

EDITORIAL

Clinical & Experimental Allergy

What's on the menu for prediction of natural history of cow's milk allergy beyond casein cocktails and epitope soup?

This editorial discusses the findings of the paper in this issue by B. Ahrens et al. [15], pp. 1630–1637.

K. M. Järvinen and J. Celestin

Division of Allergy, Asthma and Immunology, Albany Medical College, Albany, NY, USA

One of the most compelling questions for the clinician at the time of initial diagnosis of food allergy is to ascertain whether tolerance will occur over time. Knowledge of a favourable natural history, often gives great comfort to the parents of young children with food allergy. On the other hand, if the prognosis is poor, such information could provide valuable information to the clinician for strategic therapeutic planning. Such patients could be targeted for immunotherapeutic interventions, which have the potential of changing the natural course of their food allergy, if available and age appropriate.

The resolution of food allergy is variable and depends on the specific food allergen. In one earlier referral-based population study, a relatively poor outcome was observed, with only 19% of children with cow's milk allergy (CMA) becoming tolerant by age 4 [1]. However, in a more recent population-based birth cohort study of 13 019 children, 31 (57.4%) of 54 infants recovered from their IgE-CMA during the study period (4–5 years) [2]. Currently, there are no reliable predictors or biomarkers to determine when and in whom resolution of food allergy will occur. Therefore in clinical practice, periodic clinical follow up including the history of recent reactions (or lack thereof) together with the measurement of serum-specific IgE levels and skin-prick tests (SPTs), can help determine when oral food challenges are indicated to determine tolerance development.

Caseins are the major allergens responsible for CMA. They comprise about 80% of the total protein content in cow's milk. The casein fraction is comprised of α s1-, α s2-, β - and κ -casein, which correspond to 32%, 28%, 10% and 10% of the total protein content respectively. The whey fraction includes allergenic proteins such as α -lactalbumin and β -lactoglobulin among other proteins. In search for prognostic markers, James and Sampson initially reported that casein and β -lactoglobulin-specific IgE serum levels were lower initially and decreased significantly in patients who acquired tolerance in contrast with those who retained clinical sensitivity [3]. Similarly, in another study, lower levels of cow's milk-specific IgE were observed in children who became tolerant [4]. More recently, the peak cow's milk-specific IgE was found to be highly predictive of the natural history for individual patients, as subjects with levels greater than 50 kUA/L generally tend to have more persistent allergy [1]. Similarly, larger SPT wheal size has been associated with persistence of CMA [2, 5], although disputed by some [3]. Furthermore, the initially elevated serum IgG1 to IgG4 ratio observed in children with CMA was inverted in subjects with resolution of their food allergy [6]. Finally, the serum concentrations of IgG1- and IgG4-specific antibodies, and the IgE/IgG1 and IgE/IgG4 ratios for milk proteins were found to be significantly lower in the patients who are losing their milk allergy [3]. Therefore, monitoring these ratios may have some prognostic value in the natural history of CMA. It has also been shown that the development of tolerance is related to a shift towards the development of casein-specific IgA antibodies [7].

The measurement of specific IgE against allergenic (IgE-binding) portions of milk allergens (epitopes) shows promise in better differentiating subjects with favourable outcomes for milk allergy. It has been dem-

Correspondence:

Kirsi M. Järvinen-Seppo, Division of Allergy and Immunology & Center for Immunology and Microbial Diseases, Albany Medical College, 47 New Scotland Ave, MC # 151, Albany, NY, USA. E-mail: jarvink@mail.amc.edu

Cite this as: K. M. Järvinen and J. Celestin, *Clinical & Experimental Allergy*, 2012 (42) 1549–1551.

onstrated that persistence of milk allergy is associated with the presence of IgE against sequential (linear) as opposed to conformational epitopes of milk allergens [8]. Sequential epitopes are comprised of consecutive amino acids in a protein sequence, whereas conformational epitopes consist of amino acids that are brought to close proximity by protein folding from different domains of the protein. More specifically, patients with persistent milk allergy recognize a greater number of sequential milk-protein epitopes on α s1-, β - and κ -casein, major milk allergens, as compared with patients who developed clinical tolerance to milk [9]. Peptide microarray technology uses a small amount of serum and can be used to assess hundreds of epitopes at once. Parallel determination of different antibody isotypes using microarray also allows for the determination of allergen-specific IgE/IgG4 levels. Using this technology, it has been observed that an increase in the intensity of IgG4 binding to cow's milk epitopes occurred concurrently with a decrease in IgE-binding intensity among 12 patients who recovered early (by 3 years) from their CMA [10]. The authors stated that IgE and IgG4 (but not IgA) binding patterns to a panel of α s1-, α s2-, β - and κ -casein regions help predict outcomes with significant accuracy. The study by Wang et al. [11] using the same techniques, assessed milk-specific IgE and IgG4 epitope diversity and affinity of a total of 41 subjects, who in the follow-up either outgrew their allergy, retained their allergy or developed tolerance to heat-denatured (baked) milk. They reported that greater IgE epitope diversity and higher affinity were seen in those subjects with no resolution of their CMA.

