

Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children

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Abstract

Background: Oral challenges are required to establish the persistence or resolution of IgE-mediated cow's milk allergy (CMA). Determining the appropriate timing for challenging is the main difficulty. The benefit of the basophil activation test (BAT) in predicting a child's reaction to the oral challenge was evaluated and compared to the specific IgE and skin prick tests' (SPT) results.

Methods: One hundred and twelve consecutive children with CMA admitted for an oral challenge to reassess their allergy were included. Allergen-induced basophil activation was detected as a CD63-upregulation by flow cytometry.

Results: Thirty-six children (32%) had a positive oral challenge. The percentage of activated basophils in patients with a positive challenge (mean = 20.9; SD = 18.8) was significantly higher than that of patients with a negative challenge (mean = 3.9; SD = 9.8, $P < 0.0001$), and was well correlated with the eliciting dose of cow's milk ($P < 0.0001$). The BAT had an efficiency of 90%, a sensitivity of 91%, a specificity of 90%, and positive and negative predictive values of 81% and 96% in detecting persistently allergic patients. The area under the ROC curve was 0.866. These scores were higher than those obtained with SPT and IgE values, whichever positivity cut-point was chosen. Referring to a decisional algorithm combining BAT, specific IgE and SPT allowed the correct identification of 94% of patients as tolerant or persistently allergic to cow's milk proteins (CMP) in our cohort.

Conclusion: The BAT could be a valuable tool in the management of paediatric CMA in addition to specific IgE quantification and SPT, by contributing in determining whether an oral challenge can safely be undertaken.

IgE-mediated cow's milk allergy (CMA) is very common, affecting at least 2–3% of children under 1 year of age (1, 2) and requires the complete exclusion of cow's milk proteins (CMP) from the child's diet. Most children however outgrow their hypersensitivity (2). They must therefore be re-evaluated on a regular basis to detect the possible development of tolerance and thereby ensure that CMP are not eliminated from the diet for any longer than necessary.

Abbreviations

AUC, area under ROC curve; CMA, cow's milk allergy; CMP, cow's milk proteins; NPV, negative predictive value; PPV, positive predictive value; SD, standard deviation; sIgE, specific IgE.

The gold standard to discriminate between persistently allergic patients and those who have achieved tolerance is the oral challenge (3–5). These challenges are nevertheless potentially dangerous (6, 7) uncomfortable, time-consuming and expensive as they must be performed under close medical supervision. They should therefore be undertaken only when preliminary tests tend to predict a negative challenge outcome. There are nonetheless no clear and consensual clinical or biological guidelines to determine when an oral challenge can be risked. In particular, there are no consensual specific IgE (sIgE) values in predicting clinical reactivity (3, 4, 8–15). Besides, it has been pointed out that the development of clinical tolerance is not always correlated with a reduction in IgE levels (12, 16, 17). Other laboratory tests are therefore

necessary to help physicians determine with greater accuracy when oral challenges should be performed.

Basophils represent a significant effector population in allergic pathogenesis (18). Several groups developed flow cytometric approaches for assessing the activation status of these cells (19–22). Recently, the basophil activation test (BAT) was evaluated for the diagnosis of egg and peanut allergy in children (23) and for the distinction between allergic and tolerant children to heat-denatured milk (22). Basophil activation by allergen extracts can be measured *in-vitro* by the increase in the CD63 marker at the cell surface, which is concomitant with the fusion of the basophil granules to the plasma membrane (24).

The aim of this study was to examine the benefit of the BAT in predicting clinical reactivity during an oral challenge with milk in children with established CMA who were challenged to test the persistence or resolution of their allergy.

Methods

Study population

This work is a prospective study, including 112 consecutive children with a diagnosis of IgE-mediated CMA, admitted for a food challenge with cow's milk to assess the persistence or resolution of their allergy. Initial diagnosis of IgE-dependent allergy was made on the basis of a clear-cut case history of immediate IgE-mediated anaphylactic reaction (at least one episode of urticaria or of angio-oedema, occurring immediately after the first or second exposure to CMP) along with the detection of specific IgE and positive skin prick tests (SPT). Isolated atopic dermatitis was not taken into account. To assess the eventual development of tolerance, all of the patients were offered to undergo a milk challenge after evaluation by the same allergist. Approval by the Ethical Review Board of the University Hospital of Nice for carrying out this study was previously obtained.

There were 54 girls and 58 boys included, with a mean age of 3.8 (median 2.8 years; range 1.2–11.7 years). Detailed individual patients' information regarding gender, age and concomitant allergies, oral challenge outcome, BAT, sIgE and SPT results are listed in the online repository Table S1.

Oral challenge tests

Open oral challenge tests were undertaken after at least 6 months of CMP exclusion, and after 1 year of age in every case. Informed consent was previously obtained from the parents. After a preliminary labial test, increasing doses of native semi-skimmed cow's milk were administered every 30 min. The challenge was considered positive if there were skin (urticaria or angioedema), digestive (vomiting or diarrhoea), respiratory manifestations (rhino-conjunctivitis, cough or bronchospasm) and/or anaphylactic shock in the 2 h after the milk intake. Reactions were classified as mild (cutaneous or upper respiratory symptoms), moderate (angioedema or digestive) and severe (cardiovascular or lower airway symp-

toms accompanied reactions). The physician who supervised and interpreted the oral challenge was blinded to the sIgE and BAT results.

Laboratory studies

Basophil activation test

Basophil activation was estimated by flow cytometry by measuring the increase in the CD63 marker at the cell surface after an *in-vitro* incubation with controls or with milk proteins. Basophil activation test kits and cow's milk proteins were purchased from Böhmann Laboratories AG, Schönenbuch, Switzerland. The Böhmann BAG-F2 allergen consists in dried skimmed cow's milk, which was extemporaneously diluted in the stimulation buffer at the final whole protein concentrations of 100, 10 and 1 ng/ml for each test. The negative control was obtained by incubating cells with the stimulation buffer only, thereby evaluating basal CD63 expression on resting basophils. The positive control was obtained by stimulating basophils with an anti-FcεR1 IgE receptor. The technique was performed following the manufacturer's instructions and was fully described elsewhere (21). A BAT was considered positive when an increase in at least 6% of CD63-positive basophils was measured for at least one dilution of CMP, by comparison with the basal activation level measured in the negative control. This threshold of 6% was established by setting up receiver operating characteristic (ROC) curves and corresponded to the point with the highest combination of sensitivity + specificity (Fig. 1). Flow cytometric analysis of basophil activation was performed on a four-colour FACS-scan flow cytometer (FACSCalibur®, Becton Dickinson, Madrid, Spain). All of the BAT were interpreted by the same biologist, who was blinded to the oral challenge outcome and sIgE results.

