

In contrast, a ≥ 7 -mm cut-off yielded a greater specificity of 95%, but lower sensitivity of 69%.

There is no universal definition of “optimal” cut-offs. The 95% and 50% positive predictive values are commonly quoted, but these are influenced by disease prevalence and they are specific to their respective study cohorts. This may also partly explain why our findings contrast with those based primarily on pediatric studies.^{4–7} In our study, we objectively derived our optimal cut-off from the ROC curve that maximized both sensitivity and specificity. We also excluded patients with history or OFC outcomes suggestive of OAS, which can be a potential confounder. However, because our cohort was selected for suspected peanut allergy (ie, higher pretest probability), it is likely our “optimal” cut-off may not be applicable to unselected individuals.

Further larger, population-based studies are required to determine more generalizable age-specific diagnostic SPT and sIgE cut-offs for patients with suspected peanut allergy. There was a wide variation in sIgEs to whole peanut and Ara h 2, making them less useful in predicting true peanut allergy. More importantly, it is crucial to individualize risk-benefit assessment prior to OFC. We would caution against overgeneralization of specific cut-offs and recommend considering age-appropriate thresholds such as SPT ≥ 5 mm in this selected cohort during investigation of peanut allergy in adolescent and adults.

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Cross-reaction of recombinant termite (*Coptotermes formosanus*) tropomyosin with IgE from cockroach and shrimp allergic individuals



The incidence of allergic diseases appears to be increasing and poses substantial health, psychological, and economic burdens.^{1,2} In the United States, asthma affects approximately 40 million individuals, and 15 million people have food allergies. Cockroaches are considered a major class of airborne allergens that contribute to allergic disease in urban areas worldwide.³ Tropomyosin is a major allergen in cockroaches, house dust mites, and shellfish and is considered a panallergen.⁴ Cockroach tropomyosin is a 31-kDa protein with multiple IgE-binding sites that cross-react with IgE from shrimp allergic patients.

Cockroaches and termites are closely related evolutionarily within the order Blattodea, and they have some behavioral similarities.⁵ Although the German cockroach *Blattella germanica* resides in the Blaberoidea superfamily, the American cockroach *Periplaneta americana* and the Formosan subterranean termite *Coptotermes*

formosanus reside in the Blattoidea superfamily albeit within different families. Termites are a significant pest and typically live within and around urban centers, especially in the Southern United States. Termite colonies can be made up of millions of insects, which can lead to the accumulation of a high concentration of potential allergens. Humans may come into contact with termite allergens when a home is actively infested with termites that defecate potentially allergenic frass (termite droppings) or create mud tubes for travel and during termite mating season when they are attracted to light sources and swarm in populated areas.

Recently, whole body extracts from the Formosan subterranean termite *Coptotermes formosanus* have been demonstrated to cross-react with cockroach-specific antibodies and to compete for IgE binding in serum from cockroach allergic individuals.⁶ Tropomyosin contained within whole body extracts of *C formosanus* was implicated as one of the cross-reacting proteins. *C formosanus* tropomyosin (accession No. AGM32377.1) is predicted to be a 256-amino acid protein (29,354 Da) and is a member of the eukaryotic tropomyosin 1 superfamily. *C formosanus* tropomyosin is nearly identical to tropomyosin from American (99% identity to Per a 7) and German (98% identity to Bla g 7) cockroaches. It also has significant percentage identity to tropomyosins from human body lice (Ped h 7, 93% identity), house dust mite (Der f 10, 81% identity), and multiple crustacean tropomyosins, including tiger shrimp (Pen m 1, 83% identity), with which it shares sequence similarities to several mapped shrimp tropomyosin IgE epitopes.⁷

Ms Vargas and Dr Mahajan contributed equally to this work as first authors.

