Curr Allergy Asthma Rep (2017) 17: 46
DOI 10.1007/s11882-017-0716-z

Contribution of Molecular Allergen Analysis in Diagnosis of Milk Allergy

Zbigniew Bartuzi[1] · Renata Rodrigues Cocco[2] · Antonella Muraro[3] · Anna Nowak-Węgrzyn[4]

Published online: 8 June 2017
© Springer Science+Business Media New York 2017

Abstract
Purpose of Review We sought to describe the available evidence supporting the utilization of the molecular allergen analysis (MAA) for diagnosis and management of cow milk protein allergy (CMPA).
Recent Findings Cow milk proteins are among the most common food allergens in IgE- and non-IgE-mediated food allergic disorders in children. Most individuals with CMPA are sensitized to both caseins and whey proteins. Caseins are more resistant to high temperatures compared to whey proteins.
Summary MAA is not superior to the conventional diagnostic tests based on the whole allergen extracts for diagnosis of CMPA. However, MAA can be useful in diagnosing tolerance to extensively heated milk proteins in baked foods. Children with CMPA and high levels of casein IgE are less likely to tolerate baked milk compared to children with low levels of casein IgE. Specific IgE-binding patterns to casein and betalactoglobulin peptides may predict the natural course of CMPA and differentiate subjects who are more likely to develop CMPA at a younger age versus those with a more persistent CMPA. Specific IgE-binding patterns to casein and beta-lactoglobulin peptides may also predict response to milk OITand identify patientsmost likely to benefit fromOIT.

Keywords Baked milk diet · Casein · Cow milk allergy · Milk allergy · Molecular allergen analysis · Molecular diagnosis · Whey proteins

Abbreviations

CM	Cow milk
CMP	Cow milk proteins
CMPA	Cow milk protein allergy
EoE	Eosinophilic esophagitis
IgE	Immunoglobulin E
OIT	Oral immunotherapy

Introduction

Molecular allergen analysis (MAA) utilizes individual natural or recombinant allergenic components that are contained in the whole complex allergen source [1•]. By focusing on the individual major or minor allergens and controlling for allergen concentration, MAA promises a more precise (single allergen versus mix of allergens) and individualized (clinically relevant for the patient) measurement of allergen-specific IgE antibodies. As such, MAA represents a new generation of diagnostic tools that contribute to the precision medicine.

This article is part of the Topical Collection on *Immunologic/Diagnostic Tests in Allergy*

✉ Anna Nowak-Węgrzyn
anna.nowak-wegrzyn@mssm.edu

[1] Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland

[2] Allergy and Immunology Division, Department of Pediatrics, Federal University of São Paulo, São Paulo, Brazil

[3] Food Allergy Referral Centre Veneto Region, Department of Women and Child Health, Padua General University Hospital, Padua, Italy

[4] Division of Allergy and Immunology, Jaffe Food Allergy Institute, One Gustave L. Levy Place, Box 1198, New York, NY 10029, USA

MAA has been shown repeatedly to offer superior accuracy for the diagnosis of food allergy caused by plant-derived foods, in which pollen cross-reactivity is prominent [2•, 3•] [4]. Pollen-food cross-reactivity contributes to poor performance of diagnostic tests based on the whole allergen sources because these tests do not discriminate between asymptomatic immunologic cross-reactivity with pollen and true, food-specific, clinically relevant allergy [5, 6]. The pollen cross-reactive food proteins are usually of low molecular mass, very susceptible to thermal processing, and rapidly digested by enzymes in the gastrointestinal tract. Therefore their potential for inducing systemic allergic reactions is very low. They are frequently referred to as class 2 food allergens that typically induce mild symptoms (e.g., pruritus, mild swelling) upon contact with oropharyngeal mucosal membranes (Table 1). They are generally incapable of causing primary allergic sensitization in the GI tract, and oral symptoms usually follow development of significant IgE sensitization to the cross-reactive pollen. In contrast, proteins in cow's milk, hen's egg white, and seed storage proteins in nuts and seeds are referred to as class 1 food allergens that survive in the harsh gastrointestinal environment, cause sensitization, and induce systemic reactions upon ingestion [1•, 7, 8].

