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Cow's milk allergic children—Can component-resolved diagnostics predict duration and severity?

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Abstract

Background: Cow's milk allergy (CMA) affects 2% of all children. This study investigatescomponent-resolved diagnostics(CRD) to cow's milk proteins in children suspected of CMA, by correlating the level of CRD with outcome of the oral challenge. Furthermore, we evaluate the ability of serial CRD measurements to distinguish children with persistent CMA from children developing tolerance.

Methods: We included data from 78 children referred to the Allergy Centre during a 13-year period. Results from oral food challenges including threshold, severity, and sensitization data (IgE antibodies to whole milk protein, IgE components toward milk and skin prick test (SPT)) were collected. The milk allergic children were re-evaluated with sensitization data and rechallenges regularly.

Results: Thirty-nine children had negative first challenges, and 39 had positive first challenges. The positive group was rechallenged and separated into 3 groups depending on time to remission. At inclusion, children with persistent CMA had significantly larger size of SPT and higher levels of s-IgE to milk and CRD compared to the other groups. SPT wheal size was significantly larger in children with persistent CMA compared to children outgrowing CMA. Furthermore, a correlation between s-IgE level to cow's milk and casein and the severity of the allergic reaction elicited by food challenges was found.

Conclusion: Oral food challenge cannot be replaced by s-IgE to whole milk protein or milk components nor SPT in the diagnosis of CMA; however, high levels of milk components and s-IgE to milk increase the risk of a long-lasting or persisting CMA.

KEYWORDS

anaphylaxis, challenge tests, component-resolved diagnostics, cow's milk, food allergy

1 | INTRODUCTION

Cow's milk allergy (CMA) is one of the most frequent allergies in infancy, affecting approximately 2% of all children,[1,2] but with a good overall prognosis as 80% will outgrow their allergy before the age of 4.[1] A recent study has, however, questioned this early tolerance development, finding that 40% of children had persistent CMA at the age of 5 years.[3]

A diagnosis of CMA affects children and their families economically and socially, making a correct diagnosis crucial. Today, the diagnostic workup of CMA is based on case history, skin prick test (SPT), specific IgE (s-IgE), and an oral food challenge (OFC) for confirmation.[4] OFC is time-consuming, costly, and may be difficult to perform in a clinical setting. Furthermore, the OFC may be unpleasant to undergo and carry a potential risk to the child.[5] Therefore, a replacement for the OFC would be beneficial for both patients and clinicians, but due to their relatively low specificity and sensitivity, in vitro and in vivo tests have not been able to replace the OFC as the gold standard for diagnosing CMA.[6-8] In the past decades, several studies have tried to optimize decision points to discriminate between sensitization without

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clinical relevance and IgE-mediated food allergy; especially in peanut allergy, but also emerging for other foods,[9,10] component-resolved diagnostics (CRD) has been a very promising tool with the ability to discriminate allergic from tolerant patients.[11-13] However, until now, studies investigating the use of CRD in the diagnosis of CMA have shown conflicting results.[14-16]

The aim of this study was to investigate the usefulness of CRD to cow's milk proteins in children suspected of CMA, by correlating the level of CRD with the outcome of the oral challenge, including threshold and the severity of elicited symptoms. Furthermore, we evaluated the ability of serial CRD to predict longitudinal trajectories to distinguish children with persistent CMA from children developing tolerance.

2 | METHODS

This retrospective study included children suspected of CMA and referred to the Allergy Centre, Odense University Hospital, Denmark, between 2000 and 2012. Final inclusion criteria were a positive or negative oral cow's milk challenge, combined with available sensitization data, that is, SPT, s-IgE, and at least one measurement of CRD obtained within a year from the challenge date.

Children were put on elimination diet of cow′s milk prior to challenge, and those with a positive challenge continued the elimination diet, and had their CMA re-evaluated by challenge according to guidelines.[4] Median time between rechallenge was 12 months, with large individual variation [range 5-65 months].