A cohort of 15 matched control individuals devoid of CMA was included (mean age: 3.6; SD: 4.9). Basophil activation test results were negative for all of these children (mean:

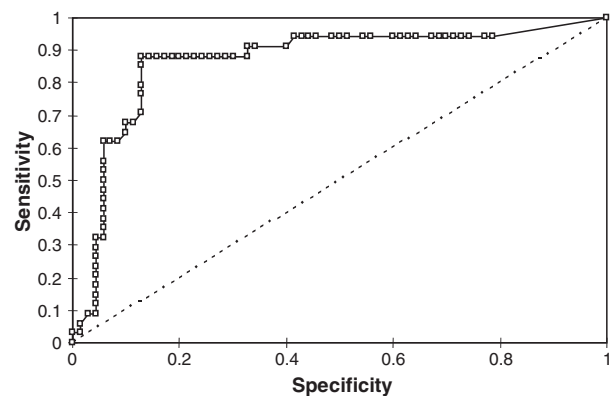


Figure 1 Receiver operating characteristic (ROC) curve for the basophil activation test (BAT) with cow's milk. Receiver operating characteristic curve for the percentage of basophils activated by cow's milk proteins (CMP). The area under ROC curve (AUC) = 0.866.

2.2; SD: 2.8) as well as sIgE levels (mean: 0.4 kUA/l; SD: 0.3). Information regarding age, gender, complete BAT results and sIgE results of this control cohort is listed in the online repository Table S2.

Specific IgE quantification

Serum samples from all patients were analysed for sIgE antibodies to cow's milk by using the ImmunoCAP technology (PHADIA AB, Uppsala, Sweden) as detailed by the manufacturer. The allergen source for the ImmunoCAP f2 used for specific IgE determinations consists in skimmed cow's milk. The detection threshold is 0.1 kUA/l.

Skin prick tests

Skin prick tests were performed in all patients but fifteen, with fresh native semi-skimmed cow's milk. Saline solution and histamine dihydrochloride (10 mg/ml; Laboratoire Stallergènes, Paris, France) were applied by means of the prick technique as negative and positive controls. Skin prick tests were read after 15 min. The mean diameters of wheals were calculated from the sum of the largest measurement across the wheal and the largest wheal measurement perpendicular to this divided by two. The SPT result was defined as positive if the mean wheal diameter was 3 mm or larger (25).

Statistical analysis

Data are expressed as means and standard deviations (SD). Receiver operating characteristics (ROC) curves were plotted with XLSTAT[®] software (Addisonsoft, Paris, France). The area under the curve (AUC) was calculated to quantify the accuracy of the BAT and to compare it with that of sIgE and SPT. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) and test efficiency (fraction of tested individuals correctly classified by the test) were calculated for the BAT, sIgE and SPT. Qualitative data (positive/negative test results) were compared using chi-squared (χ^2) and Fischer's exact tests. The correlation factor ϕ between the BAT, sIgE or SPT result and the oral challenge outcome was calculated as follows: $\phi = \sqrt{(\chi^2/n)}$. To compare the percentage of activated basophils between reactive and tolerant children, the Mann-Whitney *U*-test for nonpaired groups was used. For four independent categories (absent, mild, moderate or severe clinical reaction), the Kruskal-Wallis test was performed. All statistical analyses were performed using STATVIEW[®] software 5.0 for Windows (SAS Institute, Cary, NC, USA). A significance level of *P*-values below 0.05 was established.

Results

Among the 112 children included in the study, 36 (32%) were still allergic and had a positive oral challenge with milk. The clinical reaction involved the skin (urticaria, facial oedema) for 25 patients, the respiratory tract (cough, bronchospasm) for 13 and the gastrointestinal tract (abdominal pain, vomiting) for 11 children. Two patients had an ana-

phylactic shock. The challenge outcome was not significantly influenced by the patients' gender or mono- or multi-allergic status. A BAT was carried out for every patient, and an interpretable result was obtained for 105 (94%). Dose-response curves for each patient with the 3 CMP concentrations are displayed in Supporting Information, Fig. S1. Seven patients did not have an interpretable BAT result because of inadequate reactions with the positive control (anti-high affinity IgE receptor FcεRI) and were considered as nonresponders. This subgroup did not differ from the rest of the patient population regarding age and sex ratio. The rate of activated basophils in the BAT was not influenced by the patients' gender, age or mono- or multi-allergic status. The percentage of basophils activated by CMP in children with a positive oral challenge (mean: 20.9; SD: 18.8) was significantly higher than that of patients with a negative challenge (mean: 3.9; SD: 9.8) (Fig. 2A, B; *P* < 0.0001). Among positive challenges, a significant correlation was found between the percentage of activated basophils and the eliciting dose of milk (Fig. 2C; *P* = 0.006). The AUC for the BAT with milk was 0.866 (Fig. 1).

We speculated that the significance of a certain level of basophil activation after incubation with CMP might not be the same whether a strong or mild activation of basophils was obtained after in-vitro cross-linking of FcεRI receptors by a specific antibody in the positive control, defining high and low responders, respectively. Calculating the ratio between the specific activation obtained in response to CMP and the nonspecific activation of basophils mediated by FcεRI crosslinking allows to take into account the low or high responder status of the patient. This ratio was significantly higher in persistently reactive patients (mean: 0.58; SD: 0.39) than in tolerant patients (mean: 0.15; SD: 0.17; *P* < 0.0001). Furthermore, this ratio was extremely well correlated with the severity of the clinical reaction (Fig. 2D; *P* < 0.0001) and the eliciting dose of milk in positive oral challenges (Fig. 2E; *P* < 0.0001).

The optimal positivity threshold for cow's milk in our paediatric population was a rate of activated basophils of 6% after incubation with at least one of the three CMP concentrations. With this cut-point, the BAT was positive for 37 children and negative for 67. The BAT efficiency was 90%, the sensitivity 91%, the specificity 90%, the PPV 81% and the NPV 96% (Table 1). The concordance between the BAT and the oral challenge was excellent (*P* < 0.0001), with a correlation factor ϕ of 0.767. It can be seen in Table 1 that the other thresholds evaluated, favouring either specificity or sensitivity, were not as well correlated with the outcome of the oral challenge.

The benefit of the BAT in predicting the response to the oral challenge was then compared to that of sIgE quantification and to the SPT results. As there is no clear consensus with regard to the sIgE threshold predicting clinical reactivity, the results were presented according to the most commonly used sIgE cut-points (Table 2). The mean sIgE value was 10.6 kUA/l (SD 17.5) for persistently allergic children and 2.4 kUA/l (SD 3.5) for those who passed the challenge. Specific IgE values were highly correlated with the oral