Characteristics of CMP and Sensitization Patterns

Cow's milk (CM) is produced by the mammary glands of cows (*Bos domesticus*). Cow's milk proteins (CMP) are commonly the first foreign proteins introduced into the diet of infants who are not exclusively breast-fed because they are a source for the infant formulas, including hypoallergenic formulas based on hydrolyzed casein or whey and amino acids. CM and dairy products (e.g., cheese,

Table 1 General characteristics of common food allergens

Food allergen class	Characteristics and allergenic potential	
Class 1	•Animal or plant food (nuts, seeds) origin	
	•Water-soluble glycoproteins	
	•Molecular mass 10–70 kDa	
	•Resistant to high temperatures, gastric acid, and proteases	
	•Ubiquitous in diet	
	•Primary GI sensitization	
Food	Major allergen	Nomenclature[a]
Cow's milk	Caseins, alpha s1, alpha s2, beta, kappa	Bos d 9, 10, 11,12
	Whey proteins, e.g.,	Bos d 4
	Alpha-lactalbumin	Bos d 5
	Beta-lactoglobulin	
Hen's egg white	Ovomucoid	Gal d 1
	Ovalbumin	Gal d 2
Wheat	Omega-5 gliadin	Tri a 19
Peanut	Vicilin	Ara h 1
	Conglutin	Ara h 2
	Glycinin	Ara h3
Hazelnut	11 S globulin	Cor a 9
	2S albumin	Cor a 14
Class 2	•Plant-derived (nuts, fruits, vegetables)	
	•Highly homologous with pollen	
	•Highly heat labile, cooking reduces allergenicity	
	•Primary sensitization to airborne pollen in the respiratory tract	
Peanut	PR-10 protein	Ara h 8, cross-reacts with Bet v 1
Hazelnut	PR-10 protein	Cor a 1, cross-reacts with Bet v 1
Apple	PR-10 protein	Mal d 1, cross-reacts with Bet v 1
Carrot	PR-10 protein	Dau c 1, cross-reacts with Bet v 1
Celery	Profilin	Api g 45, cross-reacts with Bet v 2

PR-10 pathogenesis-related protein-10 family, *Bet v* allergens from birch tree pollen

[a] WHO/IUIS Allergen Nomenclature Subcommittee, www.allergen.org.

yogurt, butter) are the major nutritional source of protein, calories, and calcium for infants and young children under the age of 2 years [2•]. CMP are among the most common food allergens in all forms of food allergy and IgE- and non-IgE-mediated, eosinophilic esophagitis in all age groups. The frequency of CMP allergy (CMPA) has been estimated to range from 0.5 to 7.5% in the westernized countries [2•, 9] [10] [3•, 10, 11].

CMP are classified as class 1 food allergens, due to their resistance to digestion and heating [12–15]. They do induce sensitization via gastrointestinal tract. CMP are highly clinically cross-reactive (>90%) with milks from goat and sheep due to a high sequence homology (>80%) [16, 17]. In contrast, the laboratory and clinical cross-reactivity is very low (<5%) with milks from donkey, mare, buffalo, or camel [18] [19].

Sensitization to Individual Molecules and Its Clinical Relevance

The patterns of sensitization to the individual CMP vary significantly by study/country and age of the patients [20–22]. In general, the majority of subjects are polysensitized to several casein and whey proteins [23]. Caseins, beta-lactoglobulin, and alpha-lactalbumin are the major allergens, with over 50% of CM-allergic subjects having evidence of IgE antibodies directed at these proteins. IgE sensitization to caseins, beta-lactoglobulin, and alpha-lactalbumin is closely related, whereas IgE sensitization to bovine serum albumin (BSA) is independent of other CM proteins, and may reflect cross-reactivity with beef proteins (see Table 2) [24] [18, 25].

A large study of sensitization among Brazilian population has been performed last year, including 470 children aged 0–18 years old, homogeneously allocated according to the diagnosis of atopic diseases (food allergies, atopic dermatitis, asthma and/or allergic rhinitis, and wheezing infants) or paired controls. All children had serum-specific IgE evaluated by ImmunoCAP system (Thermo Fisher Scientific, Portage, MI, USA) for different food and inhalant allergens; almost half of the population were sensitized to cow's milk (233/470) (RC, unpublished data). Regarding specific cow's milk proteins, approximately 72, 79, 72, and 45% of 233 subjects were sensitized to alpha-lactalbumin, beta-lactoglobulin, casein, and serum bovine albumin, respectively.

Among 103 patients with confirmed diagnosis of cow's milk allergy, IgE sensitization to alpha-lactalbumin, beta-lactoglobulin, casein, and serum bovine albumin was detected in 88.7, 93.7, 86.2, and 84.2%, not different from CM-sensitized but tolerant children (n = 131; 78.7, 78, 82%, respectively), except for serum bovine albumin that was lower in tolerant children, 41.8 ($P < 0.05$).