SPT was performed on the volar surface of the forearm with a 1-mm lancet according to European Academy of Allergy and Clinical Immunology (EAACI) guidelines applying skin "prick prick" technique with pasteurized low-fat milk.[17,18] A positive SPT was defined as a mean wheal diameter ≥3 mm. A positive control and a negative control were included (Soluprick® ALK-ABELLÓ, Hørsholm, Denmark).

Serum IgE was analyzed by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). This included total IgE, s-IgE toward cow′s milk (f2) and cow′s milk component, α-lactalbumin (nBos d 4), β-lactoglobulin (nBos d 5), bovine lactoferrin (nBos d Lactoferrin), and casein (nBos d 8). Analyses were performed in accordance with the manufacturer's instructions. All patients were challenged according to EAACI guidelines by trained staff.[19] Open challenges were performed when an IgE-mediated acute reaction with objective signs was expected and/or when the child was ≤3 years. Only 3 challenge tests were performed double-blinded and placebo-controlled. Challenges were performed with commercially available pasteurized low-fat milk (fat content 1.5%). Challenge regimen and administrated doses have previously been described.[20] Symptom severity was classified into 5 groups according to Sampson et al[21] ranging from localized urticaria, nausea, abdominal pain, pruritus, OAS (group 1), generalized urticaria, angioedema and emesis (group 2), repetitive vomiting and rhinorrhea (group 3), and diarrhea and asthma (group 4) to anaphylaxis shock (group 5). All parents gave informed consent to the AC database (license no. 2008-58-0035), and the project was approved by the Danish Data Protection Agency (license no. 2012-58-0018).

2.1 | Statistics

t test: Differences between the 4 groups in levels of s-IgE were compared using the *t* test, and Spearman's rank correlation coefficient (ρ_s) was used to correlate components and thresholds. The best cutoff was found using receiver operation characteristic (ROC) curve analysis, and area under curves (AUCs) were compared using the Delong test. Spearman's rank correlation coefficient (ρ_s) was used to correlate thresholds, severity, and IgE levels of components. All calculations were performed in Stata vers. 14 (StataCorp, College Station, TX, USA) on a Windows 7 platform.

3 | RESULTS

Data from 78 children (51 boys and 27 girls) were included, where 39 had a negative first challenge (group *N*) and 39 had a positive first challenge—see flowchart (Figure 1) for descriptive data. The positive

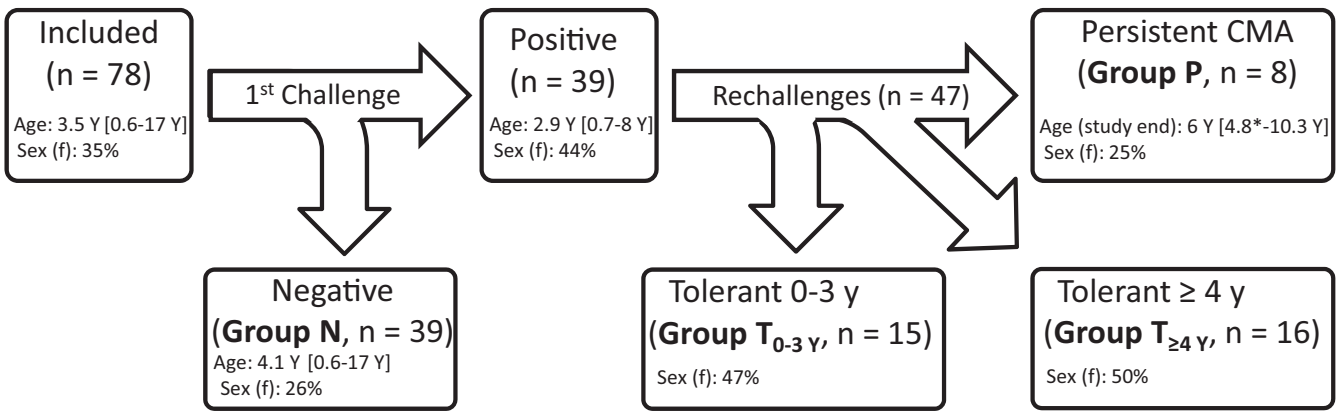

FIGURE 1 Flowchart of diagnostic algorithm and characteristics of the 125 cow's milk challenges performed at 78 children included in the study. At rechallenge, some children were challenged more than once.
*One child categorized as persistent CMA was 2.2 years at study end

