

Can a basophil activation test of cord blood predict a cow's milk allergy?

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Background: Food allergies affect 4%–6% of the pediatric population and are often present within the first 2 years of life. Cord blood cells and cytokines in high-risk infants can predict allergic problems; however, their predictive value remains unclear.

Purpose: This study aimed to determine whether a cow's milk allergy in infants can be predicted using a basophil activation test (BAT) of cord blood samples by stimulating basophils with milk protein antigens (cow's milk and casein).

Methods: We collected cord blood during the birth of 30 mother-child pairs and immediately analyzed BAT stimulated with milk protein antigens. One year later, we compared the results of those infants who developed an allergy to those who did not.

Results: We found that infants with a casein-BAT value ≥ 2.6 were 33.2 times more likely than those with a casein-BAT value < 2.6 to develop food allergy symptoms within the first year of life ($P=0.03$).

Conclusion: High casein-BAT values in cord blood may predict the development of food allergies during the first year of life. Although no association with cow's milk sensitivity has been found, casein sensitivity may indicate food allergy risk. However, further studies are required to confirm this association.

Key words: Basophil activation test, Cord blood, Food allergy, Cow's milk allergy

Meaning: Cord blood casein BAT may help identify newborns at increased risk for early-life food allergies, enabling closer monitoring and preventive strategies, although larger studies are needed for validation.

Introduction

Food allergies manifest in approximately 4%–6% of the pediatric population and frequently present symptoms within the first 2 years of life.^{1,2} The most common food antigens are cow's milk, hen's egg, wheat, peanuts, tree nuts, and seafood.^{3,4} The diagnosis of immunoglobulin E (IgE)-mediated food allergy includes detailed clinical history, skin prick test (SPT), and serum-specific IgE (sIgE) measurements; however, in suspicious cases, the diagnosis should be confirmed with oral food challenge (OFC). Nonetheless, OFC is a time- and staff-intensive procedure and carries a risk of severe reactions, which may lead to anaphylactic shock.⁵

The basophil activation test (BAT) is a flow cytometry test that measures the expression of activation markers on the surface of blood basophils. The BAT has been shown to reflect the allergic status of patients sensitized to food, inhalant, and insect venom antigens and has gained importance in eligibility for a specific therapy and monitoring of the response to therapy for an allergy.⁶ The blood sample taken is compared with the suspected antigen in a tube, which prevents patients from being exposed to the antigen under investigation. For patients and their families, it is a potentially safer and preferable test because there is no chance of an allergic reaction that could result in anaphylaxis.

Key message

Question: Can a basophil activation test (BAT) of cord blood predict a cow's milk allergy?

Finding: Infants with a high casein-BAT value were more likely to develop food allergy symptoms in the first year, whereas cow's milk BAT showed no predictive association.

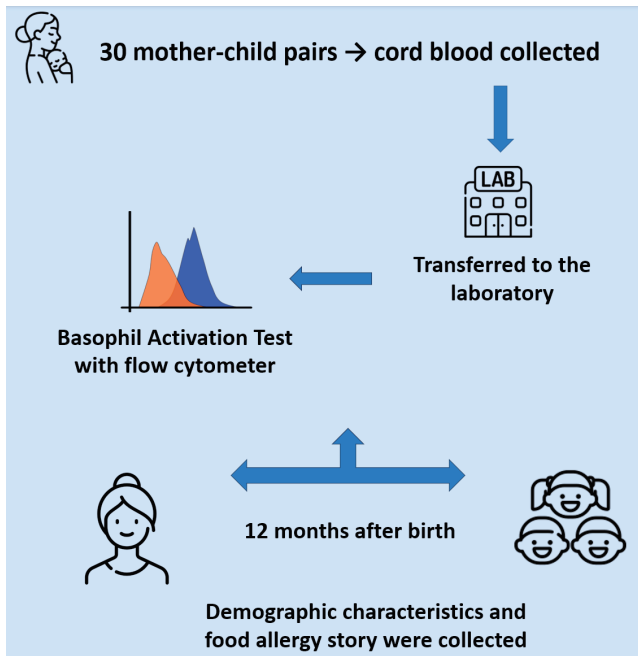
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Graphic abstract

Studies have shown that cord blood cells or cytokines in high-risk infants predict allergic diseases such as recurrent wheezing attacks later in life.⁷⁻⁹ One of the discussed cell populations in predicting allergic outcomes is basophil progenitor cells in cord blood; however, it is still unclear whether these cells have predictive value.^{8,10} It could be crucial in newborns to anticipate the food allergies that will manifest later to avoid severe reactions like anaphylaxis. The aim of this study is to determine whether cow's milk allergy (CMA) in infants can be predicted using BAT with cord blood samples by stimulating basophils with milk protein antigens (cow's milk and casein). CMA is most frequently caused by caseins, but they can also be promoted by whey proteins.¹¹ CMA is defined as a reproducible adverse reaction to one or more cow's milk component proteins.¹²