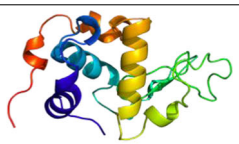
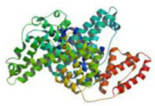
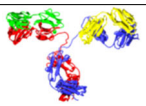
The Phenotypes of IgE-CMPA

We have reported that the majority of children with mild IgE-mediated CMPA can tolerate extensively heated CMP, e.g., in the form of baked products such as muffin [26–28]. Children with mild CMPA can safely include baked milk products in the diet and enjoy greater dietary variety and improved nutritional choices. Inclusion of dietary baked milk seems to accelerate acquisition of development of tolerance to non-heated milk, compared to strict avoidance of all forms of milk [28]. In contrast, children who react to baked milk have more severe (higher risk for anaphylaxis) and more persistent CMPA. Children with more persistent CMPA predominantly generate IgE antibodies directed against sequential CMP epitopes, especially casein. Children who tolerate baked milk and have a more mild and transient CMPA predominantly generate IgE antibodies directed against conformational CMP epitopes. Conformational protein epitopes are destroyed by high temperature, whereas sequential epitopes are not altered by high temperature [29]. In a study that investigated the patterns of IgE and IgG4 antibody binding to sequential epitopes of CMP with the use of a peptide microarray, among 41 children, those with current CMPA had increased epitope diversity compared with those who outgrew CMPA [30]. Children who tolerated baked milk products had IgE-binding patterns similar to those who had outgrown their allergy, but IgG4-binding patterns that were more similar to those of the allergic group who reacted to baked milk. Binding to higher numbers of IgE peptides was associated with more severe allergic reactions during oral milk challenge. There was no association between peptides binding IgG4 and clinical features of milk allergy. In a competitive peptide microarray assay, allergic patients demonstrated a combination of high- and low-affinity IgE binding to specific epitopes, whereas baked-tolerant subjects and those who had outgrown their CMPA demonstrated primarily low-affinity binding. In this study, greater IgE epitope diversity and higher affinity, as determined by using the peptide microarray, were associated with clinical phenotypes and severity of CMPA [30].

Persistence of Cow Milk Allergy

The majority of children with CMPA have a very favorable prognosis, with resolution by school age [31]. However, those with lifetime peak serum cow milk-specific IgE greater than 50 kU_A/L are likely to have persistent CMPA until adolescence or adulthood [32, 33]. Children with CMPA and atopic dermatitis (median age 3 years) underwent double-blind, placebo-controlled food challenges annually to evaluate for

Table 2 Allergens in cow's milk

Protein name	Allergen name	Molecular mass (kDa)	AA #	Sensitization rates among subjects with CMPA	Tertiary structure	Cross-reactivity Laboratory/clinical
Curd (coagulum)-Casein family-major allergens						
Caseins	Bos d 8	20-30			Caseins don't have a rigid tertiary structure but develop a random coil conformation stabilized by hydrophobic interactions	>85% />90% with sheep and goat milk caseins
Alpha $_{s1}$-casein	Bos d 9	23.6	199	98 [a]		
Alpha $_{s2}$-casein	Bos d 10	25.2	207	94 [a]		
Beta-casein	Bos d 11	24	209	91 [a]		
Kappa-casein	Bos d 12	19	169	91 [a]		
Whey (lactoserum)						
Alpha-lactalbumin Major allergen	Bos d 4	14.2	123; 4 disulphide bridges,70% homolgy with human alpha-lactalbumin	51		
Beta-lactoglobulin Protein family: Lipocalins Major allergen	Bos d 5	18.3; exists as a dimer	162; 2 disulphide bridges, one free cysteine; exist as isoforms A and B; binds and carries hydrophobic molecules	61		
Bovine serum albumin; Family: Serum albumins Minor allergen	Bos d 6	67	583	43		80%/15-20% wih raw beef
Immunoglobulins (mostly IgG) Family: Immunoglobulins Minor allergen	Bos d 7	160		36		
Lactoferrin Family: Trasferrins Minor allergen		800	703; forms two homologous globular dom ains named N-and C-lobes Lactoferrin exists in various polymeric forms: monomers to tetramers	35		

Source: IUIS Allergen Database, July 2015. The nomenclature derives from the Latin name of the species, e.g., *Bovis domesticus*, Bos d, whereas the number indicates the sequence in which the individual proteins has been identified as an allergen