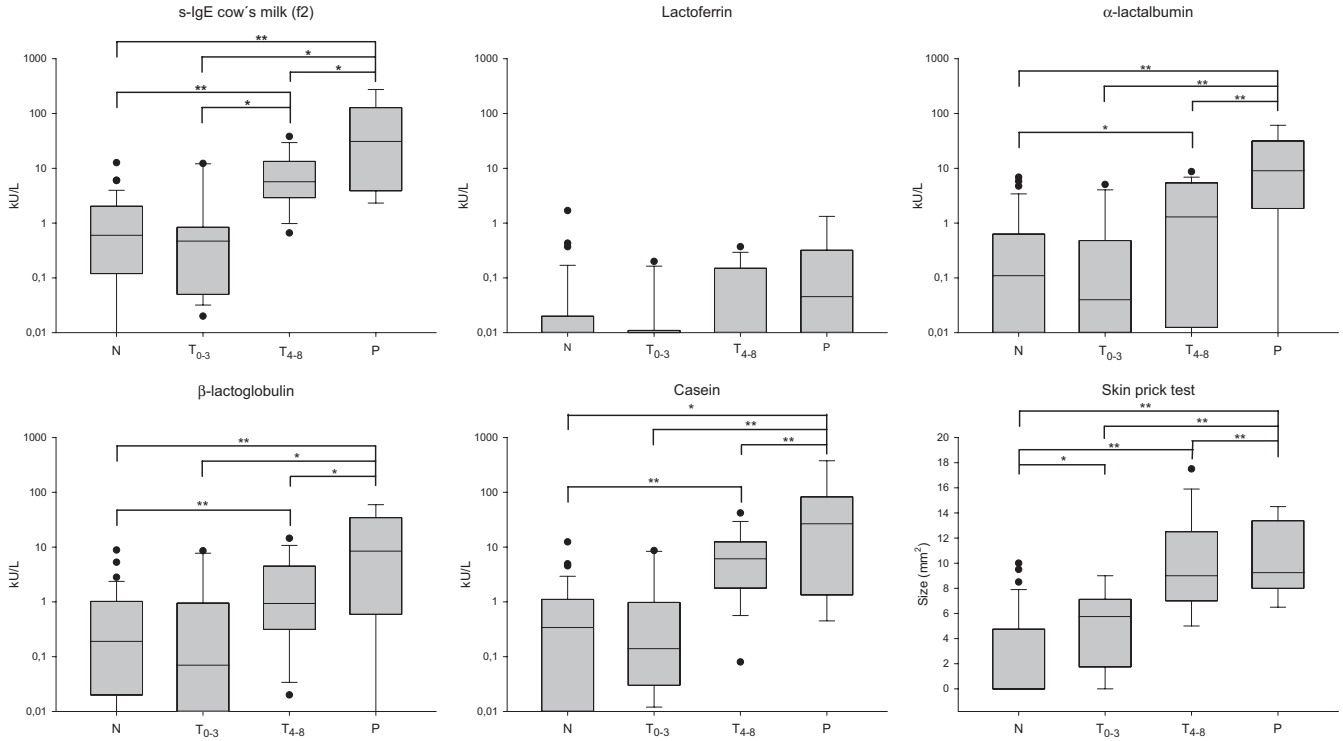

FIGURE 2 Levels of IgE to cow's milk, lactoferrin, α-lactalbumin, β-lactoglobulin, Casein, and SPT to cow's milk by challenge negative (N), early tolerance (T0-3), late tolerance (T4-8), and persistent cow′s milk allergy (P). * $P < 0{,}01$ **$P < 0{,}05$. The ordinates in KU/L are log-transformed. 0 has been set to 0.001

group was followed consecutively for up to 89 months with regular challenges, and 31 of 39 (79%) later developed tolerance to CMA, confirmed by a negative challenge. In this "tolerance-developing group," 15 children (38%) outgrew their CMA before the age of 4 years (group $T_{0\text{-}3}$, mean age at inclusion: 19,4 [range 6.5-36] months) and 16 children (41%) outgrow their CMA after the age of 4 years (group $T_{4\text{-}8}$, mean age at inclusion: 40.9 [range 6-93,9] months). The remaining 8 children (21%) had persisting CMA until study end (group *P*, mean age at inclusion was 40.5 [range 9.1-89.6] months).