Basophils are expected to be sensitized earlier for a positive result in a BAT test and its reactivity to an unexposed food is not an expected reaction in a cord blood sample, however several studies have shown that, newborns may develop sensitivity because of environmental factors and epigenetic changes during the mother's pregnancy.⁷ As not all children born to parents with allergies will develop allergic disorders, and not all children with allergies will be born to parents with atopy, this study may allow for individualized monitoring of mother and baby carrying a high risk for food allergies.

Methods

1. Study population and sample collection

The study included 30 mother-child pairs who had applied to the Akdeniz University Faculty of Medicine Hospital between May and July 2023. Pregnant women who had immunologic diseases (other than allergic diseases) or were receiving immunosuppressive treatment were excluded from the study. Maternal demographic characteristics (gestational age, number of pregnancies, week of pregnancy, delivery method, regular medication, use of vitamins and iron during pregnancy, diet during pregnancy, history of familial atopy, number of people living in the house, family education, place of residence, smoking within the house, presence of pets) were evaluated using a comprehensive set of questionnaires.

Following the birth and clamping of the umbilical cord, the maternal side of the cord was clamped, and a total of 60 mL of cord blood was collected, which was transferred within 8 hours to the laboratory for analysis.

Families were called by phone 12 months after birth, information on children's demographic characteristics (gender, birth weight, time of birth, mode of birth, feeding method, duration of breastfeeding, whether formula was given, time of starting formula, duration of formula, additional disease, vaccination status, medications used), and allergic outcomes (whether allergy symptoms developed) were obtained from questionnaires. In the survey, food allergy was defined as experiencing any rash, swelling, itching, shortness of breath, runny nose, sneezing, increased eczema symptoms, vomiting, abdominal cramps, restlessness, or mucus or blood in the stool within a few days after consuming any food and recurrence of symptoms following a rechallenge. Patients who stated that they had developed an allergy were invited to our center to perform SPT and/or sIgE. In case of rejection of the test, a history of rechallenge was considered positive for IgE-mediated food allergy, which developed in a few hours. At the end of the study, mother and baby data and cord blood results were evaluated mutually.

2. Preparation of cord blood samples and BATs

The study utilized the BÜHLMANN Flow CAST kit (Catalog No: FK-CCR-U; BÜHLMANN Laboratories AG, Switzerland) as the primary tool for BAT. The kit was used according to the manufacturer's instructions. Antigens used for stimulation included Milk (BÜHLMANN BAG-F2 Cow's Milk, BÜHLMANN Laboratories AG) and Casein (BÜHLMANN BAG-F78 Casein, BÜHLMANN Laboratories AG). Staining reagent containing anti-CCR3-PE and anti-CD63-FITC was used.

During the antigen stimulation phase of the BAT, the

recommended doses for the antigens were reviewed to identify the optimal stimulation concentration. To determine the optimal dose for this study, a range of concentrations (10, 20, 22.5, 50, and 100 ng/mL) were tested. No significant differences in positive or negative responses were observed between the 22.5 ng/mL, 50 ng/mL, and 100 ng/mL doses. Consequently, the study proceeded with the 100 ng/mL dose, which is more commonly used in the literature.^{6,13-15}

3. Test procedure

The BAT was conducted as follows (Fig. 1):

(1) EDTA (ethylenediaminetetraacetic acid)-anticoagulated whole blood was collected and mixed thoroughly by gentle inversion.

(2) Fifty microliter of the antigen solution at the selected concentration was added to appropriately labeled test tubes.

(3) Stimulation buffer (100 μ L) and 50 μ L of whole blood were then added to the tubes.

(4) The mixture was gently vortexed and incubated for 15 minutes at 37°C.

(5) Following incubation, 20 μ L of staining reagent containing anti-CD63-FITC and anti-CCR3-PE antibodies was added.

(6) The samples were lysed using prewarmed lysing reagent and centrifuged at 500 $\times$ g for 5 minutes.

(7) The cell pellet was resuspended in wash buffer, vortexed, and analyzed by flow cytometry within the same day.