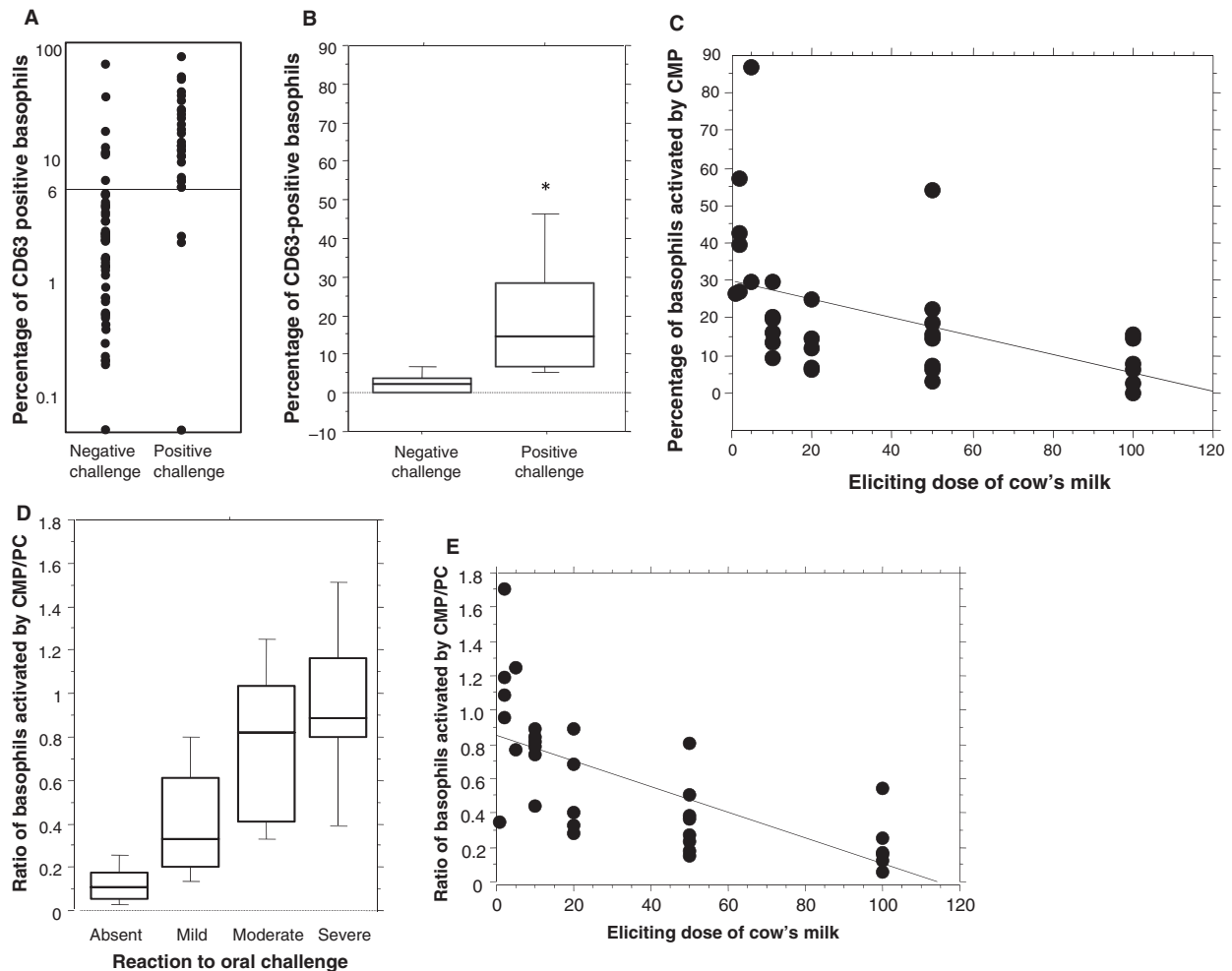


Figure 2 Distribution of basophil activation test (BAT) results for positive and negative oral challenges. (A) Individual dot plots outlining the distribution of BAT results according to oral challenge results. (B) The percentage of basophils activated by cow's milk proteins (CMP) in children with a positive oral challenge (mean: 20.9; SD: 18.8) was significantly higher than that of patients with a negative challenge (mean: 3.9; SD: 9.8) ($P < 0.0001$). (C) Correlation between the eliciting dose of milk in positive challenges and the percentage of activated basophils in the BAT with cow's milk ($P = 0.006$). (D) Correlation between the severity of clinical reactions and the intensity of basophil activation in terms of individual cellular reactivity. The severity of the clinical reaction, opposing mild (cutaneous or upper respira-

tory symptoms), moderate (angioedema or digestive) and severe reactions (cardiovascular or lower airway symptoms accompanied) was extremely well correlated with the intensity of basophil activation. Intensity of basophil activation refers to the ratio of the percentage of CD63-positive basophils after incubation with cow's milk proteins divided by the percentage of CD63-positive basophils after incubation with the positive control. Absent vs mild: $P < 0.0001$, absent vs moderate: $P < 0.0001$, absent vs severe: $P < 0.0001$, mild vs moderate: $P = 0.017$, mild vs severe: $P = 0.0006$, moderate vs severe: NS. (E) Correlation between the eliciting dose of milk in positive challenges and the intensity of basophil activation in terms of individual cellular reactivity ($P < 0.0001$). * $P < 0.05$.

challenge outcome ($P < 0.0001$). The AUC for milk sIgE was 0.758. The ROC curve for sIgE is displayed in Fig. 3A.

Skin prick tests measurements were available for 95 patients. Among persistently allergic patients, the mean wheal diameter was 10.0 mm (SD 4.0). Among those who had achieved tolerance, the mean wheal diameter was 5.8 mm (SD 3.2) ($P < 0.0001$). The AUC for SPT with milk was 0.809. The ROC curve is displayed in Fig. 3B. With a cut-point of 3 mm, the SPT were positive for 83 patients and negative for

14 patients. The correlation with the oral challenge was poor ($\phi = 0.292$, $P = 0.045$). In our cohort, the SPT had excellent sensitivity (100%) and NPV (100%) in predicting the persistence of allergy. However, the specificity was low (22%), as well as the PPV (40%) and the overall efficiency (48%).

To estimate the optimal benefit that could be obtained from the BAT in this context, different combinations of BAT, sIgE and SPT results were analysed. The sensitivity, specificity, positive predictive values (PPV), negative predic-

Table 1 Sensitivity, specificity, positive and negative predictive values (PPV and NPV), efficiency, and correlation factor ϕ with oral challenge across a spectrum of BAT cut-points (P was calculated with the Fischer's exact test)

BAT cut-off: percentage of basophils activated by CMP	Se (%)	Spe (%)	PPV (%)	NPV (%)	Efficiency (%)	Correlation factor ϕ with oral challenge	$P (\chi^2)$
2	97	51	48	97	65	0.463	<0.0001
4	91	75	63	95	80	0.612	<0.0001
5	91	82	70	95	85	0.686	<0.0001
6	91	90	81	96	90	0.752	<0.0001
8	70	89	74	86	83	0.595	<0.0001
12	64	92	78	84	83	0.586	<0.0001
15	45	94	79	79	79	0.480	<0.0001

BAT, basophil activation test; CMP, cow's milk proteins; Se, sensitivity; Spe, specificity.

Table 2 Sensitivity, specificity, positive and negative predictive values (PPV and NPV), efficiency and correlation factor ϕ with oral challenge for the most commonly used specific IgE (sIgE) cut-points and for different combinations of BAT, sIgE and skin prick tests (SPT) results (P was calculated with the Fischer's exact test)

Positivity cut-off	Se (%)	Spe (%)	PPV (%)	NPV (%)	Efficiency (%)	Correlation factor ϕ with oral challenge	$P (\chi^2)$
sIgE: 0.35 kUA/l	94	21	37	89	45	0.176	0.115
sIgE: 2 kUA/l	67	67	50	81	67	0.354	0.0005
sIgE: 5 kUA/l	44	82	57	73	68	0.339	0.0009
sIgE: 15 kUA/l	19	97	78	72	72	0.348	0.0011
sIgE with the 2 kU/l cut-off + SPT	52	68	44	74	62	0.187	0.113
BAT + sIgE with the 2 kUA/l cut-off	61	96	87	84	84	0.630	<0.0001
BAT + sIgE with the 5 kUA/l cut-off	43	96	83	77	78	0.491	<0.0001
At least 2 positive results among: BAT + SPT + sIgE with the 2 kUA/l cut-off	97	82	72	98	87	0.743	<0.0001

BAT, basophil activation test; Se, sensitivity; Spe, specificity.

tive values (NPV) and efficiency values of these combinations are exposed in Table 2.