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The open reading frame of *C formosanus* tropomyosin (accession No. KC571878.1) was synthesized and recombinantly expressed in *Escherichia coli* as a polyhistidine fusion protein to determine whether a recombinantly expressed and purified *C formosanus* tropomyosin cross-reacts with IgE from cockroach and shrimp allergic patient sera. After affinity purification, the identity of the His-tagged tropomyosin was confirmed by mass spectrometry. Circular dichroism spectra of the purified protein demonstrated a characteristic α -helical structure similar to that observed for recombinant brown shrimp (*Penaeus aztecus*) tropomyosin Pen a 1.⁸

Recognition of the recombinant termite tropomyosin by human IgE in serum from a panel of 16 cockroach, dust mite, and/or shrimp allergic individuals was evaluated by immunoblot analysis. Of the 16 donor samples tested in this work, 8 are monosensitized to dust mite, 3 are monosensitized to cockroach, 1 is polysensitized to cockroach and dust mite, 2 are polysensitized to cockroach and shrimp, and 2 are polysensitized to cockroach, dust mite, and shrimp. IgE binding to recombinant termite tropomyosin from 5 of the 16 donor samples was clearly detected. All 5 of the positive serum samples were from cockroach allergic donors. Only 2 of the 5 cockroach allergic donors whose serum samples positively reacted with termite tropomyosin were also dust mite allergic, and both had relatively high cockroach ImmunoCap scores (≥ 46.9). Serum samples from 3 cockroach allergic donors did not recognize the termite tropomyosin. Tropomyosins naturally exist as dimers, and a fraction of the recombinant protein migrated as a dimer in our immunoblots (approximately 72 kDa) and was recognized by donor serum samples. The blots were also probed with a commercially available antitropomyosin antibody that recognized the tropomyosin monomer and dimer, confirming the specificity of IgE reactivity.

The secretory response of the rat histamine-releasing cell line (RBL) loaded with IgE from shrimp allergic patient plasma was measured in response to stimulation with varying doses of termite tropomyosin and was compared to responses obtained with recombinant Pen a 1 (*P. aztecus*, brown shrimp). Humanized RBL cell monolayers, expressing the human Fc ϵ RI subunit, were grown overnight in 24-well plates. Cells were sensitized with 20% plasma from a shrimp allergic individual for 2 hours and stimulated with varying doses of Pen a 1 or termite tropomyosin at 37°C for 30 minutes. β -Hexosaminidase release was measured as described previously,⁹ and the percentage of histamine release was calculated after subtraction of spontaneous release. A dose-dependent increase in degranulation responses was observed with termite tropomyosin and was comparable to that seen with Pen a 1 stimulation (Fig 1). Decreased signal at higher tropomyosin concentrations is likely attributable to changes in average tropomyosin-antibody aggregate size and the formation of monovalent allergen–receptor complexes, which limit crosslinking and reduce signal.

Characterizing new allergens is essential to an increased understanding of the unique properties that confer allergenic potential to some proteins and to help identify factors that contribute to allergic disease. Tropomyosin is a panallergen common to shellfish and insects, and several studies have demonstrated IgE cross-reactivity between cockroach and shrimp tropomyosin.^{4,10} Cockroaches and termites are within the same order,⁵ and there is IgE cross-reactivity between cockroach and termite extracts.⁶ This is the first study, to our knowledge, to demonstrate directly that a recombinant termite tropomyosin cross-reacts with IgE from both cockroach and shrimp allergic patients. We are not aware of patients with an allergy specifically to termites. However, we have observed evidence of occupationally induced allergy to termites (C.P.M., 2016, oral, unpublished data). Exposure to termites may act as a sensitizing agent through airborne contact, skin contact, or oral routes in infested areas. Globally, termites are also a source of food and are increasingly being considered as an alternative source of nutrition in the Western world. Our results suggest that termite