4. Positive control

The kit includes 2 positive controls: anti-Fc ϵ RI monoclonal antibody and N-formyl-methionyl-leucyl-phenylalanine (fMLP). The anti-Fc ϵ RI monoclonal antibody mimics the antigen-induced cross-linking of IgE receptors, while fMLP serves as a non-specific basophil activator. According to the manufacturer's protocol, activation of at least 10% of basophils with either control indicates the test is evaluable. Internal evaluations have shown that the anti-Fc ϵ RI control achieves robust activation rates, ensuring the reliability of the assay.

Flow cytometric analyses were performed using the BD Accuri C6 flow cytometer. Data analysis was performed using a flow cytometer equipped with a 488-nm argon laser. Basophilic cells were selected out of the lymphocyte population using anti-CCR3. CD63 expression was assessed as an indicator of basophil activation, and results were expressed as the percentage of CD63-positive basophils among the total gated basophils. The BAT score was defined as the percentage of activated basophils to total gated basophils [$100 \times (\text{CD63} + \text{basophils}) / (\text{total gated basophils})$].

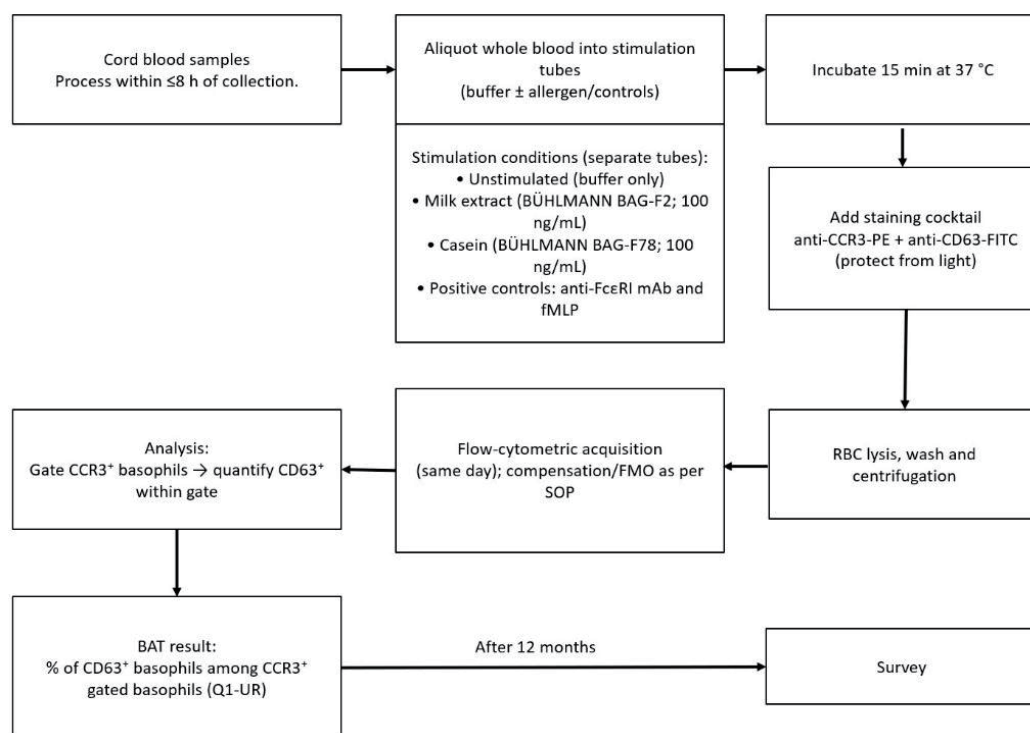


Fig. 1. Workflow of basophil activation test assay. fMLP, formyl-methionyl-leucyl-phenylalanine; FITC, fluorescein isothiocyanate; FMO, fluorescence minus one; SOP, standard operating procedure; RBC, red blood cell; BAT, basophil activation test; Q1-UR, quadrant 1-upper right; CCR3, C-C motif chemokine receptor.

5. Ethical approval

The study was approved by the ethics board (KAEK-2022/321) at the University of Akdeniz, Turkey.

6. Statistics analysis

All statistical analyses were performed using SPSS ver. 16.0 (SPSS Inc., USA). Descriptive statistics for the qualitative variables are expressed as frequencies and percentages. Univariate analyses for potential prognostic factors such as sex and birth weight were performed using the chi-square and Fisher exact test. Variables with *P* values of <0.200 were included in the multivariate analysis (binary logistic regression analysis). Associations were expressed as hazard ratios and 95% confidence intervals (CIs). Statistical significance was set at *P*<0.05.