Discussion

Predicting when a child becomes tolerant to CMP and when to offer a food challenge remains one of the most important and difficult issues of CMA management. This work aimed to evaluate the benefit of a functional cellular test, the BAT, regarding this issue.

Our results first show that the BAT is feasible even for very young children, as the test was interpretable in every case but seven. As recommended by Ebo et al. (19) and Boumiza et al. (26), we determined the optimal cut-point for BAT with CMP by ROC curve analysis. The optimal 6% cut-point calculated for our young paediatric population is very close to the ones established by Ocmant et al. (23) for egg (5%) and peanut (9.1%) in children and by Erdmann et al. (27) for celery (6.3%), hazelnut (6.7%) and carrot (8.9%) in an adult population. However, this threshold might vary across populations, as is the case for sIgE cut-points.

With an efficiency of 90% and an AUC of 0.866, this study highlights the high performance of the BAT in helping to determine the appropriate moment to carry out an oral challenge with cow's milk. In particular, the good NPV

(96%) implies that it can be relied upon in deciding to undertake a challenge with a minimum of danger. Likewise, we believe that the good PPV (81%) indicates that oral challenges should be performed with great precautions or altogether postponed when the BAT is positive, particularly when associated with high sIgE levels.

The good correlations between BAT and clinical reactions stem from the fact that the principle of BAT closely reflects the physiopathology of IgE-mediated allergy. CD63 expression is indeed intimately related to the phenomenon of basophil degranulation. In resting basophils, CD63 is anchored to the intracellular granules and only barely expressed on the surface membrane, both in healthy subjects and in allergic patients. Upon challenge with specific allergens that cross-link Fc ϵ RI and induce granules exocytosis, CD63 becomes expressed at the cell surface with a high density on the activated basophils. CD63 detection by flow cytometry is therefore highly specific of IgE-mediated basophil activation.

As pointed out by Kleine-Tebbe et al. (28) and De Weck et al. (29), individual basophil outcome depends on the cellular reactivity defined as the maximal cellular response after optimal stimulation. To take this factor into account, we calculated for each patient, the ratio between the percentage of CD63-positive basophils after incubation with CMP and that obtained with the positive control (anti-Fc ϵ RI), considering

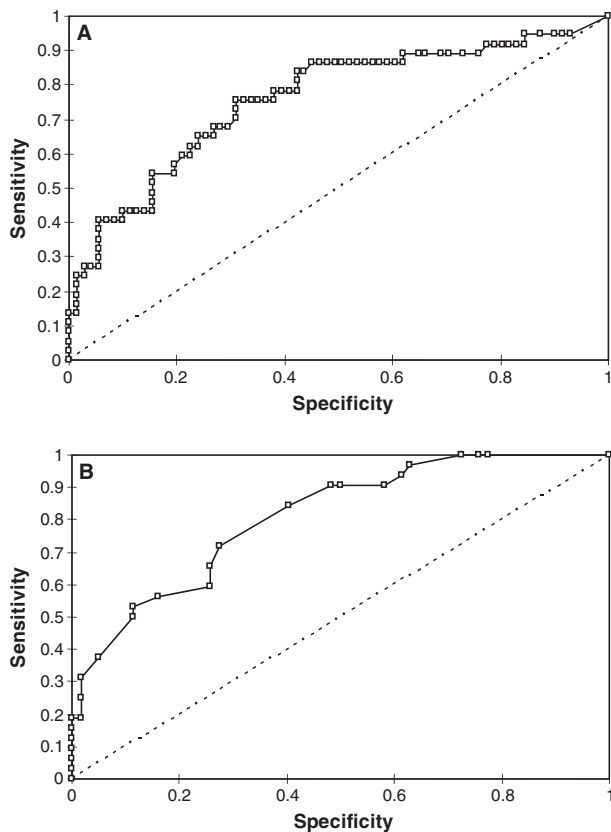


Figure 3 Receiver operating characteristic (ROC) curves for specific IgE (sIgE) and skin prick tests (SPT) with cow's milk. (A) ROC curve for cow's milk sIgE. The area under ROC curve (AUC) = 0.758. (B) ROC curve for SPT with cow's milk. AUC = 0.809.

that this was the optimal available stimulation (29). Interestingly, we noted a statistical correlation between this ratio and the oral challenge outcome, in terms of absence/presence of clinical reaction (AUC of 0.857) but also in terms of reaction severity (Fig. 2D) and of the eliciting dose of milk in positive challenges (Fig. 2E).

To date, the biological test on which practitioners commonly rely to determine the appropriate moment to undertake a challenge is the sIgE quantification. In our study population, sIgE levels were well correlated with the challenge outcomes ($P < 0.0001$). The 50% PPV of sIgE > 2 kUA/l established by Perry et al. (13) was also verified in our cohort. We then compared the BAT and sIgE efficiencies. We showed that the BAT was better correlated with the oral challenge outcome than were the sIgE levels. Moreover, this remained true independently of the elicited cut-point for either BAT or sIgE, as attested by the ROC curves and AUC. This superiority could be explained by the fact that the measurement of CD63-expression on basophils during a functional assay more closely reflects the physiopathology of IgE-mediated allergy than serum sIgE levels, which are an indirect reflection of sensitization. Likewise, in our cohort SPT were not very useful for predicting the loss

of symptomatic allergy to cow's milk. This is in accordance with what was previously published (30, 31).

All of this suggests that adding the BAT to the allergic work-up significantly improves the estimation of the patients' allergic status. For instance, relying on an algorithm combining clinical data, SPT results, sIgE levels and BAT results (Fig. 4), we were able to correctly identify 94% of the newly tolerant children as well as 94% of the persistently allergic patients.

In our study, seven children (6%) had noninterpretable BAT results, being nonresponders. This is a well-known limitation of the BAT assay (24, 29, 32). These patients were not retested as we did not wish to subject them to a new blood sampling for research purposes. As it was impossible to give these noninterpretable tests either a positive or negative value, they were excluded from the main statistical analyses, as is permitted in such cases (33) and was performed elsewhere (22, 23).

We obtained ten discordant results between clinical outcome and BAT. Seven were positive BAT results without any clinical reaction during the challenge. Cow's milk proteins were reintroduced in the diet, and none of these children experienced any allergic reaction in the following months. These seven patients were the same age (3.9 years, DS 3.4) and sex ratio boy/girl (0.75) than the tolerant children who had a negative BAT [3.3 years, standard deviation (SD) 2.4; sex ratio of 0.80]. However, they were higher BAT responders as the average percentage of CD63-positive basophils in the positive control of these seven patients was 66.8% (SD 16.1) vs 45.2% (SD 25.8). Moreover, they had higher sIgE levels: 5.4 kUA/l (SD 6.0) vs 2.2 kUA/l (SD 3.0) and SPT results: 7.4 mm (SD 1.7) vs 4.3 mm (SD 2.5) than the tolerant patients who had a negative BAT result. This discrepancy between positive BAT result and oral tolerance probably stems from the fact that the BAT is performed *in-vitro* on isolated basophils exclusively and does not take into account all of the regulatory mechanisms involved in the induction of clinical tolerance *in-vivo*, on a gastrointestinal or on a systemic level. For instance, the study conducted by Wanich et al. (22) indeed demonstrates that basophil responsiveness can be inhibited by an autologous factor present in serum, which they hypothesized to be allergen-specific IgG. Recent studies have also supported the hypothesized role for allergen-specific regulatory T-cells in the spontaneous resolution of milk allergy (20, 34).