[a] Percentage of those sensitized to casein Bos d 8

resolution of CMPA [34]. Casein-specific and beta-lactoglobulin-specific IgE, IgG, IgG1, and IgG4 antibody concentrations were analyzed in all patients at regular intervals. In the patients who became clinically tolerant to CM, the IgE-specific antibody concentrations and IgE/IgG-specific ratios for both milk proteins were lower initially and

decreased significantly with time, in comparison with those in the group who remained allergic to CM. The concentrations of IgG1- and IgG4-specific antibody to casein and the IgE/IgG1 and IgE/IgG4 ratios for both casein and beta-lactoglobulin were significantly lower in the patients who became tolerant to CM. No differences in the IgG-specific concentrations were observed in either group at any of the time points, consistent with poor correlation between food-specific IgG and clinical manifestations of food allergy [2•, 35]. Monitoring casein-specific and beta-lactoglobulin-specific IgE concentrations and IgE/IgG ratios may help predict which patients will develop tolerance to cow milk [34]. In another Finnish study, serum samples at the time of diagnosis (mean age, 7 months), 1 year later, and at follow-up (mean age, 8.6 years) were obtained from 11 patients with persistent IgE-mediated CMPA at age 8 to 9 years and 12 patients who became tolerant to CM by age 3 years [36]. The binding of IgE, IgG4, and IgA antibodies to sequential epitopes derived from five major CMP was measured with a peptide microarray-based immunoassay. IgE epitope-binding patterns were stable over time in patients with persistent CMA, whereas binding decreased in patients who became tolerant to CM early. Among patients who became tolerant to CM early, the signal of IgG4 binding increased and that of IgE decreased over time. IgE and IgG4 binding to a panel of alpha(s1)-, alpha(s2)-, beta-, and kappa-casein regions predicted outcome with significant accuracy. Development of tolerance to CM is associated with decreased epitope binding by IgE and a concurrent increase in corresponding epitope binding by IgG4.

Cow Milk Allergens in Eosinophilic Esophagitis

Cow milk is the single most common food allergen in children and adults with eosinophilic esophagitis (EoE) [37]. A subset of patient with CM-responsive EoE has evidence of IgE sensitization to CMP, although it is generally accepted that IgE antibodies do not play a role on pathophysiology of EoE. In a recent study, adults (n = 46) and children (n = 51) with EoE underwent skin prick testing and serum measurement (whole and diluted) of IgE antibodies specific for aeroallergens, food extracts, and individual CM component allergens by ImmunoCAP and Immuno Solid-Phase Allergen Chip [38]. At all ages, there was a higher prevalence of sensitization to food extracts by ImmunoCAP than by skin prick testing. ImmunoCAP assays for specific milk allergens gave positive IgE antibody results in 31 of 34 sera. The correlations between specific IgE antibody to alpha-lactalbumin (Bos d 4) or beta-lactoglobulin (Bos d 5) and milk extract were strong (R = 0.89 and 0.76, respectively; $P < 0.001$). The IgE responses in cow's milk-sensitized patients with EoE are frequently directed against whey proteins Bos d 4 and Bos d 5.

The Effect of Extensive Heating on CMP Stability and Allergenicity

CMP contain both conformational and sequential IgE-binding epitopes. Children with persistent CMPA have been shown to predominantly generate IgE antibodies directed against sequential casein epitopes [39, 40]. Extensive heating, e.g., baking, affects the allergenicity of CM protein, with caseins being more resistant to heating compared to whey proteins that are susceptible to heating [29]. Heating of beta-lactoglobulin results in formation of the intermolecular disulfide bonds and binding to other food proteins that result in a reduced allergenicity of beta-lactoglobulin [41]. The majority (70–80%) of the CM-allergic children tolerate CM as an ingredient in the baked products [26, 42] [43]. Reactivity to baked milk is a marker of a more severe and more persistent CM allergy [26, 28] [44]. Inclusion of the baked products containing CM into the diet of children with CM allergy appears to accelerate development of tolerance to unheated CM and this approach has become an accepted clinical practice [3•, 28, 45]. High levels of specific IgE antibodies directed against casein are predictive of clinical reactivity to baked milk [42, 46].

Identifying Patients At Risk for Severe Adverse Reactions to Milk Oral Immunotherapy

Currently there are no proven therapies to cure food allergy [2•, 47]. Oral immunotherapy (OIT) is currently the most actively investigated immunomodulatory treatment for milk allergy. The response to OIT varies among individual patients with a subset experiencing frequent systemic adverse reactions and failure to desensitize. Peptide microarrays have been utilized to study antibody responses to specific epitopes on caseins and whey proteins in children undergoing OIT with milk. Among 32 Finnish children (6–17 years of age) with CMPA, 26 children successfully completed OIT and six children discontinued OIT due to adverse reactions [48]. IgE binding to CM peptides (α_{s1}-, α_{s2}-, β-, and κ-caseins, and β-lactoglobulin) decreased and IgG4 binding increased following the OIT in children who achieved desensitization. Compared with OIT successes, OIT failures (who discontinued OIT due to adverse reactions) developed increased quantities and affinity of epitope-specific IgE antibodies and a broader diversity of IgE and IgG4 binding, but less overlap in IgE and IgG4 binding to CM peptides.