No prior effect-size estimates were available for cord blood BAT in the literature; therefore, no a priori sample-size calculation was performed. After data collection, we computed post hoc power for the association between casein BAT (≥ 2.6 vs. <2.6) and 12-month food allergy symptoms using G*Power v3.1.9.7 (exact test for 2 independent proportions, 2-sided; $\alpha=0.05$; $n=9$ vs. 21; control proportion 0.38).

Results

A total of 30 mother-child pairs were included in the study. Demographic characteristics of the study participants are shown in Tables 1 and 2. Postnatal age at the time the survey was carried out had a mean of 13.6 ± 1.5 months. All of the cases were born with normal birth weights, and most underwent cesarean section delivery (Table 1). The majority of them were breastfed but not exclusively, mostly for at least 6 months. Formula was also used in about one-third of the cases, primarily during the first 3 months (Table 1). Allergic symptoms mostly emerged after the 6th month of birth (Table 1).

Mother, father, and sibling atopy rates were 26.7%, 13.3%, and 13.3%, respectively. Of the mothers, just 17% were taking regular medication, and 60% had been exposed to smoke during pregnancy. Only a small group of cases (17%) had pets at home (Tables 1 and 2).

It was found that 9 of the cases (30.0%) developed symptoms of food allergy, and 8 of them had CMA. Two of the cases with CMA were diagnosed with a history of CMA and SPT (Table 3). Six cases had a convincing history of allergic reactions following the ingestion of cow's milk protein; however, only 3 of them gave consent for SPT and no evidence of sensitization observed (casein was not included in any of the SPTs performed in our city in that period) (Table 3).

We compared the 2 groups, cases developing allergies

or not, with univariate analysis (Table 4). Variables with *P* values of <0.200 were cases fed with formula, atopy in the family, nutrition type during pregnancy, smoking during pregnancy, neutrophil-to-lymphocyte ratio (NLR), and

Table 1. Demographic characteristics of the studied infants (n=30)

Characteristic	Value
Sex	
Female	15 (50.0)
Male	15 (50.0)
Birth weight (g)	3,227 $\pm$ 440
Type of birth	
Vaginal delivery	8 (26.7)
Caesarean section	22 (73.3)
Age at the time of survey (mo)	13.6 $\pm$ 1.5
Nutrition	
Breastmilk	
No	2 (6.7)
Yes	28 (93.3)
Duration of breastfeeding (mo)	
≤ 6	4 (13.3)
>6	26 (86.7)
Formula	
No	20 (66.7)
Yes	10 (33.3)
When the formula was first introduced?	
≤ 12 wk	8 (80.0)
>13 wk	2 (20.0)
Duration of formula use (mo)	
≤ 6	5 (50.0)
>6	5 (50.0)
Vaccination	
Incomplete	2 (6.7)
Age-appropriate	28 (93.3)
Drug use	
Vitamin D	30 (100)
Iron	26 (86.7)
Probiotics	5 (16.7)
Other drugs	3 (10.0)
Siblings	
No	10 (33.3)
Yes	20 (66.7)
Sibling atopy	
No	16 (53.3)
Yes	4 (13.3)
Food allergy in the family	
No	19 (63.3)
Yes	11 (36.7)
Allergy symptom	
No	21 (70.0)
Yes	9 (30.0)
Age of symptom onset (mo)	
<6	3 (33.3)
≥ 6	6 (66.6)

Values are presented as number (%) or mean $\pm$ standard deviation.

casein-BAT score. As the median values of cow's milk BAT and casein-BAT were 4.1 (0.1-24.6) and 2.6 (0.1-15.0), we compared the 2 groups according to these median levels (Table 4). No difference was found for cow's milk BAT ($P=0.69$);

Table 2. Demographic characteristics of studied infants' parents (n=30)

Characteristic	Value
Maternal age (yr)	30.1 (21.0–38.0)
Maternal atopy	
No	22 (73.3)
Yes	8 (26.7)
Maternal education	
Secondary education and less	15 (50)
High school and higher	15 (50)
No. of pregnancies	
1	7 (23.3)
≥ 2	23 (76.7)
Maternal smoking during pregnancy	
No	26 (86.7)
Yes	4 (13.3)
Passive smoke exposure during pregnancy	
No	12 (40)
Yes	18 (60)
Vaccination during pregnancy	
No	6 (20)
dT	24 (80)
Nutrition during pregnancy	
Healty	25 (83.3)
With additives	5 (16.7)
Regular medication of the mother	
No	25 (83.3)
Yes	5 (16.7)
Father atopy	
No	26 (86.7)
Yes	4 (13.3)
Father education	
Secondary education and less	16 (53.4)
High school and higher	14 (46.6)
Siblings	
No	10 (33.3)
Yes	20 (66.7)
Siblings atopy	
No	16 (53.3)
Yes	4 (13.3)
Place of residence	
City center	14 (46.7)
Rural	16 (53.3)
No. of people in the house	
<4	9 (30)
≥ 4	21 (70)
Keeping of pet	
No	25 (83.3)
Yes	5 (16.7)