Lastly, three patients had negative BAT results despite an indisputable IgE-mediated reaction upon challenging. Among these three patients, two had mild cutaneous or nose-throat clinical reactions but the third had a sudden and severe reaction with bronchospasm and anaphylactic shock after having tolerated 37 ml of milk. These three patients were younger than the persistently allergic children who had a positive BAT, with an average age of 2.2 years (SD 0.2) vs 4.6 years (SD 2). Furthermore, they were not as good BAT responders as allergic children with a positive BAT: the average percentage of CD63-positive basophils in the positive control was 35% (SD 12.8) vs 57.7% (SD 22.8). Moreover, they had lower sIgE levels: 2.9 kUA/l (SD 2.2) vs 11.3 kUA/l (SD 18.9). The SPT had remained very large in

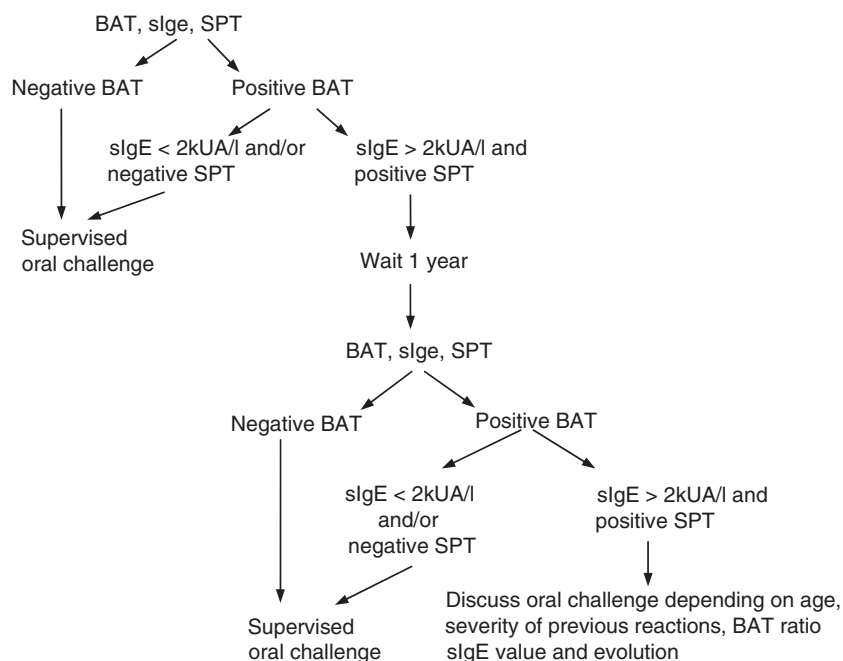


Figure 4 Decisional algorithm taking into account basophil activation test (BAT), specific IgE and skin prick tests (SPT) results and clinical elements.

both groups of patients: 9.3 mm (SD 2.6) and 10 mm (SD 4.2) respectively. We do not currently have an explanation for these false-negative BAT results. We can hypothesize that an alternative mechanism of anaphylaxis could be involved, with mast cells being the predominant cell type for these allergies. Although basophils and tissue mastocytes both mediate IgE-driven functions in a similar way, there is increasing evidence that these two cell types are quite different and that basophils are not 'circulating mastocytes'. Recent studies have demonstrated the role of basophils in the regulation of adaptative responses and in particular in the initiation of Th2 response (35–38) and highlighted the diversity of mast cell function as well as the importance of the mucosal microenvironment and innate immunity in the development of food allergy or tolerance (39–42). Unfortunately, postreaction sera were not available and tryptase could not be measured in these patients. Because of these false-negative results, and in particular because of the patient who reacted severely, we believe that the BAT should be considered as a new tool to help dictate whom to challenge, but could not in itself lead to a radical change in challenging methods (such as home challenging). Indeed, even if occurring with a low incidence (1/112 patient, 0.89%), it appears that potentially severe reactions cannot be totally excluded despite a negative BAT result, and medical supervision is still mandatory. However, it is noteworthy to mention that new protocols are being developed, with techniques requiring very small blood samples and with higher sensitivity (J. Sainte-Laudy, personal communication). The possibility of starting challenges at a higher dose in children with a negative BAT as in children with a positive BAT but with low rates of activated basophils and low BAT ratio could be considered, as an excellent correlation was found between these BAT results and the eliciting dose of

positive challenges ($P = 0.006$). This possibility must however be validated in other studies.

Conclusion

Altogether, this study indicates that the BAT is a valuable tool in helping to decide when an oral challenge can safely be undertaken in the follow-up of IgE-mediated CMA. It could systematically be performed along with the determination of sIgE and SPT. A negative BAT would incite the clinician to plan a challenge, whereas a positive BAT should lead to greater caution, particularly when associated with high sIgE values. We therefore believe that the amount of unnecessary, costly and dangerous positive challenges that could be avoided justifies the extra expense of this functional test.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Dose-response curves for each patient with the 3 CMP concentrations. (a) False negative BAT results, (b) true negative BAT results, (c) false positive BAT results, (d) true positive BAT results.

Table S1. Patients admitted for an oral challenge with cow's milk: gender, age, concomitant allergies, oral challenge outcome, BAT, specific IgE and skin prick tests results.

Table S2. Gender, age, concomitant allergies, BAT, and specific IgE to cow's milk in 15 control patients.