Twenty-five Spanish CM-allergic children (median age 5.1 years) underwent CM-OIT and were classified as low, moderate, and high risks according to the number of allergic reactions (safety), time required to achieve desensitization (efficacy), and need of premedication. IgE and IgG4 peptide microarray immunoassay was performed using a library of overlapping peptides of CMP (α_{s1}-, α_{s2}-, β-, and κ-caseins,

and β-lactoglobulin) at baseline, after oral desensitization, and 6, 12, and 24 months of follow-up [49]. CM OIT induced a rapid increase in IgG4 binding and a gradual decrease in IgE binding to sequential peptide epitopes. High-risk patients recognized a statistically significantly higher number of peptides in caseins (IgE binding) at all the times studied. Similar but less pronounced changes were observed for IgG4-binding peptides. Clustering analysis grouped together the high-risk patients; 13 regions of caseins significantly differed between groups of patients regarding binding of IgE antibodies. Bioinformatics analysis selected two sets of 16 IgE-binding peptides at baseline that predicted safety of CM-OIT (R(2) = 0.858) and efficacy (R(2) = 0.732), respectively.

These results suggest that the detailed analysis of IgE and IgG4 binding to specific informative CM peptides might predict whether CM OIT will be tolerated successfully and improve the safety of CM OIT.

Potential Role of MAA in the Diagnosis of CMPA—What Is the Evidence?

At this time, there is no convincing evidence that MAA is superior to the whole-extract-based tests for the diagnosis of unheated milk, although studies have identified casein as the most predictive major milk allergen in children with CMPA [20, 50] [51].

However, the differential susceptibility of CMP fractions, with casein being more resistant than whey proteins, to extensive heating is the basis for utilizing MAA for the purpose of the diagnosis of allergy to extensively heated (baked) CMP [29] [29].

In the largest study to date, baseline immunologic data from 225 patients evaluated for tolerance to baked milk with an oral food challenge (OFC) were analyzed [46]. At baseline, serum samples were collected from subjects to measure CM-, casein-, and β-lactoglobulin-specific IgE as well as casein- and β-lactoglobulin-specific IgG_4 antibody concentrations with the ImmunoCAP system (Thermo Fisher Scientific, Portage, MI). The levels of specific IgE to CM, casein, and β-lactoglobulin were significantly higher in baked milk-reactive patients compared to baked milk-tolerant subjects ($P < 0.05$). Casein- and β-lactoglobulin-specific IgG_4 levels did not differ significantly between subjects reactive and tolerant to baked milk. Casein-specific IgE had a significantly greater accuracy for predicting baked milk reactivity, compared to specific IgE to CM ($P = 0.01$) and to β-lactoglobulin ($P = 0.02$), shown in Fig. 1. All patients with undetectable levels of specific IgE to casein ($n = 25$, 11.1%) tolerated baked milk. Various cutoff levels of specific IgE to CM, casein, and β-lactoglobulin have been analyzed. Specific IgE levels representing the 95% specificity of the tests were chosen as the positive decision points and levels representing the 95% sensitivity as the negative decision points. These values were chosen because they are independent of the disease prevalence. The positive decision point for reactivity to baked milk was 20.2 kU_A/L for casein-specific IgE (UniCAP). In practice, this means that patients having casein-specific IgE antibodies greater than 20.2 kU_A/L are unlikely to pass a baked milk challenge and baked milk products should be avoided. In contrast, a concentration lower than 0.94 kU_A/L (negative decision point) indicates a very low risk of reacting to baked milk, even though the patient might react to regular milk. When giving equal weight to sensitivity and specificity, the optimal cutoff point was casein IgE of 4.95 kU_A/L. The authors concluded that based on the large cohort of children with CMA, quantitative measurements of casein-specific IgE antibodies (by UniCAP) are useful in the management of CMA. Combined with clinical history and the expertise of the physician, the use of cutoff decision points for casein IgE could identify the optimal candidates for baked milk OFCs and improve management of children with suspected CMA.

