

ORIGINAL ARTICLE

Clinical Allergy

The Basophil Activation Test reduces the need for a food challenge test in children suspected of IgE-mediated cow's milk allergy

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Abstract

Background: The gold standard for the diagnosis of cow's milk allergy is the Double-Blind Placebo-Controlled Food Challenge (DBPCFC) test. However, disadvantages of the DBPCFC are the potential risk of anaphylactic reactions, the time-consuming procedure and high costs.

Objective: The aim of this study was to determine the reliability of the Basophil Activation Test (BAT) both for the initial diagnosis of cow's milk allergy in children and for the determination of tolerance in children with cow's milk allergy.

Methods: Ninety-seven BATs and cow's milk-specific IgE (sIgE) tests were performed in 86 infants/young children, suspected of (persistent) cow's milk allergy, who were qualified for an in-hospital DBPCFC. The BAT was performed with cow's milk extract and the purified major allergens casein, α -lactalbumin, β -lactoglobulin. Basophil activation was determined by CD63 upregulation measured by flow cytometry. The BAT results were compared to the DBPCFC outcomes.

Results: Based on unequivocal DBPCFC and BAT result combinations (80%), the BAT had a sensitivity and specificity of 100% (CI: 86%-100% and 68%-100%, respectively) in IgE-sensitized children (41% of the tested children). All non-IgE-sensitized children (59%) had a negative DBPCFC and BAT, except for five patients. These latter showed delayed and relatively mild symptoms in the DBPCFC with a negative BAT, supporting a non-IgE-mediated allergy in these children.

Conclusions and Clinical Relevance: The BAT seems reliable and cost-effective to diagnose patients with an IgE-mediated cow's milk allergy. In IgE-sensitized patients, a BAT might replace a DBPCFC. For non-IgE-sensitized patients presenting with mild symptoms, we propose to consider a (double-blind) extended (time) challenge test at home.

1 | INTRODUCTION

The most common food allergy during infancy is cow's milk allergy. The pathophysiological mechanism of cow's milk allergy is

commonly subdivided into IgE (type 1) and non-IgE-mediated types. Cow's milk allergy is capable of inducing a multitude of adverse reactions in children, which may involve organs such as the skin, gastrointestinal tract or respiratory system, ranging from mild to

life-threatening symptoms (anaphylaxis). Clinical practice suffers from variability in symptoms that appear less pronounced and an unclear relationship with ingestion of cow's milk.¹ Cornerstone of the diagnosis of cow's milk allergy is still a thorough medical history followed by a Double-Blind Placebo-Controlled Food Challenge (DBPCFC) test.² However, the disadvantages of the DBPCFC are the potential risk of an anaphylactic reaction, the time-consuming procedure and the high costs. Detection of specific IgE (sIgE) (for cow's milk extract or major milk components) or skin prick tests (SPT) may be of help in the diagnosis of cow's milk allergy. However, these tests mainly show if a patient is sensitized, as the relationship with a clinically relevant allergy is generally low.²⁻⁵ As such a safe, simple and robust in vitro test is urgently needed. In recent years, research focused on functional in vitro assays measuring activation of the patient's basophils after exposure to the allergen. In particular, the Basophil Activation Test (BAT) showed to be promising. Activation of basophils via the IgE-receptor leads to an increase in surface markers (CD63 and CD203c), which level of expression is measured by flow cytometry.⁶ So far, the BAT was mainly used for determining the degree of cow's milk tolerance in proven cow's milk allergic patients.⁷⁻¹¹

The aim of our study was to investigate the reliability of the BAT both for the initial diagnosis of cow's milk allergy in infants and for determination whether the cow's milk allergic child has outgrown the allergy. Consequently, the theoretical degree of reduction of challenge tests was assessed. In contrast to most previous studies, we used the DBPCFC, considered the gold standard, as reference test (unless an unmistakable serious allergic reaction to cow's milk accidentally occurred at home).

2 | MATERIALS AND METHODS

2.1 | Study design and settings

This is a prospective study covering the time period January 2011–May 2015. Data were collected at the Rijnstate Hospital in Arnhem, the Netherlands. This is a large secondary care in- and outpatient clinic. The procedures followed were in accordance with the ethical standards of the local feasibility committee, the Medical Research Involving Human Subjects Act (WMO) and the principles of the declaration of Helsinki (59th WMA General assembly, Seoul, October 2008) of the World Medical Association. This study was approved by the institutional medical ethical committee (nr 741-291210).