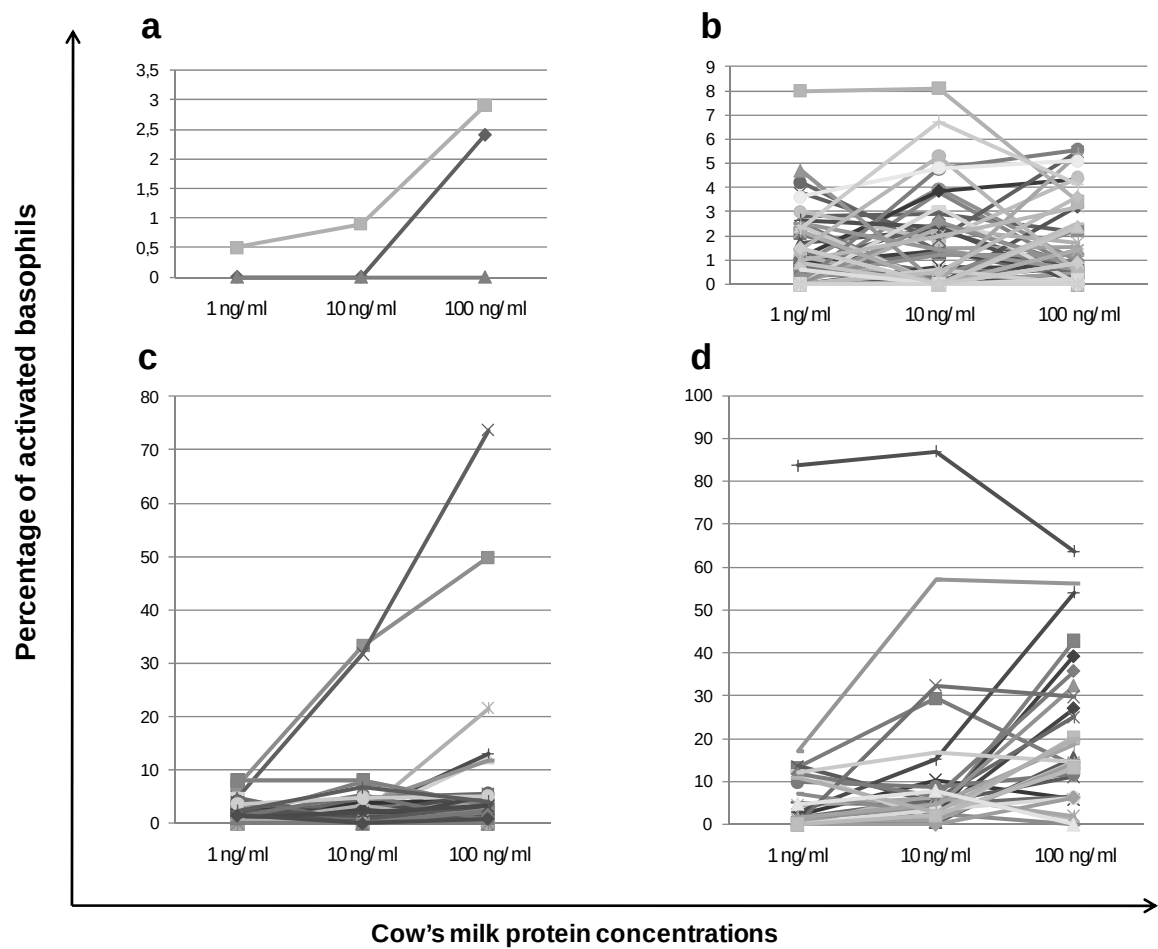
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ONLINE REPOSITORY TABLE 1

BENEFIT OF THE BASOPHIL ACTIVATION TEST IN DECIDING WHEN TO REINTRODUCE COW'S MILK IN ALLERGIC CHILDREN

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Online repository table 1: Patients admitted for an oral challenge with cow's milk: gender, age, concomitant allergies, oral challenge outcome, BAT, sIgE and skin prick tests results

	Sex	Age	BAT							Oral challenge	sIgE (kUA/L)	Skin prick test (mm)	Other IgE-mediated food allergies and aeroallergen sensitizations
			Result	NC	PC	CMP 100 ng/l	CMP 10 ng/l	CMP 1 ng/l	CD63/PC ratio				
1	F	1.7	Positive	5.0	41.1	54.8	38.3	12.11	1.33	Negative	5.53	NA	
2	M	4.3	Non responder	1.2	0.7	0.7	0.48	1.43		Positive: urticaria (at 50 ml)	4.53	NA	
3	F	3.2	Non responder	0.3	2.6	2.5	0.73	0.96		Negative	4.12	NA	
4	F	4.2	Positive	8.1	20.5	35.2	10.57	4	1.71	Positive: bronchospasm, angio-oedema (at 2 ml)	14.4	7	
5	M	2.3	Negative	4.1	65.5	2.4	3.2	1.2	0.05	Negative	4.13	7	Egg, soy, kiwi
6	F	1.4	Negative	2.5	20.8	2.5	1.58	1.74	0.23	Negative	7.62	6	
7	F	2.1	Negative	1.9	33.3	2.4	2	4	0.07	Negative	1.18	NA	
8	M	1.4	Negative	0.0	9.8	0.5	2.23	1.71	0.05	Negative	0.85	0	Egg
9	F	1.4	Negative	0.7	14.1	0.2	0.47	0.47	0.20	Negative	0	3	
10	F	3.5	Positive	37.8	67.8	80.6	39.54	22.7	1.19	Positive: bronchospasm (at 2 ml)	8.4	5.5	Egg, peanut, lupin, soy Cat, dog
11	F	1.5	Non responder	0.6	3.7	2.2	2.75	1.08		Negative	0.38	0	
12	F	1.4	Positive	1.0	77.6	13.3	2.05	2.6	0.17	Negative	2.72	6.25	
13	M	1.6	Negative	2.7	21.0	4.8	5.57	5.44	0.23	Negative	< 0.1	5.5	Egg
14	F	3.4	Negative	0.9	25.4	1.8	4.85	1.75	0.07	Negative	0.62	0	Egg, mustard, hazelnut
15	F	1.8	Negative	10.1	35.7	8.2	5.63	8.02	0.23	Negative	1.22	4.5	
16	F	2.8	Negative	7.1	22.8	7.7	10.82	4.57	0.34	Negative	0.79	8.5	
17	M	6.8	Positive	1.9	46.3	17.5	2.33	2.94	0.38	Positive: urticaria (at 50 ml)	14.6	8.5	Egg, mustard, kiwi Acari, cat, dog
18	M	2.8	Negative	1.6	87.2	2.3	4.09	2.3	0.03	Negative	3.5	6	Egg
19	F	3.0	Negative	1.0	36.7	5.4	4.86	2.1	0.15	Negative	< 0.1	7	
20	M	4.8	Positive	0.2	12.6	6.0	10.58	2.33	0.84	Positive: abdominal pain, vomiting and urticaria (at 10 ml)	8.66	NA	
21	M	4.9	Positive	0.6	77.8	25.7	6.45	2.25	0.33	Positive: urticaria (at 20 ml)	16.1	18	Egg, mustard Birch pollen
22	F	2.0	Negative	0.8	25.6	1.3	2.13	1.61	0.05	Negative	1.71	6	
23	M	5.8	Negative	0.5	44.2	2.0	1.98	0.64	0.05	Negative	10.1	NA	