In another study, 35 children underwent open challenges to baked milk and 29 (83%) passed [43]. Compared with those who passed, children who failed were younger (median age,

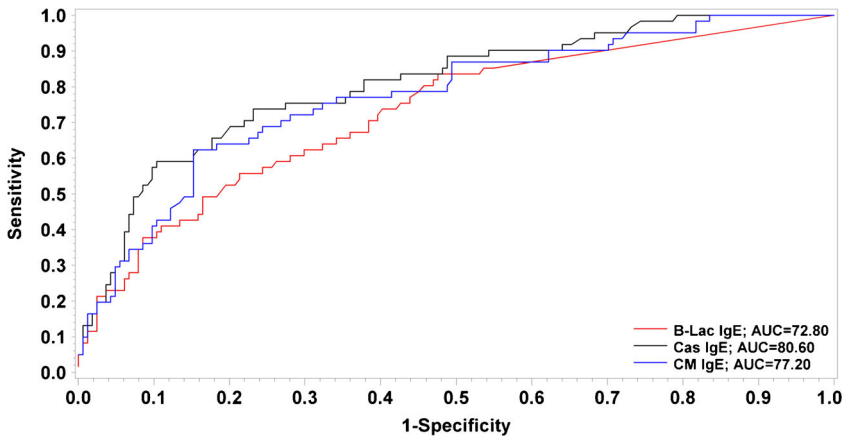


Fig. 1 Combined cohort of milk-allergic patients [42]. Detection of casein-specific IgE for the diagnosis of CMPA. Predicted probabilities of the baked milk challenge outcome and serum-specific IgE concentrations to cow's milk, casein, and β-lactoglobulin. Full ROC curves for the three predictors tested (cow's milk-, casein-, and β-lactoglobulin-specific IgE) in the combined cohort of milk allergic patients ($n = 225$)

8.9 and 3.7 years, respectively; $P = 0.02$). Children with a milk skin prick test (SPT) wheal less than 12 mm were more than 90% likely to pass a baked milk challenge, and no child with milk SPT wheal less than 7 mm failed a baked milk challenge. A casein SPT wheal ≤9 mm, a milk serum IgE (sIgE) level of 1.0 kU_A/L, and a casein sIgE level of 0.9 kU_A/L had a 90% positive predictive value for passing baked milk challenge.

Among 132 children who were initially classified as baked milk-reactive or baked milk-tolerant or having "outgrown milk allergy" based on the results of oral food challenges, significant differences across the clinical groups were observed for median casein- and milk-specific IgE levels, casein-specific IgG_4 levels, and casein IgE/IgG_4 ratios; milk-specific to non-specific basophil activation ratio, median basophil reactivity, and spontaneous basophil activation (CD203c expression after stimulation with RPMI); and milk SPT wheal diameters [42]. Casein- and milk-specific IgE level, milk-specific basophil reactivity, and milk SPT wheal diameter were all significantly greater among baked milk-reactive children compared to baked-tolerant children. When receiver operating curves were generated, casein IgE had the largest area under the curve indicating a greater diagnostic accuracy for tolerance to baked milk compared to CM-IgE, CM-skin prick test wheal diameter, and milk-specific basophil reactivity.

Collectively, these data support the utility of measuring IgE to casein to determine tolerance to baked milk. It should be, however, noted that the diagnostic accuracy of MAA for baked milk allergy is inferior compared with MAA for peanut or tree nuts [1•, 4]. This is explained by a less distinct difference between the allergenicity of casein versus whey proteins compared to the difference between the allergenicity of birch pollen cross-reactive proteins (Ara h 8) versus storage seed proteins, e.g., Ara h 2 [1•].

Conclusions

CMP remain one of the most common food allergens in infancy and childhood in IgE- and non-IgE-mediated food allergic disorders. Most individuals with CMPA are sensitized to both caseins and whey proteins. MAA is not superior to conventional diagnostic tests based on the whole allergen extracts for diagnosis of CMPA. However, MAA can be useful in diagnosing tolerance to extensively heated milk proteins in baked foods, reflective of the higher susceptibility of whey proteins compared to caseins to high temperature. Children with CMPA and high levels of casein IgE are less likely to tolerate baked milk compared to children with low levels of casein IgE. Specific IgE-binding patterns to casein and beta-lactoglobulin peptides may predict the natural course of CMPA and differentiate subjects who are more likely to develop CMPA at a younger age versus those with a more persistent CMPA. Specific IgE-binding patterns to casein and beta-lactoglobulin peptides may also predict response to milk OIT and identify patients most likely to benefit from OIT. More studies are needed to fully understand the application and limitations of MAA for CMPA diagnosis and management.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance

1.• Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI molecular allergology user's guide. Pediatr Allergy Immunol. 2016;27(Suppl 23):1–250. **The most comprehensive resource for molecular allergen analysis to date, addressing both food and inhalant allergens.**

2.• Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008–25. **The official guidelines of EAACI addressing the diagnosis of food allergy.**

3.• Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, et al. Food allergy: a practice parameter update—2014. J Allergy Clin Immunol. 2014;134(5):1016–25 e43. **The current AAAAI practice parameter for food allergy addressing the diagnosis of food allergy.**

4. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90–8.