3.1 | IgE levels at first challenge

We found no difference in IgE levels for α-lactalbumin, β-lactoglobulin, bovine lactoferrin, casein, and s-IgE to cow′s milk between the challenge negative group (*N*) and those children who developed tolerance before 4 years of age ($T_{0\text{-}3}$) at first challenge. Only the SPT showed significant differences ($P \leq .01$) between group N (mean SPT 2.3 mm) and group $T_{0\text{-}3}$ (mean SPT 5.2 mm). Six children with a positive challenge had no detectable s-IgE, and all of these developed early tolerance ($T_{0\text{-}3}$). Only one of these had SPT >3 mm to cow's milk. S-IgE to cow′s milk (f2) was significantly higher in the group who developed late tolerance compared to the group who developed early tolerance. No differences were found between the IgE levels for α-lactalbumin, β-lactoglobulin, bovine lactoferrin, casein, and for SPT between the group who developed tolerance late and the group who developed early tolerance. Children with persistent CMA (*P*) had significantly larger size of skin prick test and higher levels of s-IgE to milk, and all measured components except from lactoferrin than all the other groups (*N*, $T_{0\text{-}3 \text{ and }} T_{4\text{-}8}$) at inclusion. IgE levels for cow′s milk, α-lactalbumin, β-lactoglobulin, and casein were lower in the challenge negative group (*N*) than the group that developed late tolerance ($T_{4\text{-}8}$). This significant difference was also found for skin prick test size (Figure 2). Lactoferrin did not show any significant differences between any of the groups.

3.2 | Diagnostic values of IgE and SPT at time of challenge

We performed ROC curve analyses to test the ability of s-IgE to cow′s milk, components to cow′s milk, and SPT to discriminate between milk allergic and non-allergic children. The ROC curve results show that the casein and s-IgE to cow′s milk are the superior predictors of the outcome (as positive/negative) of the challenge test. The f2 (cow′s milk) has an optimal cutoff value of ≥3.64 kU/L with a sensitivity of 63% and a specificity of 87%. The IgE to casein has a cutoff value of ≥2.33 kU/L with a sensitivity of 61% and a specificity of 83%. A specificity of 100% was obtained by applying cutoff levels ≥26.1 kU/L for cow′s milk and 16.8 kU/L for casein with a corresponding sensitivity of 16% and 21%, respectively. There was no significant difference between area under curve (AUC), except for lactoferrin, that had significantly lower AUC.

The result indicates that IgE to casein and cow′s milk is performing with similar accuracy in a clinical setting. It also shows the limitations

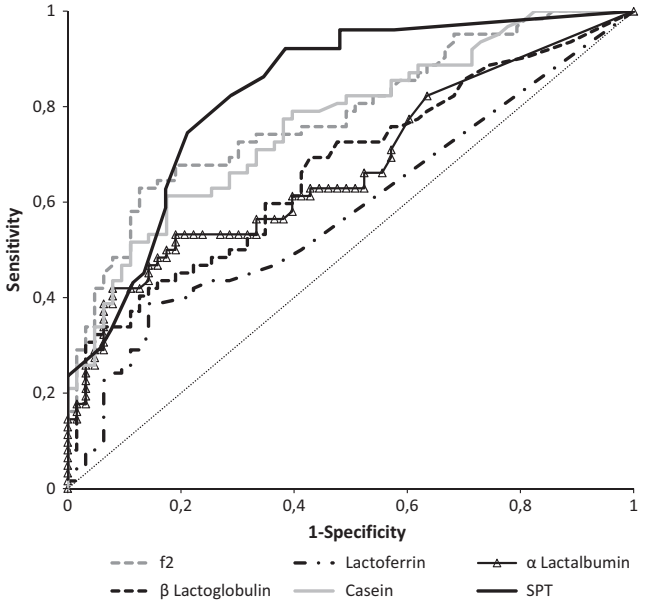

FIGURE 3 ROC curve for s-IgE and SPT for cow's milk, lactoferrin, α-lactalbumin, β-lactoglobulin, and casein

of both cow′s milk and Casein when it comes to differentiate between positive and negative challenges (Figure 3).