24	M	1.8	Negative	2.7	34.7	1.9	2.47	1.9	0.06	Negative	0.82	6.5	
25	F	1.5	Negative	1.5	23.1	0.7	0.51	1.49	0.03	Negative	0.58	0	
26	F	3.4	Positive	6.2	21.8	19.3	8.31	5.87	0.89	Positive: bronchospasm, urticaria, abdominal pain, sneezing (at 10 ml)	32.9	9.25	Egg
27	M	5.8	Negative	5.4	86.9	5.6	6.75	9.57	0.06	Negative	3.19	8.5	Egg, peanut, mustard, pistachio, nut Cat
28	M	2.6	Negative	1.0	10.3	1.5	2.34	1.86	0.14	Negative	4.04	6	Acari, cat
29	F	1.4	Negative	0.7	87.8	1.6	1.97	1.14	0.02	Negative	< 0.1	0	
30	M	2.7	Negative	4.6	62.9	3.1	2.65	5.7	0.05	Negative	0.53	0	Peanut Birch pollen
31	F	1.8	Negative	1.5	19.7	4.7	1.52	0.41	0.24	Negative	2.5	0	Egg
32	F	2.6	Negative	2.5	20.8	2.9	1.58	1.74	0.27	Negative	0.34	5.5	Pine tree pollen
33	M	6.3	Positive	8.5	76.3	72.3	95.47	92.17	1.25	Positive: bronchospasm, angio- oedema (at 5 ml)	8.97	NA	Acari
34	F	7.9	Negative	2.8	31.3	3.4	3.3	4.1	0.11	Negative	0.43	7	Egg, peanut, fish, hazelnut
35	M	8.9	Negative	1.0	29.8	1.7	1.73	0.55	0.06	Negative	0.9	NA	Acari
36	M	4.8	Negative	2.5	56.3	2.3	1.96	0.32	0.04	Negative	1.36	9	Egg, fish, nut, hazelnuts, lens
37	M	4.1	Positive	25.0	85.0	81.0	82	42	0.96	Positive: urticaria + angio- oedema (at 2 ml)	100	7	Cockroach
38	M	5.9	Positive	1.1	9.2	7.5	5.9	6.2	0.82	Positive: urticaria + angio- oedema (at 10 ml)	13.4	10.5	Egg, peanut, celery
39	M	7.1	Positive	2.9	38.8	42.2	4.8	1.3	1.09	Positive: bronchospasm and anaphylactic shock (at 2 ml)	4.52	14.5	Egg Cat, dog
40	M	3.7	Negative	2.4	57.1	6.8	4.2	5.4	0.12	Negative	0.57	6	Egg
41	F	2.0	Negative	3.2	33.9	2.2	0.61	2.34	0.06	Negative	7.21	10.5	Egg
42	M	2.4	Positive	42.0	92.3	55.8	71.4	55.2	0.77	Positive: bronchospasm, urticaria, rhinoconjunctivitis (at 2 ml)	16	18	
43	M	2.4	Negative	3.2	25.3	2.1	2.6	3.2	0.14	Negative	0.28	4.5	
44	F	6.2	Negative	7.1	26.7	3.7	6.1	9.6	0.14	Negative	13.5	0	Egg
45	M	3.7	Positive	7.8	66.2	9.1	14.93	2.33	0.23	Positive : urticaria (at 50 ml)	1.65	8	Egg, peanut, whey, corn, soy
46	F	1.5	Negative	4.7	95.3	2.7	4.9	4.7	0.03	Negative	0.16	0	

47	M	5.9	Positive	10.5	29.2	21.7	15.37	24.04	0.74	Positive: bronchospasm, abdominal pain (at 5 ml)	3	5	Acari
48	M	7.2	Positive	6.6	45.2	8.5	12.84	10.94	0.28	Positive: bronchospasm, urticaria (at 20 ml)	< 0.1	7	Egg, mustard, pistachio Acari, birch and olive tree pollen
49	M	11.7	Negative	0.3	26.4	2.5	0.63	0.64	0.15	Negative	0.97	0	Egg, fish Acari, cat, dog, olive tree pollen
50	M	3.7	Negative	7.8	40.1	6.5	7.2	10.2	0.16	Negative	1.64	NA	Egg, mustard, fish, whey, hazelnut Cat, dog
51	F	5.9	Positive	14.2	38.2	26.0	23	24	0.68	Positive: bronchospasm, abdominal pain (at 5 ml)	1.71	9.5	
52	M	2.4	Negative	5.8	64.9	11.3	7.68	9.62	0.17	Negative	0.88	0	
53	M	1.6	Positive	2.7	69.5	76.4	34.4	7.5	1.10	Negative	16.8	7	
54	M	8.4	Positive	1.7	69.1	55.8	16.83	4.55	0.81	Positive: urticaria (at 50 ml)	1.08	9.5	Egg, peanut
55	M	1.9	Positive	6.8	59.3	3.8	9.19	13.9	0.23	Positive: urticaria, diarrhea (at 50 ml)	< 0.1	NA	
56	F	4.2	Negative	3.0	66.9	4.7	5.3	1.9	0.07	Negative	1.4	6	Egg, mustard, whey Acari, cat
57	F	9.5	Negative	7.8	83.4	13.4	12.56	4.29	0.16	Negative	7.4	NA	Egg, mustard
58	M	6.6	Positive	9.5	64.8	24.0	10.44	21.86	0.37	Positive: urticaria, abdominal pain, vomiting (at 50 ml)	1.2	20.5	Egg
59	F	5.9	Negative	6.4	69.6	5.8	8.71	9	0.08	Negative	0.92	9.5	
60	M	6.6	Positive	9.1	82.7	44.8	15.59	20.92	0.54	Positive : urticaria, rhinoconjunctivitis (at 100 ml)	19.6	6.5	
61	M	1.7	Positive	1.9	76.7	28.3	3.3	2.2	0.35	Positive : urticaria (at 1 drop)	1.12	9	Egg
62	M	4.7	Negative	1.9	56.8	3.1	3.1	2.2	0.05	Negative	5.81	8.5	Egg
63	M	4.6	Negative	1.1	46.6	0.0	4.3	0	0.00	Negative	0.3	9.5	Nut, pistachio
64	M	4.6	Negative	2.8	30.4	0.7	1.6	1.4	0.02	Negative	0.17	0	
65	F	2.9	Positive	1.3	66.5	33.7	5.4	2.6	0.51	Positive: angio-oedema and cough (at 50 ml)	0.59	7.5	
66	F	1.6	Positive	4.2	77.5	25.8	6	6.4	0.33	Negative	6.14	10	
67	F	8.1	Negative	1.2	48.3	0.7	1	0.3	0.01	Negative	1.37	NA	
68	F	1.5	Negative	12.0	48.6	11.4	11.75	16.7	0.23	Negative	6.11	4	
69	M	1.9	Negative	0.2	28.5	0.9	0.4	0.9	0.03	Negative	7.85	8.5	
70	F	1.4	Negative	2.3	38.3	3.7	2.1	4.5	0.10	Negative	0.55	4.5	

