ($\kappa = 0.8497$), the sensitivity and specificity were high in this sample. An observed disagreement with the reference test of 5.88 and 8.82%, respectively, for cSPT and ffSPT translates into 3.9 to 5.9% discordant tests.

DISCUSSION

In reference to the initial goal of investigation of the performance characteristics of SPTs with beef, the main findings of this study were that a positive cSPT result is a sensitive diagnostic method, whereas a negative SPT outcome with fresh beef excludes IgE-mediated BA. From a clinician's viewpoint, the properties of the two SPT methods are unusually high for a skin test. What is remarkable is that a commercial preparation should prove so sensitive a diagnostic assay and that fresh beef should be such perfect screening test material. When the ROC curves of cSPTs and ffSPTs are analyzed, the areas under the curves show similar trade-offs between sensitivity and false-positive results. For a child with BA and from a public health perspective, however, a second finding is that both source materials must be used to reduce to 20.68% the proportion of patients who will need to undergo DBPCFC. Thus, for allergists in secondary care, "it is not yet time to stop skin testing"³² in the workup of BA. The question of how these findings can be generalized is addressed elsewhere in this Supplement, inasmuch as concordance with the reference test used to calculate the efficiency of a diagnostic method requires epidemiologic data for extrapolation to the overall pediatric population.

In a clinical setting, evaluating how the selection of source material (native and processed) affects the performance characteristics of SPTs has implications for construction of the diagnostic workup of BA (Fig 2). In this series, a dual SPT workup, with performance of ffSPT first and then cSPT, leads to substantial savings in diagnostic work and patient's time in the hospital. If the process could be further streamlined, it could also become a step toward the standardization of procedures and materials in the workup of food allergy in chil-

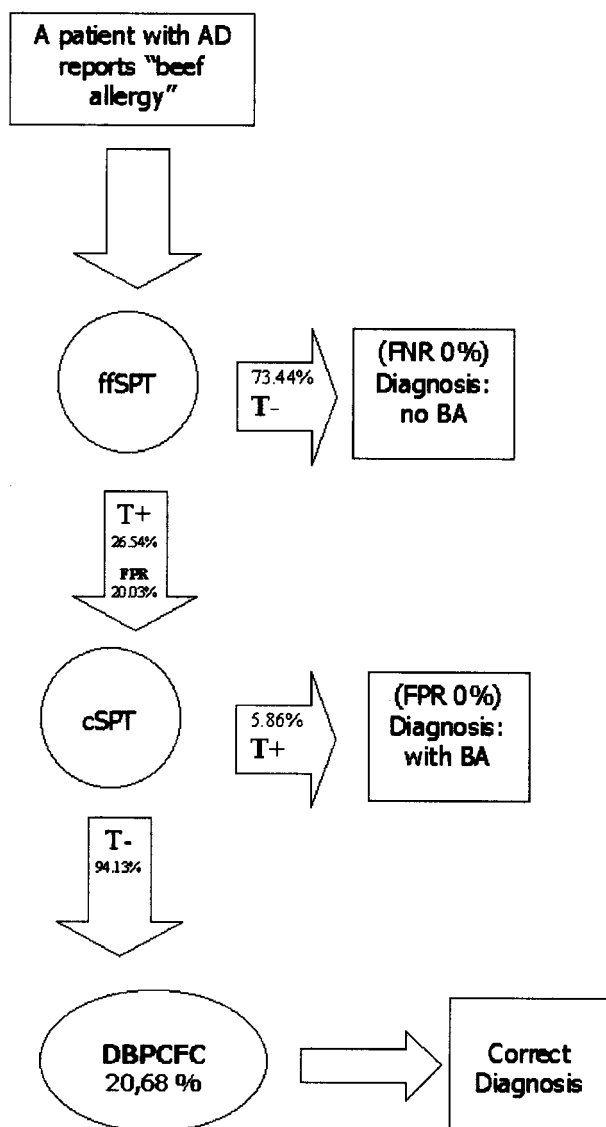


Figure 2. Flowchart for the workup of BA. AD, atopic dermatitis; FNR, false-negative rate; FPR, false-positive rate; T-, negative test result; T+, positive test result.

dren.^{28,33} For instance, the time before interpretation and thus diagnostic and operator time may be reduced by half if both cSPT and ffSPT were performed together. The additional cost of a DBPCFC mobilizes public health resources for at least two consecutive days, as legally prescribed in the regional health authority guidelines used in Milan.³⁴ Without factoring the human and social costs involved, the patient's financial investment may be an estimated US\$560. In a population of 34 children, a reduction of 79.32% in the need to perform food challenges translates into a reduction of costs from US\$19,040 to \$15,103.

The question of whether SPTs may reduce the need for oral food challenges in children with BA is complex and involves

epidemiologic analysis as well as statistical methods used to interpret the predictive accuracies of these tests and challenges in the population. In this study, we were able to quantify only some of the implications of using native or processed allergens and the answer seems to be that a qualified improvement is contingent on the specific allergenic composition of the source material chosen for extracts. With ffSPTs, however, the false-positive rate remains high and the specificity is low. Thus, they are less useful than cSPTs for excluding the presence of BA. Yet, in one study,³⁵ tests with native bovine proteins were found to have high specificity, and much lower sensitivity to beef was found in another study.²⁸ Food challenges could confirm only 11 of 89 cases (12.36%). This study did not address the issue of cutoff points. The allergen extract used (Greer Laboratories) consisted of frozen beef processed with acetone and subjected to air- and heat-drying cycles. It was stored at -15° to -12° C before extraction (M. White, Greer Laboratories, personal communication, 1997). Myosin, tropomyosin, and actin, which feature large in the extract we analyzed, have been found to have only moderate relevance for BA in children, although they are frequent primers of sensitization.³⁶ In our study, BA was confirmed in all children primed by the Greer allergen extract. The Bayer extract, which approximated native beef in composition and BSA content at semiquantitative analysis, could be considered quasi-standardized.³⁷ In this study, 20.69% of cases needed oral food challenges after skin testing, and testing first with the native allergen also reduced the number of SPTs necessary to reach a diagnosis. The main drawback of using native material, however, is the unpredictability of the response hypersensitive patients may have to various proteins. In vitro assays (radioallergosorbent tests) provide an alternative, although the influence of these diagnostic methods on the workup of BA is an unanswered research question. In addition, a need exists for consideration of the influence of processing on the accuracy of allergens because extract composition and presentation, and hence skin test performance characteristics, may be confounded by these factors. The lack of consensus on nomenclature, of uniform reporting of statistics, and of clinical practice guidelines based on these criteria continues to hamper our ability to communicate and manage the patient with ARBP in a multidisciplinary context.

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Requests for reprints should be addressed to:
 Alessandro Fiocchi, MD
 Department of Child and Maternal Medicine
 The Melloni Hospital
 Via Melloni, 52
 Milan 20123, Italy
 E-mail: allerg@tin.it