2.2 | Study population

Eighty-six clinically suspected cow's milk allergic children who were eligible for a DBPCFC test were prospectively enrolled during the study period. Patients were seen at the outpatient clinic shortly after the presentation of symptoms of cow's milk allergy.

Eighty percent of the DBPCFC tests were performed in an initial diagnostic work-up, with the remaining done in follow-up diagnosis to determine persistence or resolution of a previously established

cow's milk allergy. Clinical evaluation consisted of a detailed medical history and complete physical examination. Most children were already breastfed with their mothers on a cow's milk free diet or were fed with cow's milk free formula. Symptoms referred by the children in the IgE cow's milk-sensitized group were skin and mucus related (urticaria, increase in eczema, swelling of the lip), respiratory tract related (throat tightness and shortness of breath accompanied by signs of bronchus obstruction) and gastrointestinal related (nausea and vomiting). The non-IgE cow's milk-sensitized group reported mostly delayed and mild gastrointestinal symptoms or symptoms such as fussing, abdominal discomfort, irritable behaviour and crying.

Inclusion criteria were a DBPCFC and an IgE blood test to investigate the allergic status of the child. At the start of this study, measurement of cow's milk sIgE was not a standard test used for screening for cow's milk allergy under the age of 4 years (Discussion). Parents gave informed consent to both a DBPCFC and blood tests (sIgE and BAT).

In 90% of the cases, both a DBPCFC and blood tests were performed. In 10%, only blood tests were executed because 8% of children had been (unintended) exposed to cow's milk at home and suffered from a severe systemic reaction, that is, Sampson score 3–4, or it was anticipated that repeated exposure during a DBPCFC was too hazardous. As such we labelled them as cow's milk allergic, that is, as positive open provocations. For 2% of the children, an open provocation at home was performed without any adverse reaction.

A serum sample was used for sIgE determination, and an EDTA blood sample was obtained for the BAT. The BAT was performed within 24 hours after blood collection. People who performed the BAT were blinded to the provocation test results and vice versa.

2.3 | Double-Blind Placebo-Controlled Food Challenge (DBPCFC) test

Children underwent an in-hospital DBPCFC according to the national paediatric guideline "Diagnosis of cow's milk allergy in children in the Netherlands".¹² A challenge result was labelled either positive, negative or doubtful. A challenge result was regarded positive when symptoms occurred on the verum day, whereas no symptoms were noticed on the placebo day. Symptoms had to be identical to the symptoms at initial presentation. A challenge was labelled negative when no symptoms occurred on both test days or when symptoms occurred on the placebo day with no symptoms on the verum day. At last, a challenge was considered doubtful when none of the before-mentioned conclusions could be drawn. These doubtful DBPCFC outcomes (9.7%) were excluded from calculation of the diagnostic performance of the BAT. Our standardized protocol fulfilled the criteria described in the ESPGHAN Guideline: Diagnosis and management of CMPA.¹³

2.4 | Specific IgE measurement

Specific IgE (sIgE) for cow's milk extract and the purified major components, casein, β -lactoglobulin and α -lactalbumin were measured on

an ImmunoCAP 250 with allergen caps of ThermoFisher (Uppsala, Sweden) according to the manufacturer's instructions. Patients with sIgE to cow's milk extract and at least one component of ≥ 0.35 kU/L were considered as IgE sensitized.

2.5 | Basophil Activation Test

The BAT was performed with the Flow2-CAST kit and dry-powdered cow's milk extract and purified components (casein, α -lactalbumin, β -lactoglobulin) of Bühlmann (Basel, Switzerland) according to the instructions of the manufacturer. Basophils were selected based on CCR3 (CD193)/SSC and activation was determined by the level of CD63 expression measured by flow cytometry (FACS-Canto II, BD Biosciences).¹⁴ Stimulation of basophils (15 minutes at 37°C) with an anti-Fc ϵ R1 IgE receptor antibody was used as positive control (<12% difference in CD63-positive basophils between positive and negative control (no stimulation) was supposed to be a non-responder). The percentage of non-responders in our study was 13.4%, which is in accordance with literature data (6%-17%).⁶ Concentrations up to 400 ng/mL (1, 10, 100, 200, 400) for cow's milk and casein and up to 200 ng/mL (10, 50, 100, 200) for β -lactoglobulin and α -lactalbumin were tested in order to reach a plateau phase in the dose-response curve for most patients. The optimal cut-off point was determined by generating a ROC curve with the highest degree of basophil reactivity (CD63% positive basophils (corrected for the negative control)) in the dose-response curve and the DBPCFC test result. Doubtful DBPCFC and inconclusive BAT (non-responder) results were necessarily excluded from the ROC analysis. However, the inconclusive BAT results were taken into account when assessing the clinical capacity of the BAT and its effect on the reduction of DBPCFCs.

