IMMULITE® 2000 and Cow's Milk Allergy: A Clinical Perspective

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As a pediatric facility, Lisbon's Dona Estefânia Hospital takes a special interest in the area of food allergy, particularly that associated with cow's milk, the most frequent food allergy in Portugal. Heavily reliant on food challenge tests for diagnosis and follow-up, the hospital's Immunoallergy Department has turned to in vitro allergy testing in an effort to decrease the need for this risky practice. The department recently conducted a study to compare milk-specific IgE values obtained by IMMULITE® 2000 Allergy and UniCAP® in a subpopulation of pediatric patients with immediate symptoms of IgE-mediated cow's milk allergy (CMA). According to the study, both instruments demonstrated essentially the same performance, but investigators advised against using the methods interchangeably. Their findings also suggest that published specific IgE cutoff values for CMA are not only population specific, but method specific as well.

CMA

Cow's milk is one of the most significant food allergens worldwide, and approximately 2.5 percent of children are believed to suffer from CMA. The frequency of CMA in Portugal is highlighted by a food allergy distribution report on 414 Portuguese patients (ages 0 – 18 years) with food allergies, who were consecutively observed over the course of one year in Dona Estefânia Hospital's Immunoallergy Department (Figure 1). Cow's milk was responsible for almost half (46%) the cases of food allergies in this population, followed by egg (29%) and fish (7%).

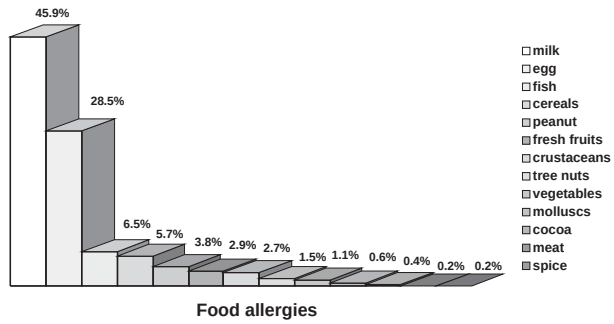


Figure 1.

CMA is more prevalent in the first years of life. While this disease is usually self-limiting, with a transient clinical course, it appears to be persistent in some cases. The self-limiting aspect of this condition is demonstrated through observations of 134 children by the Immunoallergy Department between 1993 and 1996. Most of the cases (92%) experienced CMA for less than 36 months, while the disease continued for a longer duration in approximately 8% of patients (Figure 2).

Duration of disease in 134 children with CMA observed between 1993 and 1996 (Immunoallergy Dept, Dona Estefânia Hospital, Lisbon)

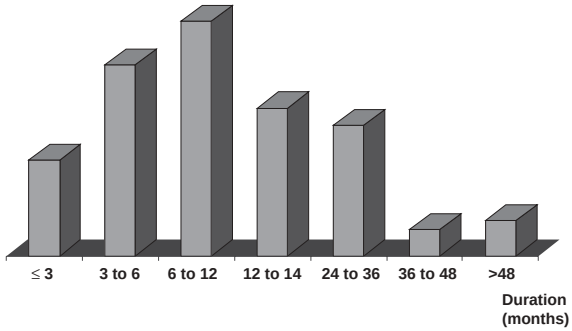


Figure 2.

A heterogeneous disease, CMA occurs in various forms including immediate IgE-mediated reactions, which typically manifest as urticaria or wheezing; delayed IgE-mediated reactions, which often lead to symptoms such as eczema; non-IgE-mediated reactions, which result in a number of enteropathies; and some mixed disorders. Routine diagnosis of CMA is based on clinical history and skin prick testing (SPT). SPT is highly sensitive; however, its positive predictive value is limited. Consequently, when diagnosing CMA or any other food allergy, the hospital's Immunoallergy Department always employs the food challenge test, which is considered to be the gold standard.