Values are presented as median (range) or number (%).
dT, diphtheria and tetanus toxoid

however, there was a significant statistical difference in the comparison of casein-BAT, with casein BAT ≥ 2.6 present in 8 of 9 infants (88.9%) who developed food allergy symptoms and in 8 of 21 (38.1%) of those without symptoms ($P=0.017$). With one exception, all cases that developed an allergy exhibited a casein-BAT score of 2.6 or higher. The case with a low casein-BAT score was followed for recurrent wheezing and flare-up of atopic dermatitis, and the rest of the allergic cases were followed up for allergic skin lesions (Table 3). Since the receiver operating characteristic (ROC) analysis did not identify a statistically significant cutoff value for casein-BAT (area under the curve=0.651, $P=0.197$), it was not possible to determine an optimal threshold for classification. Therefore, casein-BAT values were dichotomized based on the median and included as a categorical variable in the logistic regression analysis.

Risk factors were determined by creating a regression model using formula use, nutrition during pregnancy, family atopy, smoking during pregnancy, NLR, and casein-BAT. It has been found that cord blood casein-BAT score of 2.6 or higher increases the risk of developing food allergy symptoms in the first year of life by 33.2 times ($P=0.03$) (Table 5).

We computed a *post hoc* power for the observed association between casein BAT ≥ 2.6 (vs. <2.6) and 12-month food allergy symptoms using GPower v3.1.9.7 (exact unconditional test for 2 independent proportions, 2-sided; $\alpha=0.05$; $n=9$ vs 21; control proportion=0.38); the achieved power ($1-\beta$) was 0.93.

Discussion

Because intrauterine factors are considered to play a role in the developmental origins of many diseases, including food allergies, there has been interest in analyzing cord blood for possible biomarkers of atopic disease.^{7,8,16} Finding abnormalities in many of the cellular and genetic components of cord blood in children who are at risk of atopy is the main objective of biomarker research. Several possible cord blood biomarkers of atopic risk have been identified, such as level of total IgE, decrease in the ratio of T-helper cell type 1 (Th1) to type 2 (Th2), low interleukin (IL)-10 (a Th1-related cytokine), increased CCL22 (a Th2-related chemokine), decreased frequency and function of Tregs, and lower levels of IL-12 and IL-15, which have been associated with allergic diseases, such as atopic dermatitis, food allergy, and recurrent wheeze.¹⁶⁻²⁰ However, the findings have been inconclusive at the moment, and there is particularly a lack of evidence regarding prospective studies.

Table 3. Infants with allergies

No.	Recurrent allergic reactions	Onset age (mo)	SPT/sIgE	History of cow's milk allergya	Cow's milk BAT	Casein BAT
1	Wheezing, atopic dermatitis flare-up	10	NA	Positive	0.10	0.10
2	Atopic dermatitis flare-up	2	Negative	Positive	2.4	5.6
3	Urticaria	11	Negative	Negative (strawberry)	2.5	2.6
4	Urticaria	10	NA	Positive	4	2.6
5	Urticaria, angioedema	10	Banana, apple	Positive	4.5	4.3
6	Atopic dermatitis flare-up	7	NA	Positive	7.4	7.3
7	Urticaria	6	Cow's milk, egg	Positive	8.5	7.8
8	Urticaria	3	NA	Positive	9.3	9.1
9	Urticaria	5	Cow's milk, egg	Positive	12.1	14.2

SPT, skin prick test (not containing casein); sIgE, specific immunoglobulin E; BAT, basophil activation test; NA, not tested due to family refusal.

^aAll cases demonstrated erythema postchallenge.

Allergic reactions and anaphylaxis are the result of mast cell and basophil activation and degranulation. Results from both humans and mice have shown that basophils are present in circulation from birth; however, mast cells emerge only in the skin during embryonic stages, which appear to be immature and unable to respond to IgE until after birth.^{21,22} In neonates, basophils are more readily available cells in the peripheral blood than mast cells and are, therefore, more accessible for testing even in the cord blood.^{23,24} This advantage of basophils in neonates and the relationship of BAT with various allergic diseases provided the rationale in our research for studying BAT in cord blood.^{5,8} While neonatal basophils theoretically need to be sensitized before they can respond to casein, further studies are needed to determine how basophils of some of our cases had a response to casein. Even though it is unknown if basophils are sensitized with sIgE before birth, studies have shown that basophils are highly sensitive to some antigens and have a high level of FcεRI expression *in utero*.^{25,26} Understanding how BAT functions in this context could yield valuable insights into the development of allergic conditions later in life.