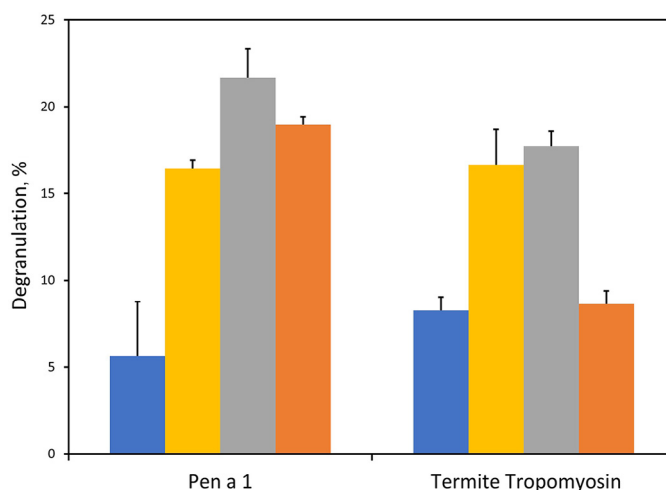


Figure 1. Purified recombinant *Coptotermes formosanus* tropomyosin causes dose-dependent degranulation. RBL-2H3 cells were primed overnight with plasma from a shrimp allergic donor followed by washing of monolayers with Hanks buffer and stimulation with varying doses (blue [0.001 μ g/mL], yellow [0.01 μ g/mL], gray [0.1 μ g/mL], and orange [1 μ g/mL]) of recombinant Pen a 1 or termite tropomyosin. Cells were incubated at 37°C for 30 minutes for facilitating degranulation. Aliquots of supernatant were added to substrate solution (1.3 mg/mL of p-nitrophenyl-N-acetyl- β -D-glucosamine in 0.1 M citrate, pH 4.5) and incubated for 60 minutes, and the reaction was stopped with 0.2 M glycine (pH 10.7). Percentage of degranulation was evaluated by quantifying the color development at 405 nm using a spectrophotometer and normalizing to total enzyme content measured in cells lysed with 1% Triton X-100. The release observed in absence of any stimulant (spontaneous) was subtracted from each experimental value.

tropomyosin may pose a threat to those with cockroach or shellfish allergies because of cross-reactivity and may contribute as a sensitizing agent in geographic areas infested with termites.

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Ethanol-induced urticaria caused by sensitization to acetic acid



Alcoholic beverages contain ethanol, usually known as alcohol. The adverse effects of alcohol consumption are well known, but there are reactions, such as urticaria and anaphylaxis, that are rare and less known. The etiopathogenic mechanism of these reactions is not clear and seems to be multifactorial. We describe a patient who developed urticaria after drinking alcoholic beverages likely because of acetic acid allergic sensitization.

A 40-year-old atopic woman with recurrent episodes of acute urticaria with severe facial angioedema for the last 2 years was referred to our outpatient allergy clinic. Symptoms developed 10 minutes after drinking alcoholic beverages (beer, wine, champagne, vermouth) and disappeared after treatment with methylprednisolone and diphenhydramine. Previously, she tolerated all alcoholic beverages and currently tolerates nonalcoholic beer, vinegar, fruit, and foods. Symptoms were not related to drug administration or nonsteroidal anti-inflammatory drug use.

Skin prick test results were positive to pollens, cat, dog, and rabbit. The results of tests to other allergens, including mites, molds, latex, palm profilin, peach lipid transfer protein, *Anisakis* simplex, and foods (eggs, milk, fish, shellfish, nuts, fruits, and grape), were all negative. The results of prick tests to beer, wine, pure ethanol (99.9% vol/vol), and metabisulfite were negative, but the results for acetic acid 10% were positive (negative in 8 control patients). Serum tryptase levels were within the normal range, and the total IgE level was 76 kU/L.