CMA therapy is based on a strict avoidance diet, free of any dairy products, for a period of months to years, depending on the evolution of the allergy. Patients are followed throughout the duration of the disease with periodic food challenges to determine the point at which milk tolerance is achieved. Once the results of these food challenges become negative, the patient can resume a normal diet.

Pitfalls of the Food Challenge

Although the food challenge test is considered to be the gold standard, there are a number of problems associated with this test. It is dangerous for patients to undergo, owing to the risk of reaction. Therefore, it must always be performed in a hospital under experienced supervision. The food challenge is also very time consuming to perform, as it requires the administration of progressively increasing quantities of milk, beginning with 1 drop or 1 mL at intervals of 20 to 30 minutes. Doses may be increased until they reach a normal serving size. Throughout the testing period, the patient is monitored for any reactions over a period of several hours. These repeated food challenges may have a negative

psychological impact on children, due to the feelings of fear and anxiety experienced during testing and the disappointment when a challenge is positive. Furthermore, positive food challenges could theoretically have an adverse effect on the immunological mechanisms for achieving food tolerance. By giving children food to which they react, the challenge test may stimulate the immune response and delay tolerance.

Quantitative Allergen-specific IgE

There is clearly a great need for improved diagnostic methods to better identify allergic patients, predict tolerance achievement and decrease the administration of potentially positive challenge tests. In cases of IgE-mediated allergy, quantitative serum allergen-specific IgE offers appealing possibilities as a clinical tool. It may allow clinicians to do any or all of the following for patients with IgE-mediated allergy:

- Identify clinically relevant sensitization—not just the presence of sensitization
- Evaluate effectiveness of allergen avoidance measures
- Monitor evolution of allergic disease
- Obtain prognostic information.

Validation Study

Since different in vitro methods for allergen-specific IgE have become available, questions have inevitably emerged regarding performance comparability, including whether or not results obtained by different methods can be used interchangeably. It is apparent that there remains a strong need for careful evaluation of these methods. After the introduction of IMMULITE 2000 Allergy into the hospital's Immunoallergy Department, investigators at Dona Estefânia Hospital began validating IMMULITE 2000 Allergy by comparing its clinical performance and other characteristics to those of their previous method (UniCAP). One element of this process included a comparative study expressly designed to validate IMMULITE 2000 Allergy cow's milk-specific IgE results against values obtained with UniCAP in a group of patients and controls.

Sample Selection

For the purposes of this study, the investigators focused on a relatively homogeneous sample population for whom specific IgE measurements could have meaningful results. They selected a subgroup of 31 pediatric patients (ages 1 – 9 years; mean 4.3 years) with IgE-mediated CMA manifesting with immediate-type symptoms. All patients were in CMA follow-up at the hospital.

Each had been previously diagnosed by a clinical history indicative of CMA and a positive SPT and challenge test to milk. In addition, all individuals displayed immediate-type symptoms, such as urticaria, wheezing, vomiting and diarrhea, within a few minutes to a few hours after milk ingestion.

The Control Group

The controls consisted of recently stored serum samples from 19 "unhealthy" children (ages 2 – 14 years; mean 6.4 years) with atopic dermatitis and other allergic symptoms, such as asthma and rhinitis. These patients had no history of CMA.

Investigators selected "unhealthy" controls since a previous study had already provided data using healthy controls. Furthermore, this control group would be more representative of patients encountered in the usual clinical setting. Using serum of atopic dermatitis patients was justified since these patients often have high serum levels of total IgE. As false-positive results occur frequently in populations with high serum IgE, the investigators recognized that this control group would also enable them to test method specificity.

Methods

During the study, all patients from the sample group underwent a milk challenge. The outcome of these tests revealed that 22 out of 31 patients remained symptomatic, while nine had become milk tolerant. (Data from these nine individuals were excluded from the analysis comparing patients and controls.)

Serum milk-specific IgE measurements were obtained using both IMMULITE 2000 Allergy and UniCAP on the same blood sample for each patient. The results were considered positive if values were ≥ 0.35 kU/L. SPT with milk extract (IPI-ASAC Spain) was considered positive if the mean wheal diameter was >3 mm.