BAT evaluates blood basophils following their exposure to specific antigens or controls.^{6,27} When activated, basophils increase the expression of various activation markers on their surface, which can be quantified using flow cytometry.⁶ The most commonly utilized marker for assessing basophil activation is CD63, a lysosomal-associated membrane protein. CD63 is initially located on the membrane of secretory lysosomes within the basophil but becomes expressed on the cell's plasma membrane during degranulation, which makes it the most frequently used marker to assess basophil activation.⁶ Despite its use to diagnose and monitor allergic diseases, BAT has several limitations. As well as the standardization issue, it also requires large volumes of fresh blood and needs to be processed immediately. In our study, we studied 30 mother-child pairs who consecutively applied to our center for birth and gave informed consent for collecting

the cord blood samples to study BAT and to conduct a questionnaire at the end of the first year. Initially, our study aimed to evaluate responses to cow's milk and casein using the BAT test and examine the potential development of CMA during follow-up. However, our findings revealed that BAT by cow's milk was not linked to subsequent milk or food allergies. Instead, we discovered that BAT specifically triggered by casein was associated with a higher likelihood of developing food allergies later on. This may be important in cases where close monitoring for food allergies is required, and earlier introduction of complementary foods may be considered in view of tolerance development.

Although cord blood analysis is a popular method of study to measure allergy risk in later stages of life, to our knowledge, the BAT has never been used as a method in cord blood. Although the use of BAT in clinical practice is promising, there is still a need for standardization.⁵ Even though some efforts have been made, the main unmet need is to establish a cutoff value for each antigen.²⁸ Although we were unable to determine a cutoff value for casein-BAT, we showed on the basis of the median value that food allergies occurred more frequently in the first year in people with casein-induced basophil activation values of 2.6 and above. It is noteworthy that one patient who developed an allergy exhibited a score lower than 2.6 and had a history of recurrent wheezing and flare-ups of atopic dermatitis. In contrast, the remaining allergic cases had a score of 2.6 and above, and all were followed up for allergic skin lesions. Basophils play an important role in the pathogenesis of allergic skin lesions and the high BAT score in the cord blood of these patients suggests that basophil sensitivity may be present from birth. Other cases with high casein-BAT scores did not have allergies in the study period; however, these patients will be monitored longer to see if they develop allergies.

Out of the 9 cases where allergies developed, SPT was conducted in 5 instances. The allergens were identified in 3 of these SPTs (hen's egg, strawberry, banana, and cow's

Table 4. Univariate analysis of development of food allergy symptoms (n=30)

Variable	Group 1: Symptom: no (n=21)	Group 2: Symptom: yes (n=9)	P value
Sex			0.43 ^{a)}
Female	9 (42.9)	6 (66.7)	
Male	12 (57.1)	3 (33.3)	
Birth weight (g)	3,260 (2,440–4,000)	3,220 (2,073–4,000)	0.73 ^{b)}
Mode of delivery			
Vaginal delivery	5 (23.8)	3 (33.3)	0.67 ^{a)}
Caesarean section	16 (76.2)	6 (66.7)	
Breastmilk			0.52 ^{a)}
No	1 (4.8)	1 (11.1)	
Yes	20 (95.2)	8 (88.9)	
Formula			0.11 ^{a)}
No	16 (76.2)	4 (44.4)	
Yes	5 (23.8)	5 (55.6)	
Mother age (yr)	30.3 (21.0–38.0)	30.5 (29.0–38.0)	0.46 ^{b)}
No. of pregnancies			1.00 ^{a)}
1	5 (23.8)	2 (22.2)	
≥2	16 (76.2)	7 (77.8)	
Smoking during pregnancy			0.07 ^{a)}
No	20 (95.2)	6 (66.7)	
Yes	1 (4.8)	3 (33.3)	
Place of residence			1.00 ^{a)}
City center	10 (47.6)	4 (44.4)	
Rural	11 (52.4)	5 (55.6)	
Passive smoking during pregnancy			0.42 ^{a)}
No	7 (33.3)	5 (55.6)	
Yes	14 (66.7)	4 (44.4)	
Pet at home			1.00 ^{a)}
No	17 (81.0)	8 (88.9)	
Yes	4 (19.0)	1 (11.1)	
No. of people in the house			0.68 ^{a)}
<4	7 (33.3)	2 (22.2)	
≥4	14 (66.7)	7 (77.8)	
NLR			0.11 ^{a)}
≤0.33	13 (61.9)	2 (22.2)	
>0.33	8 (38.1)	7 (77.8)	
Cow's milk BAT			0.69
≤4.1	11 (52.4)	4 (44.4)	
>4.1	10 (47.6)	5 (55.6)	
Casein BAT			0.017^{a)}
<2.6	13 (61.9)	1 (11.1)	
≥2.6	8 (38.1)	8 (88.9)	

Values are presented as median (range) or number (%).