3.3 | Correlation between serology, threshold, and severity of the allergic reaction at OFC

No significant differences were found when looking at the correlation between the threshold and the level of s-IgE to cow′s milk and the components, whereas the size of the SPT correlated to threshold of the challenge test (*R*s: 0.32 *P* < .01). Threshold was neither correlated to the severity of the allergic reaction nor to the duration of the disease ($T_{0\text{-}3}$, $T_{4\text{-}8}$, *P*). Severity of a positive reaction was graded and classified into 5 groups according to Sampson et al[21] whereas a negative challenge was denoted with severity = 0. For all challenges (n = 125), we found a correlation between the levels of s-IgE to cow′s milk (*R*s = 0.53, *P* < .05), casein (R_s = 0.49, *P* < 0.05), and the severity of the allergic reaction during oral food challenge. By only including positive challenges (n = 86), the positive correlation between levels of s-IgE to cow′s milk (*R*s = 0.26, *P* < .05) and for casein (*R*s = 0.25, *P* < .05) was reduced (Table 1). Large individual variations within each group were observed.

4 | DISCUSSION

In this retrospective chart review including 78 children suspected of cow's milk allergy during a period of 12 years, we collected data of outcome from challenge tests and s-IgE. To our knowledge, only a few studies[3,14–16,22,23] have been performed looking at the natural course of cow's milk allergy including component-resolved diagnostics, trying to find factors predicting which patients outgrowing their allergy. In this study, we looked at the IgE levels at the first challenge test and compared it with time to remission. We found a significant difference between the levels of s-IgE to cow′s milk, α-lactalbumin, β-lactoglobulin, and casein, and the time to remission, thus enabling distinguishing children with persistence of cow′s milk allergy from the groups outgrowing the cow′s milk allergy and the non-allergic group. Skin prick test (SPT) could separate all groups, except those with early tolerance ($T_{0\text{-}3}$) from those with late tolerance ($T_{4\text{-}8}$); however, these two groups could be distinguished by their s-IgE to cow′s milk level. These findings are in accordance with the prospective study performed by T. Vanto et al[14] In this study, T. Vanto was able to separate children developing tolerance from children with persisting CMA on the basis of the level of IgE to cow′s milk, casein, β-lactoglobulin, and SPT.

This is, to our knowledge, the first published study, which has demonstrated that CRD using ImmunoCAP can segregate children outgrowing their CMA at early age from children outgrowing their CMA later in childhood, thus predicting the natural course of the CMA. Ahrens et al investigated the ability of s-IgE to cow′s milk and CRD by ImmunoCAP to predict remission of CMA in children. They found a significant difference when looking at s-IgE to cow's milk, but not at the level of casein. Interestingly, the study showed enhanced likelihood of becoming tolerant if the microarray-based IgE to alpha-lactalbumin, beta-lactoglobulin, α s1-casein and κ-casein were low.[16]

Based on the 125 performed challenges, ROC curves analysis was applied to diagnostic values and ideal cutoff. Best serological predictor for clinical CMA was s-IgE toward cow′s milk and casein, with cutoffs at ≥3.64 kU/L and ≥2.33 kU/L, respectively (AUC: 78%/77%,

TABLE 1 Diagnostic values and correlation to threshold/severity for s-IgE and skin prick test (SPT) for cow′s milk, lactoferrin, α-lactalbumin, β-lactoglobulin, and casein