Results

The distribution of patient results appeared very similar for each method, revealing a large dispersion of values for both IMMULITE 2000 Allergy and UniCAP. Results for controls were much lower, with a few being positive.

Cow's milk-specific IgE

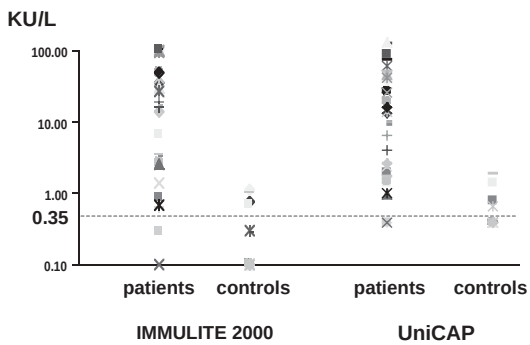


Figure 3.

A side-by-side comparison of results for the CMA patient (sample) group indicated that UniCAP values tended to be lower than IMMULITE 2000 Allergy values within the same samples (Figure 4).

Cow's milk-specific IgE: patient result comparison

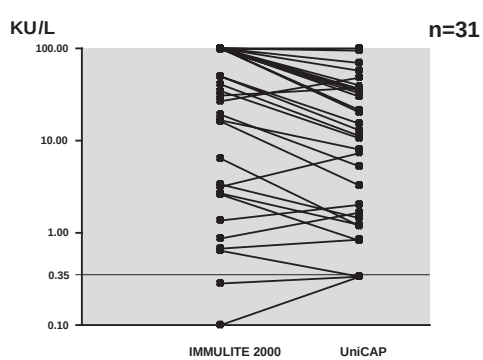


Figure 4.

A regression analysis of all 50 serum samples demonstrated good agreement between the two methods, with a correlation coefficient of 0.80 (Figure 5).

Correlation between methods

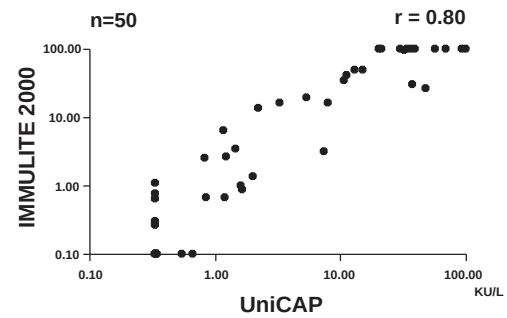


Figure 5.

When results for the 50 samples were distributed by class, both methods paralleled each other in all classes except Class 6 (Figure 6), in which values obtained by IMMULITE 2000 Allergy predominated. A detailed analysis of results by class displayed six patients who were categorized as Class 4 by UniCAP and Class 6 by IMMULITE 2000 Allergy. Three other patients were categorized as Class 6 by IMMULITE 2000 Allergy and Class 5 by UniCAP. Overall, however, the two methods demonstrated a total agreement of approximately 90%.

Cow's milk-specific IgE: distribution by class

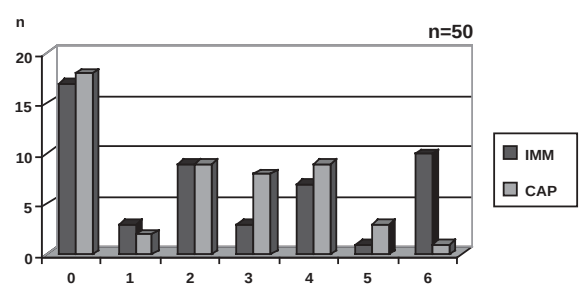
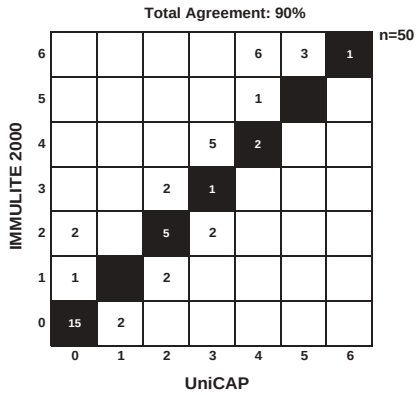


Figure 6.