NLR, neutrophil-to-lymphocyte ratio; BAT, basophil activation test.

^{a)}Fisher exact test. ^{b)}Mann-Whitney *U* test.

Boldface indicates a statistically significant difference with *P*<0.05.

milk). We could not show the relationship between allergies and casein due to the absence of casein in SPT tests in 5 cases and no SPT in 4 cases; however, the relationship between casein-BAT and food allergy may be important in showing that these cases are likely to be predisposed to food allergies from birth.

The most important limitation of the study is the low number of cases, which prevented us from making sub-

group evaluations such as the type of food allergy or the type of symptoms. Therefore, the results presented should be interpreted with caution and require further validation. The second limitation: we were unable to identify a significant cutoff value in the ROC analysis since we used the median casein-BAT value in the logistic regression analysis as a categorical variable. With a larger number of patients, the casein-BAT test can be standar-

Table 5. Multivariate analysis of an infant's risk of developing food allergy symptoms in the first year of life

Variable	HR (95% CI)	P value
Formula		
No	1	
Yes	2.2 (0.1–38.9)	0.605
Smoking during pregnancy		
No	1	
Yes	2.2 (0.1–77.5)	0.670
NLR		
≤0.33	1	
>0.33	5.5 (0.3–100.3)	0.254
Casein BAT		
<2.6	1	
≥2.6	33.2 (1.3–868.1)	0.035

HR, hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; BAT, basophilic activation test.

Boldface indicates a statistically significant difference with $P<0.05$.

dized, and a cutoff value can be obtained for a valid use. One can speculate as to why the BAT with cow's milk does not yield the same results as casein. BAG-F2 contains multiple milk proteins, and the relative representation of casein epitopes may be lower compared with BAG-F78. Furthermore, in complex extracts, conformational changes or masking effects may reduce the accessibility of casein epitopes, which may explain why casein BAT was positive despite a lack of significant reactivity to cow's milk extract. Lastly, we conducted a single-center, prospective pilot feasibility study to obtain preliminary association signals. Because post hoc power is driven by the observed effect size and our estimates have wide CIs, these results should be viewed as hypothesis-generating and used to inform a priori calculations for future larger studies. The higher frequency of food allergy detected in our study was probably due to a selection bias consisting of patients admitted to a tertiary center.

In conclusion, a high casein-BAT value in cord blood may be predictive for food allergy that may develop in the first year of life or even later. Antigen-specific BAT evaluation in cord blood may pave the way for changes in the lifestyle, environmental factors, and diet of allergic children if validated in a larger number of patients.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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References

1. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120:638-46.
2. Elghoudi A, Narchi H. Food allergy in children-the current status and the way forward. *World J Clin Pediatr* 2022;11:253-69.
3. Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics* 2009;124:1549-55.
4. Burks AW, Jones SM, Boyce JA, Sicherer SH, Wood RA, Assa'ad A, et al. NIAID-sponsored 2010 guidelines for managing food allergy: applications in the pediatric population. *Pediatrics* 2011;128:955-65.
5. Santos AF, Riggioni C, Agache I, Akdis CA, Akdis M, Alvarez-Perea A, et al. Eaaci guidelines on the diagnosis of IgE-mediated food allergy. *Allergy* 2023;78:3057-76.
6. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice. *Allergy* 2021;76:2420-32.
7. Gallant MJ, Ellis AK. What can we learn about predictors of atopy from birth cohorts and cord blood biomarkers?. *Ann Allergy Asthma Immunol* 2018;120:138-44.
8. Junge KM, Hörnig F, Herberth G, Röder S, Kohajda T, Rolle-Kampczyk U, et al. The LINA cohort: Cord blood eosinophil/basophil progenitors predict respiratory outcomes in early infancy. *Clin Immunol* 2014;152:68-76.
9. Soti AL, Usemann J, Schaub B, Frey U, Latzin P, Fuchs O. Can biomarkers in umbilical cord blood predict atopic disease at

school age? *Pediatr Res* 2021;89:389-92.