Protein microarray technology allows for the measurement of specific IgE against multiple molecules, i.e. individual allergens (such as β -lactoglobulin) as opposed to measuring IgE to the whole food (as with cow's milk), making molecular diagnosis or component-resolved diagnostics (CRD) possible. Nowadays, a growing number of recombinant and purified molecules can be evaluated. This approach may provide a diagnostic and prognostic platform for identifying food-allergic subjects from those with sensitization based on cross-reactivity. It may also help predict the severity of clinical reactions based on sensitization to proteins with greater risk of severe reactions, and the natural history of food allergy. As an example, Nicolaou et al. [12] compared the sensitization profiles of peanut-allergic and peanut-tolerant children. They identified the peanut component Ara h 2 as the most important predictor of clinical peanut allergy. Information of CRD microarray technology was also compared against oral food challenges in the diagnosis of cow's milk ($n = 85$) or egg ($n = 60$) allergy by Ott et al. [13]. Although the performance characteristics of CRD are similar to established

methods of IgE detection, the small amount of blood required for analysis, which could be obtained from capillaries, might be a lucrative alternative for young patients. However, another study reported that in children with milk allergy ($n = 58$), the same technology can help predict oral food challenge results [14]. Compared to serum-specific IgE testing by ImmunoCAP (ThermoFischer Scientific), it may provide better negative predictive value in casein-specific IgE-positive children with levels lower than the 95% predictive value, when the diagnosis is in doubt. Therefore, it may reduce the number of oral food challenges required to confirm or rule out tolerance.

Using the CRD approach, the current article is the first attempt to predict the prognosis of milk allergy based on the determination of total IgE, cow's milk-specific IgE and specific IgG and IgG4 to α -lactalbumin, β -lactoglobulin and casein done by fluorescence enzyme immunoassay using the ImmunoCAP 250 System[®] (ThermoFischer Scientific, ImmunoDiagnostics Division, Portage, MI, USA; former Phadia, Uppsala, Sweden) [15]. They also utilized allergen microarray analysis to measure IgE to natural purified cow's milk allergens by ImmunoCAP ISAC[®]134 IgE assay (VBC-GENOMICS, Vienna, Austria; Phadia, Uppsala, Sweden). They conducted a retrospective analysis of 52 children with challenge-confirmed CMA who were followed over time for tolerance development. They found that the likelihood of outgrowing CMA correlates with a low level of cow's milk-specific IgE as well as low levels of specific IgE to α -lactalbumin, β -lactoglobulin, κ -casein and α s1-casein, but not with specific IgG and IgG4 levels to α -lactalbumin, β -lactoglobulin and total casein. They concluded that cow's milk-specific IgE is a good prognostic marker for persistence of CMA, and that CRD does not add to the prognosis of CMA especially in light of its increased cost. Another group recently included a mathematical model to predict the age of tolerance development to cow's milk, utilizing IgE levels to several milk components [16]. This approach currently does not allow for the prediction of individual patient's tolerance development.

In conclusion, judging from the present literature, the best predictive marker for resolution of CMA that is currently available to us still appears to be the "good old" cow's milk-specific IgE. Although measurement of IgE epitope-binding intensity, diversity or affinity to caseins using peptide microarray shows promise as a tool in identifying patients with less favourable prognosis, this method is still under investigation and available for research purposes only. The data so far suggest that CRD adds more cost, but does not necessarily provide more information to the prognosis of CMA, although studies in different populations of patients might prove otherwise. Until better prognostic biomarkers become available, the peak and trend of specific

IgE in addition to SPT size and clinical acumen may give a reasonable estimate of one's chances of outgrowing milk allergy. Clinical signs of poor prognosis include severe symptoms at presentation, multiple sensitizations and early onset of asthma and allergic

rhinitis, reaction to a small amount of milk and young age at the time of the first reaction [2, 5].

Conflict of interests: The authors declare no conflicts of interests.

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