5. Nowak-Wegrzyn A, Szajewska H, Lack G. Food allergy and the gut. Nat Rev Gastroenterol Hepatol. 2016;14:241.

6. Sicherer SH, Sampson HA. Peanut allergy: emerging concepts and approaches for an apparent epidemic. JAllergy ClinImmunol. 2007;120(3):491–503.

7. Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H. Allergens are distributed into few protein families and possess a restricted number of biochemical functions 10. J Allergy Clin Immunol. 2008;121(4):847–52.

8. Breiteneder H, Mills EN. Molecular properties of food allergens. J Allergy Clin Immunol. 2005;115(1):14–23.

9. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and Management of Food Allergy in the United States: summary of the NIAID-Sponsored Expert Panel Report. J Allergy Clin Immunol. 2010;126(6):1105–18.

10. Schoemaker AA, Sprikkelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history

of challenge-proven cow's milk allergy in European children—EuroPrevall birth cohort. Allergy. 2015;70:963.
11. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy. 2014;69(8):992–1007.
12. Kattan JD, Cocco RR, Jarvinen KM. Milk and soy allergy. Pediatr Clin N Am. 2011;58(2):407–26.
13. Mendonca RB, Franco JM, Cocco RR, de Souza FI, de Oliveira LC, Sarni RO, et al. Open oral food challenge in the confirmation of cow's milk allergy mediated by immunoglobulin E. Allergol Immunopathol. 2012;40(1):25–30.
14. Cocco RR, Jarvinen KM, Han N, Beyer K, Sampson HA. Mutational analysis of immunoglobulin E-binding epitopes of beta-casein and beta-lactoglobulin showed a heterogeneous pattern of critical amino acids between individual patients and pooled sera. Clin Exp Allergy. 2007;37(6):831–8.
15. Cocco RR, Jarvinen KM, Sampson HA, Beyer K. Mutational analysis of major, sequential IgE-binding epitopes in alpha s1-casein, a major cow's milk allergen. J Allergy Clin Immunol. 2003;112(2):433–7.
16. Jarvinen KM, Chatchatee P. Mammalian milk allergy: clinical suspicion, cross-reactivities and diagnosis. Curr Opin Allergy Clin Immunol. 2009;9(3):251–8.
17. Bencharitiwong R, Giavi S, Vereda A, Ibanez MD, Jarvinen KM, Papadopoulos NG, et al. Heating does not decrease immunogenicity of goat's and ewe's milk. J Allergy Clin Immunol Pract. 2013;1(4):418–21.
18. Restani P, Beretta B, Fiocchi A, Ballabio C, Galli CL. Cross-reactivity between mammalian proteins. Ann Allergy Asthma Immunol. 2002;89(6 Suppl 1):11–5.
19. Sicherer SH. Clinical implications of cross-reactive food allergens. J Allergy Clin Immunol. 2001;108(6):881–90.
20. Tsabouri S, Douros K, Priftis KN. Cow's milk allergenicity. Endocr Metab Immune Disord Drug Targets. 2014;14(1):16–26.
21. Garcia-Ara MC, Boyano-Martinez MT, Diaz-Pena JM, Martin-Munoz MF, Martin-Esteban M. Cow's milk-specific immunoglobulin E levels as predictors of clinical reactivity in the follow-up of the cow's milk allergy infants. Clin Exp Allergy. 2004;34(6):866–70.
22. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, et al. Specific-IgE levels in the diagnosis of immediate hypersensitivity to cow's milk protein in the infant. J Allergy Clin Immunol. 2001;107:185–90.
23. Fiocchi A, Dahdah L, Albarini M, Martelli A. Cow's milk allergy in children and adults. Chem Immunol Allergy. 2015;101:114–23.
24. Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. Curr Opin Allergy Clin Immunol. 2009;9(3):265–9.
25. Martelli A, De Chiara A, Corvo M, Restani P, Fiocchi A. Beef allergy in children with cow's milk allergy; cow's milk allergy in children with beef allergy. Ann Allergy Asthma Immunol. 2002;89(6 Suppl 1):38–43.
26. Nowak-Wegrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin Immunol. 2008;122(2):342–7.
27. Nowak-Wegrzyn A, Fiocchi A. Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity. Curr Opin Allergy Clin Immunol. 2009;9(3):234–7.
28. Kim JS, Nowak-Wegrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. J Allergy Clin Immunol. 2011;128(1):125–31.