10. Fernandes R, Kusel M, Cyr M, Sehmi R, Holt K, Holt B, et al. Cord blood hemopoietic progenitor profiles predict acute respiratory symptoms in infancy. *Pediatr Allergy Immunol* 2008;19:239-47.
11. Lin HY, Shyur SD, Fu JL, Lai YC. Whey and casein specific IgE and the cow's milk challenge test for atopic children. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1998;39:99-102.
12. Giannetti A, Toschi Vespasiani G, Ricci G, Miniaci A, di Palma E, Pession A. Cow's milk protein allergy as a model of food allergies. *Nutrients* 2021;13:1525.
13. Nucera E, Pecora V, Buonomo A, Rizzi A, Aruanno A, Pascolini L, et al. Utility of basophil activation test for monitoring the acquisition of clinical tolerance after oral desensitization to cow's milk: pilot study. *United European Gastroenterol J* 2015;3:272-6.
14. Ruinemans-Koerts J, Schmidt-Hieltjes Y, Jansen A, Savelkoul HFJ, Plaisier A, van Setten P. The basophil activation test reduces the need for a food challenge test in children suspected of IgE-mediated cow's milk allergy. *Clin Exp Allergy* 2019;49:350-6.
15. Doña I, Ariza A, Fernández TD, Torres MJ. Basophil activation test for allergy diagnosis. *J Vis Exp* 2021;(171). doi: 10.3791/62600.
16. Hinz D, Simon JC, Maier-Simon C, Milkova L, Röder S, Sack U, et al. Reduced maternal regulatory T cell numbers and increased T helper type 2 cytokine production are associated with elevated levels of immunoglobulin E in cord blood. *Clin Exp Allergy* 2010;40:419-26.
17. Allam JP, Zivanovic O, Berg C, Gembruch U, Bieber T, Novak N. In search for predictive factors for atopy in human cord blood. *Allergy* 2005;60:743-50.
18. Tadaki H, Arakawa H, Sugiyama M, Ozawa K, Mizuno T, Mochizuki H, et al. Association of cord blood cytokine levels with wheezy infants in the first year of life. *Pediatr Allergy Immunol* 2009;20:227-33.
19. Herberth G, Heinrich J, Röder S, Figl A, Weiss M, Diez U, et al. Reduced IFN-gamma- and enhanced IL-4-producing CD4+ cord blood T cells are associated with a higher risk for atopic dermatitis during the first 2 yr of life. *Pediatr Allergy Immunol* 2010;21(1 Pt 1):5-13.
20. Lehmann I, Herberth G. Cord blood immune status: predicting health or allergy? *Allergy* 2012;67:445-8.
21. Hayashi C, Sonoda T, Nakano T, Nakayama H, Kitamura Y. Mast-cell precursors in the skin of mouse embryos and their deficiency in embryos of Sl/Sld genotype. *Dev Biol* 1985;109:234-41.
22. Honda Y, Ono S, Honda T, Kataoka TR, Egawa G, Kitoh A, et al. Murine neonatal skin mast cells are phenotypically immature and minimally sensitized with transplacentally transferred IgE. *J Allergy Clin Immunol* 2019;144:617-20.e5.
23. Hibbert J, Strunk T, Nathan E, Prosser A, Doherty D, Simmer K, et al. Composition of early life leukocyte populations in preterm infants with and without late-onset sepsis. *PLoS One* 2022;17:e0264768.
24. Dhakal M, Miller MM, Zaghouani AA, Sherman MP, Zaghouani H. Neonatal basophils stifle the function of early-life dendritic cells to curtail Th1 immunity in newborn mice. *J Immunol* 2015;195:507-18.
25. Szépfalusi Z, Todoran L, Elsässer S, Jagdt B, Wank H, Urbanek R. Cord blood leucocytes/basophils produce and release sulfidoleucotrienes in response to allergen stimulation. *Clin Exp Allergy* 1999;29:382-7.
26. Wada T, Toma T, Shimura S, Kudo M, Kasahara Y, Koizumi S, et al. Age-dependent increase of IgE-binding and FcεR1 expression on circulating basophils in children. *Pediatr Res* 1999;46:603-7.
27. Bergmann MM, Santos AF. Basophil activation test in the food allergy clinic: its current use and future applications. *Expert Rev Clin Immunol* 2024;20:1297-304.
28. Chirumbolo S. Major pitfalls in BAT performance may be caused by gating protocols and CD63% cut off evaluation. *Cytometry A* 2014;85:382-5.

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