Concordance



5 discordant results:

1 patient

pos. by IMMULITE 2000, neg. by UniCAP
neg. challenge

4 controls

2 pos. by UniCAP (class 1), 2 pos. by IMMULITE 2000 (class 2)

Figure 7.

When comparing the 22 patients with active CMA (positive challenge test during study) against the 19 controls, four false-positive results were obtained by each method and there were no false-negative results. Both methods demonstrated identical performance in this population, with 100% sensitivity, 78.9% specificity, 100% negative predictive value and 84.6% positive predictive value. The specificity was quite good, particularly since the controls consisted of atopic dermatitis patients.

In summary, the comparison study between IMMULITE 2000 Allergy and UniCAP indicates the following:

- Higher values obtained by IMMULITE 2000 Allergy
- No interference with clinical performance for most discrepancies between the two methods, as they occur in the upper classes in this population
- Good correlation and agreement between both methods
- Very high sensitivity and good specificity for both methods, given the high probability of false positives in the control group
- Same performance demonstrated by both methods

Published Decision/Cutoff Values

The clinical utility of quantitative specific IgE is currently under investigation, particularly in the case of food allergy. Many investigators have been studying the performance of different cutoff values. Two particularly notable studies have each identified very unique cutoff values for the diagnosis and follow-up of CMA. The reason for the disparity lies in the usage of different sample populations, which appear to dictate their own distinct clinical cutoff levels. Sampson (2002), for example, identified 15 kU/L as the IgE concentration most predictive of clinical reactivity to cow's milk (specificity 94%, positive predictive value 95%).[1] This cutoff value was obtained using the UniCAP method on older children exhibiting atopic dermatitis as the primary manifestation of food allergy. In contrast, Garcia-Ara et al. (2001) determined a set of four cutoff levels for clinical CMA, and indicated that specific IgE values > 5 kU/L demonstrated 99% specificity and 95% positive predictive value for CMA patients.[2] While this cutoff value was also obtained using the UniCAP method, the sample population consisted of infants, under the age of 1 year, with immediate symptoms.

This group of investigators did not wish to establish their own cutoff level, as their study population was not large enough, but they were curious as to how the two methods would perform in this population at some of the published cutoff levels.

Indeed, identical performance was obtained between the two methods when cutoff levels for IMMULITE 2000 Allergy were increased (Tables 1 and 2).

	Cutoff (kU/L)	Sens.	Spec.	NPV	PPV
UniCAP	2.5	90.9%	88.9%	80%	95.2%
IMMULITE 2000	5	90.9%	88.9%	80%	95.2%

Table 1.

	Cutoff (kU/L)	Sens.	Spec.	NPV	PPV
UniCAP	15	63.6%	100%	52.9%	100%
IMMULITE 2000	35	63.6%	100%	52.9%	100%

Table 2.

This observation was not surprising, considering that the published cutoff values (2.5, 5 and 15 kU/L) were established with the UniCAP method, and that IMMULITE 2000 Allergy values are higher than those of UniCAP. These results demonstrate that published cutoff levels for CMA are not just specific to a population, but also to the assay method used. Such findings highlight the need for caution when using these decision points for clinical application.

Conclusion

The results of this study indicate that both in vitro methods offer the same clinical performance; however, specific-IgE values obtained by these methods should not be used interchangeably.

References

1. Sampson HA. Improving in-vitro tests for the diagnosis of food hypersensitivity. Curr Opin Allergy Clin Immunol 2002;2:257-61.

2. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, Martin-Munoz F, Reche-Frutos M, Martin-Esteban M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cows' milk protein in the infant. J Allergy Clin Immunol 2001;107:185-90.

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