29. Bloom KA, Huang FR, Bencharitiwong R, Bardina L, Ross A, Sampson HA, et al. Effect of heat treatment on milk and egg proteins allergenicity. Pediatr Allergy Immunol. 2014;25:740.
30. Wang J, Lin J, Bardina L, Goldis M, Nowak-Wegrzyn A, Shreffler WG, et al. Correlation of IgE/IgG4 milk epitopes and affinity of milk-specific IgE antibodies with different phenotypes of clinical milk allergy. J Allergy Clin Immunol. 2010;125(3):695–702.
31. Savage J, Sicherer S, Wood R. The natural history of food allergy. J Allergy Clin Immunol Pract. 2016;4(2):196–203.
32. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol. 2007;120(5):1172–7.
33. Wood RA, Sicherer SH, Vickery BP, Jones SM, Liu AH, Fleischer DM, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol. 2013;131(3):805–12.
34. James JM, Sampson HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. J Pediatr. 1992;121(3):371–7.
35. Gocki J, Bartuzi Z. Role of immunoglobulin G antibodies in diagnosis of food allergy. Postepy dermatologii i alergologii. 2016;33(4):253–6.
36. Savilahti EM, Rantanen V, Lin JS, Karinen S, Saarinen KM, Goldis M, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. J Allergy Clin Immunol. 2010;125(6):1315–21.
37. Furuta GT, Katzka DA. Eosinophilic Esophagitis. N Engl J Med. 2015;373(17):1640–8.
38. Erwin EA, Tripathi A, Ogbogu PU, Commins SP, Slack MA, Cho CB, et al. IgE antibody detection and component analysis in patients with eosinophilic esophagitis. J Allergy Clin Immunol Pract. 2015;3(6):896–904.
39. Chatchatee P, Jarvinen KM, Bardina L, Vila L, Beyer K, Sampson HA. Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow's milk allergic patients. Clin Exp Allergy. 2001;31(8):1256–62.
40. Chatchatee P, Jarvinen KM, Bardina L, Beyer K, Sampson HA. Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: differences in patients with persistent and transient cow's milk allergy. J Allergy Clin Immunol. 2001;107(2):379–83.
41. Lorenz AR, Scheurer S, Vieths S. Food allergens: molecular and immunological aspects, allergen databases and cross-reactivity. Chem Immunol Allergy. 2015;101:18–29.
42. Ford LS, Bloom KA, Nowak-Wegrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. J Allergy Clin Immunol. 2013;131(1):180-6–e1-3.
43. Bartnikas LM, Sheehan WJ, Hoffman EB, Permaul P, Dioun AF, Friedlander J, et al. Predicting food challenge outcomes for baked milk: role of specific IgE and skin prick testing. Ann Allergy Asthma Immunol. 2012;109(5):309–13.
44. Mehr S, Turner PJ, Joshi P, Wong M, Campbell DE. Safety and clinical predictors of reacting to extensively heated cow's milk challenge in cow's milk-allergic children. Ann Allergy Asthma Immunol. 2014;113(4):425–9.
45. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. Clin Exp Allergy. 2014;44(5):642–72.
46. Caubet JC, Nowak-Wegrzyn A, Moshier E, Godbold J, Wang J, Sampson HA. Utility of casein-specific IgE levels in predicting reactivity to baked milk. J Allergy Clin Immunol. 2013;131(1):222-4–e1-4.
47. Gernez Y, Nowak-Wegrzyn A. Immunotherapy for food allergy: are we there yet? J Allergy Clin Immunol Pract. 2017;5(2):250–72.
48. Savilahti EM, Kuitunen M, Valori M, Rantanen V, Bardina L, Gimenez G, et al. Use of IgE and IgG4 epitope binding to predict

the outcome of oral immunotherapy in cow's milk allergy. Pediatr Allergy Immunol. 2014;25(3):227–35.

49. Martinez-Botas J, Rodriguez-Alvarez M, Cerecedo I, Vlaicu C, Dieguez MC, Gomez-Coronado D, et al. Identification of novel peptide biomarkers to predict safety and efficacy of cow's milk oral immunotherapy by peptide microarray. Clin Exp Allergy. 2015;45(6):1071–84.
50. D'Urbano LE, Pellegrino K, Artesani MC, Donnanno S, Luciano R, Riccardi C, et al. Performance of a component-based allergen-microarray in the diagnosis of cow's milk and hen's egg allergy. Clin Exp Allergy. 2010;40(10):1561–70.
51. Gjesing B, Osterballe O, Schwartz B, Wahn U, Lowenstein H. Allergen-specific IgE antibodies against antigenic components in cow milk and milk substitutes. Allergy. 1986;41(1):51–6.