After written consent, an open oral challenge test with beer (5.5% vol/vol) was performed. Fifteen minutes after a 330-mL cumulative dose, the patient experienced pharyngeal and otic pruritus, with generalized urticarial, facial swelling, and edema in the soft palate that resolved within 2 hours after therapy. The results of open challenge tests with nonalcoholic beer and potassium metabisulfite were negative. Finally, a single-blind, placebo-controlled challenge with ethanol (99.9% vol/vol) was performed. Placebo consisted of oral administration of 100 mL of concentrated and sugary fruit juice. Progressive doses of 1 mL, 5 mL, and 10 mL of ethanol dissolved in 100 mL of juice were administered to the patient. Twenty minutes after the last dose (cumulative dose, 16 mL), she experienced a similar reaction to the previous one, without changes in tryptase levels. After avoiding alcohol intake the patient remains asymptomatic.

Alcohol is mainly metabolized in the liver and converted to acetaldehyde by the enzyme alcohol dehydrogenase. Later, aldehyde dehydrogenase transforms it into acetate, which is finally converted into water and carbon dioxide in the citric acid cycle.

Adams et al¹ published an in-depth review of adverse reactions to alcoholic drinks.¹ Although the exact prevalence of these reactions is unknown, skin reactions after ingestion of alcoholic beverages are relatively frequent. Patients with skin diseases, such as chronic urticaria, mastocytosis, or carcinoid syndrome, can

experience exacerbation of skin symptoms after alcohol ingestion. In addition, the occurrence of facial flushing caused by alcohol-induced vasodilatation attributable to a genetic enzymatic defect in metabolism (ALDH2 polymorphism) is also frequent, particularly in those of Asian descent.

Isolated cases of immunologic urticaria or even anaphylaxis have been reported. Most of them are attributable to an allergic sensitization to additives or contaminant contents in wine (papain, metabisulfite, grapes, eggs, milk) or beer (barley, hop, *Sacharomyces*, malt). Only some primary reactions to ethanol itself have been published.

In 1968, Hicks² reported the first case of anaphylaxis after alcoholic drinks attributable to ethanol, as demonstrated by the challenge test. In 1976, Karvonen and Hannuksela³ described a case of urticaria induced by alcoholic beverages with positive provocation test results to ethanol and acetic acid. In 1983, Przybilla and Ring⁴ reported the first case of alcohol-induced anaphylaxis with positive skin prick test results to acetic acid. Since then, several similar cases have been published with positive skin prick test result to acetic acid and positive challenge test results to ethanol.^{5–10} We have reviewed these publications, finding 11 cases, with detailed clinical characteristics summarized in Table 1.

These reactions mainly occur in nonatopic middle-aged women. Frequently, symptoms are severe (67% anaphylaxis) and appear a few minutes after the ingestion of any sort of alcoholic drinks. These patients usually tolerate vinegar (58%). Positive skin test results to ethanol, acetaldehyde, or alcoholic beverages were not detected in any of the patients.

Currently, the etiopathogenic mechanism remains unknown. An increase in tryptase levels after challenge tests has been reported in some cases, and a possible IgE-mediated mechanism has been suggested by several authors, although ethanol is unlikely to act as an allergen because of its low molecular weight.

None of the patients with positive prick test results to acetic acid had positive skin test results to acetaldehyde, ethanol, or other tested alcoholic drinks. Sensitization to acetaldehyde has not been demonstrated by cutaneous tests in any of the reported patients. These patients had acetic acid–positive cutaneous test results that were correlated with positive challenge test results to ethanol. Instead, they seem to be of a specific nature because we have not found positive cutaneous test results in controls, not even in those with flushing-like reactions to alcohol. Therefore, it seems likely that acetic acid is the metabolite responsible for the sensitization.

Acetic acid or its ionized form acetate is found in vinegar (3%–5% vol/vol), which is primarily responsible for its sour taste and smell. It is absorbed, almost unchanged, by diffusion. Then, acetic acid is oxidized in tissues to generate adenosine triphosphate. Allergic reaction to acetic acid has been demonstrated by challenge test only in a patient who needed a dose of 100 g of 4% acetic acid.³ However, most of the studied cases tolerate vinegar, including our patient.