3 | RESULTS

3.1 | Study population; baseline characteristics and DBPCFC results

In total, 97 BATs were performed for 86 patients (51 boys and 35 girls). Nine patients were included more than once due to a prior doubtful DBPCFC test ($n = 1$) outcome or one or more times a follow-up ($n = 8$) of their cow's milk allergy. Table 1 shows the baseline characteristics and DBPCFC test outcomes of IgE and non-IgE-sensitized children at the time of inclusion in the study.

3.2 | Basophil Activation Test vs DBPCFC test

Based on ROC analysis, the highest combination of sensitivity and specificity, 83% and 100%, respectively (AUC 0.90), was obtained when a gradual up-regulation to $\geq 9\%$ CD63-positive basophils was found with increasing allergen concentration for cow's milk and at least one milk component. This threshold value (achieved independently of the allergen concentration) was considered a positive BAT

TABLE 1 Characteristics of the study participants

	IgE-sensitized patients N = 36	Non-IgE-sensitized patients N = 61
Sex (%)		
Male	53	64
Female	47	36
Age (y)		
Median (IQR)	2.7 (1.1-3.8)	0.9 (0.6-1.3)
Range	0.5-11.9	0.3-3.0
sIgE cow's milk (kU/L)		<0.35
Median (IQR)	5.2 (1.4-12.9)	-
Range	0.40-64.1	-
sIgE casein (kU/L)		<0.35
Median (IQR)	2.2 (0.6-5.6)	-
Range	< 0.35-74.6	-
sIgE β -lactoglobulin (kU/L)		<0.35
Median (IQR)	1.0 (0.5-2.7)	-
Range	<0.35-30.3	-
sIgE α -lactalbumin (kU/L)		<0.35
Median (IQR)	1.3 (0.5-3.5)	-
Range	<0.35-12.9	-
DBPCFC		
Positive	18	5
Negative	9	45
Doubtful	1	9
(Unintended) Open provocation		
Positive	8	0
Negative	0	2

IQR, interquartile range.

outcome. For four patients with a positive BAT result for cow's milk extract, the milk components could not be measured due to insufficient blood sample. These patients were nevertheless considered to have a positive BAT outcome.

3.2.1 | IgE cow's milk-sensitized patients

Twenty-four sIgE cow's milk-sensitized patients (0.49-64.1 kU/L) with a positive BAT had a positive DBPCFC. Figure 1A shows the BAT dose-response curves for cow's milk and Table 2 shows the sIgE values and BAT results for cow's milk extract and the individual allergens. Most cow's milk allergic patients showed a positive BAT at a cow's milk concentration <100 ng/mL while a minor part ($n = 9$) ≥ 100 ng/mL (Figure 1A). The results in Table 2 show that a positive BAT for cow's milk extract is always accompanied by $\geq 9\%$ CD63 upregulation of at least one individual allergen component, confirming that the BAT for cow's milk has a high specificity. However, performing a BAT for individual allergens does not have much added value for diagnosis in daily clinical practice.