Components	Best cutoff	Sens. (%)	Spec. (%)	AUC	% Corr. Diag.	Threshold (R_s)	*P*-value*	Severity(R_s)	*P*-value**
f2	≥3.64 kU/L	62.9	87.3	0.78	75.2	0.07	<.01	0.26	<.05
Lactoferrin	≥0.08 kU/L	38.7	85.7	0.58	62.4	0.02	.20	0.19	.13
α-lactalbumin	≥0.77 kU/L	53.2	81.0	0.67	67.2	0.07	<.01	0.14	.26
β-lactoglobulin	≥1.59 kU/L	43.6	84.1	0.67	64.0	0.11	<.01	0.15	.23
Casein	≥2.33 kU/L	61.3	82.5	0.77	72.0	0.06	<.05	0.25	<.01
SPT	≥5.00 mm	92.2	61.5	0.83	75.9	0.32	<.01	0.22	.12

*To threshold (in mL cow's milk).
**To severity measured by Sampson score 1-5.[18]

specificity 87%/83%, and sensitivity: 63%/61%, respectively). The remaining IgE components did not improve or add any clinical value.

Ott et al have described a specificity of IgE to cow's milk (*f*2) of 81.4% and a sensitivity of 51.4% but at a cutoff level of 8.1 kU/L and concluded that the clinical usefulness of the sum of components for milk using a microarray (ISAC™) (specificity: 83.7, sensitivity: 59.5) is the same as using ImmunoCAP.[24] Identifying children at risk of anaphylaxis (meaning type 1 allergic reaction to cow's milk) versus children with minor reactions, Cingoloni et al found that the ImmunoCAP for casein performed better than the other components (cutoff: 1.8 kUA/L, specificity: 0.77, sensitivity: 0.65). These findings are close to the results reported in our study.[25]

We were also able to establish cutoff levels for s-IgE cow's milk and to casein with a specificity of 100% (26.1 kU/L and 16.8 kU/L, respectively) on the cost of reduced sensitivity (16% and 21%, respectively). Ott et al[24] describe a cutoff level of 66.90 kU/L with a specificity of 99%. This means that our data are in accordance with studies conducted previously when looking at AUC, specificity, and sensitivity, but the decision points vary within the populations.[15,26,27]

From a clinical point of view, s-IgE tests cannot sufficiently discriminate between tolerant and allergic children, making the challenge test necessary. The s-IgE to casein can be used equally to s-IgE to milk (*f*2) but is not superior to s-IgE to milk, and at the time being no single laboratory test confirms the diagnosis of cow's milk allergy. As the IgE decision points vary from one population to another, the decision points cannot be transferred between populations.

To our knowledge, only one study [25] has investigated the correlation between severity of adverse reactions to milk and IgE levels of milk components, but surprisingly excluded children with most severe case histories from oral challenges. In line with other allergens (reference 9,12), we found that the severity could be predicted on group level by s-IgE to milk (*f*2) and casein, in line with finding from other allergens, meaning higher IgE to cow's milk and casein increases the risk of a severe allergic reaction, however it's speculative whether this correlation is transferable to individual level in real-life settings. It is important to notice, that despite this correlation, challenge tests are still safe to perform. We challenged patients with very high levels of IgE, without eliciting any severe reactions (grade 5—anaphylactic shock).

Surprisingly, in our study, only 38% gained tolerance before the age of 4 years and 21% were continuously allergic at study end. This finding might be due to selection bias, as we only included patients from a tertiary center. Therefore, our results ideally have to be confirmed in a prospective study including children referred from clinical practitioners.

5 | CONCLUSION

Our data indicate that neither s-IgE antibodies to whole milk protein nor IgE components are able to replace the oral food challenges as the gold standard in the diagnosis of cow's milk allergy. We have shown that a high level of IgE to specific cow's milk proteins, casein, α-lactalbumin, and β-lactoglobulin, increases the risk of a long-lasting or persisting cow's milk allergy, which is also reflected in whole milk protein (s-IgE to milk). We found a correlation between the level of s-IgE to cow's milk and casein and the severity of the allergic reaction. The findings could be included in daily practice when evaluating cow's milk allergic children.

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