71	F	2.8	Negative	2.6	21.0	0.9	2.1	2.4	0.04	Negative	4.43	8.5	Egg
72	M	8.1	Non responder	2.4	2.0	1.0	2.1	2.9		Positive: urticaria (at 50 ml)	8.09	8	
73	F	1.4	Negative	1.0	50.7	1.6	2.4	3.5	0.03	Negative	1.09	0	
74	F	2.2	Negative	3.8	56.7	2.2	2.5	1.2	0.04	Negative	0.18	7.5	Egg, peanut, mustard, whey
75	F	1.8	Negative	2.6	21.0	0.9	2.1	2.4	0.10	Negative	4.43	8.5	Egg
76	F	2.6	Positive	5.0	44.0	34.8	37.4	5.3	0.79	Positive: rhinoconjunctivitis (at 10 ml)	4.87	4.5	
77	M	2.1	Negative	0.7	86.7	1.4	3.3	0.6	0.02	Negative	0.58	9.5	
78	F	1.4	Negative	1.6	44.4	5.0	9.7	10.6	0.24	Negative	4.43	0	
79	M	1.7	Negative	5.9	39.4	6.8	4.8	7.4	0.19	Negative	0.39	9	
80	F	9.1	Non responder	2.0	3.4	1.0	2.1	2.9		Negative	0.18	9.5	Egg, peanut, fish, hazelnut
81	M	1.4	Negative	2.3	38.3	3.7	2.1	4.5	0.10	Negative	0.55	4.5	
82	F	1.4	Positive	0.8	62.0	15.2	1.6	1.8	0.25	Positive: rhinoconjunctivitis and vomiting (at 50 ml)	4.27	7	
83	F	1.5	Negative	4.4	49.6	3.6	5	4.1	0.07	Negative	< 0.1	7	
84	M	1.2	Negative	0.7	20.6	1.2	6	2.1	0.06	Negative	0.95	6.5	
85	M	1.7	Negative	1.0	69.0	6.4	1.1	0.6	0.09	Negative	10.2	7	Egg
86	M	1.8	Positive	4.0	67.8	10.2	7.4	3.6	0.15	Positive: urticaria (at 50 ml)	0.23	7.5	
87	M	2.2	Negative	21.9	98.2	24.4	13.2	19.1	0.25	Negative	0.2	10	Egg
88	F	5.9	Positive	0.5	70.3	19.0	5	2.2	0.27	Positive : sneezing, abdominal pain, urticaria, rhinitis (at 50 ml)	24.5	11.5	Egg, nut Acari
89	F	2.2	Negative	2.9	42.7	5.3	2.9	2.3	0.12	Positive : urticaria (at 50 ml)	0.38	11.5	
90	F	11.7	Positive	1.5	67.8		9.3	2.5	0.14	Negative	1.13	6.5	Egg, seafood Acari, cat, dog
91	F	2.1	Negative	4.5	34.6	3.2	2	3	0.09	Negative	0.2	7	Egg, peanut, nut
92	F	1.7	Positive	2.9	44.2	17.6	19.6	15	0.40	Positive: urticaria (at 10 ml)	1.1	9.5	Peanut, mustard, hazelnut
93	M	1.4	Negative	0.2	20.6	3.8	0.7	0	0.18	Negative	0.78	5	
94	M	5.0	Negative	0.9	21.0	1.1	0.5	0.75	0.10	Negative	NA	6.5	Acari, dog
95	F	4.4	Negative	5.8	94.8	8.2	5.4	6.7	0.09	Negative	NA	4	
96	M	2.4	Positive	0.0	85.2	12.9	2	2.1	0.15	Negative	0.24	NA	
97	F	5.4	Non responder	0.1	1.9	0.95	0	0		Positive: urticaria and angio-oedema (at 2 ml)	8.59	13.25	Egg, mustard
98	M	2.4	Negative	0.2	20.2	3.1	1.1	0.7	0.15	Positive: bronchospasm and anaphylactic shock (at 50 ml)	4.08	6.5	Egg, almond, hazelnut

99	F	5.0	Negative	6.6	82.6	9.8	8.6	7.6	0.12	Negative	2.32	4.5	Fish Pollen
100	F	5.8	Negative	8.7	51.2	5.7	6.9	4.6	0.11	Negative	NA	NA	Egg, fish, lens Acari, cypress pollen
101	M	7.1	Positive	7.5	49.1	19.3	11.1	4.8	0.39	Negative	NA	NA	Egg, mustard
102	M	6.9	Positive	1.9	50.0	21.8	4.8	12.8	0.44	Positive: urticaria, vomiting, rhinitis, conjunctivitis, cough (at 10 ml)	3.78	17	Acari, cat, dog
103	M	3.0	Negative	1.1	76.3	6.2	5.9	4.7	0.08	Negative	0.91	NA	Egg
104	M	1.7	Non responder	0.8	3.2	1.4	0.8	0.2		Negative	0.43	6.5	Egg, whey
105	F	3.2	Positive	3.2	59.0	9.5	1.6	1.9	0.16	Positive: urticaria, pruritus, abdominal pain (at 100 ml)	1.66	10	Egg
106	F	1.6	Negative	2.9	98.9	7.0	9.6	5.2	0.07	Negative	1.15	12	Egg
107	M	9.7	Negative	2.1	76.0	1.1	1	2.7	0.81	Negative	1.08	7	Egg, peanut
108	F	5.8	Positive	6.2	21.8	19.3	8.31	5.87	0.89	Positive: bronchospasm, urticaria, abdominal pain, sneezing (at 20 ml)	23.8	9.5	Egg
109	M	3.8	Positive	4.6	75.0	2.2	12.4	9.4	0.17	Positive : cough, sneezing, rhinitis (at 100 ml)	5.46	13.5	Acari
110	F	1.8	Negative	7.2	24.5	3.8	4.7	3.8	0.16	Negative	0.99	4.5	
111	M	1.7	Negative	5.9	39.4	6.8	4.8	7.4	0.19	Negative	0.39	9	
112	M	1.9	Negative	1.1	42.2	0.6	0.3	0.3	0.01	Positive: urticaria (at 10 ml)	4.13	10	

Footnote: PC = Positive control; NC = Negative control; NA = Not available

The gender of each patient, the age at the time of the oral challenge, the BAT results with the 3 CMP concentrations, the oral challenge outcome, the specific IgE value for whole cow's milk and the prick test results are recorded. The CD63/PC ratio corresponds to the ratio of the highest percentage of activated basophils obtained with CMP divided by the percentage of activated basophils obtained in the positive control.

ONLINE REPOSITORY TABLE n°2

BENEFIT OF THE BASOPHIL ACTIVATION TEST IN DECIDING WHEN TO REINTRODUCE COW'S MILK IN ALLERGIC CHILDREN

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Online repository table 2: Gender, age, concomitant allergies, BAT, and sIgE to cow's milk in 15 control patients

	Sex	Age	BAT							sIgE (kUA/L)
			Result	NC	PC	CMP 100 ng/l	CMP 10 ng/l	CMP 1 ng/l	CD63/PC ratio	
1	M	0.7	Negative	1.8	27.1	3.5	3.2	3.2	0.34	0.71
2	F	3.4	Negative	0.9	25.4	1.8	4.85	1.75	0.07	0.62
3	M	2.1	Negative	0.7	86.7	1.4	3.3	0.6	0.02	0.58
4	M	1.4	Negative	0.0	9.8	0.5	2.23	1.71	0.05	0.85
5	F	0.9	Negative	0.7	14.1	0.2	0.47	0.47	0.20	< 0.10
6	F	0.9	Negative	1.5	23.1	0.7	0.51	1.49	0.03	0.58
7	F	0.9	Negative	0.6	17.7	2.2	2.75	1.08	0.09	0.38
8	M	0.5	Negative	2.3	38.3	3.7	2.1	4.5	0.10	0.55
9	F	0.8	Negative	1.6	44.4	5.0	3.7	2.6	0.24	0.70
10	M	2.0	Negative	12.0	77.0	16.2	11.8	11.3	0.07	NA
11	M	8.1	Negative	1.1	33.4	0.8	0.8	0.6	0.00	0.26
12	M	5.3	Negative	3.7	96.8	3.0	5.4	4	0.02	< 0.10
13	F	2.2	Negative	1.8	79.8	0.8	1	0.6	0.02	< 0.10
14	F	17.8	Negative	1.5	74.5	1.2	2.6	3	0.02	0.15
15	F	7.1	Negative	13.0	65.8	9.8	4.6	7.8	0.00	0.12

Footnote: PC = Positive control; NC = Negative control; SI = skin indices; NA = Not available

The gender of each patient, the age at the time of the oral challenge, the BAT results with the 3 CMP concentrations, the oral challenge outcome, the specific IgE value for whole cow's milk and the prick test results are recorded. The CD63/PC ratio corresponds to the ratio of the highest percentage of activated basophils obtained with CMP divided by the percentage of activated basophils obtained in the positive control.