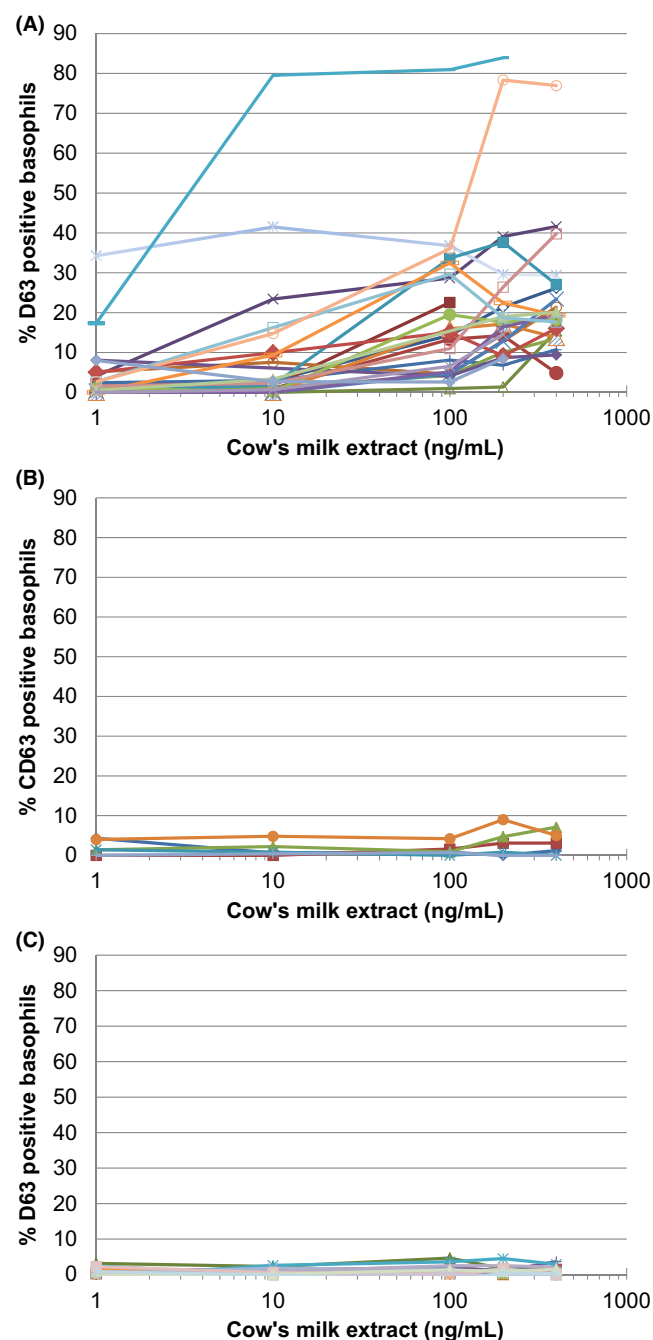


FIGURE 1 Basophil activation dose-response curves for IgE-sensitized children with a positive ($n = 24$) DBPCFC test (A) and negative ($n = 8$) DBPCFC (B) and for non-IgE-sensitized children with a negative DBPCFC ($n = 40$) (C). The percentage of CD63-positive basophils (corrected for the negative control) is plotted against the concentration of cow's milk extract (log-scale)

Eight sIgE-sensitized patients (0.51–5.2 kU/L) with a negative BAT had a negative DBPCFC outcome (Figure 1B). Three IgE-sensitized patients were non-responder in the BAT and one IgE-sensitized patient was both a non-responder in the BAT and had a doubtful DBPCFC (Table 3).

3.2.2 | Non-IgE cow's milk-sensitized patients

All non-IgE-sensitized patients had a negative BAT (Figure 1C) and nearly all a negative DBPCFC, except for five patients. These five children showed mostly delayed and only mild gastrointestinal symptoms in the DBPCFC test. Seven non-IgE-sensitized patients had a doubtful DBPCFC outcome, seven patients were non-responder in the BAT and two patients were both a non-responder in the BAT and had a doubtful DBPCFC (Table 3).

Table 3 also shows that the percentage of doubtful DBPCFC outcomes and inconclusive BATs (non-responders) is 9.7% and 13.4%, respectively. This shows that both tests suffer from “data missing due to clinical practise” to the same extent.

Based on unequivocal DBPCFC and BAT result combinations (80%), the BAT had a sensitivity and specificity of 100% (CI: 86%–100% and 68%–100%, respectively) in IgE-sensitized children (41% of the tested children) (Table 3 (bold printed edge of the contingency table)). This 100% sensitivity and specificity was obtained independently whether the BAT was applied for the initial diagnosis or follow-up of cow's milk allergy. For the follow-up patients, a negative or positive BAT result corresponded to resolution and persistence of cow's milk allergy, respectively.

Based on the 100% sensitivity and specificity of the BAT in IgE-sensitized patients and taking into consideration the observed percentage inconclusive BATs (non-responders), a theoretical reduction in challenge tests in our population (with a conclusive provocation test result) is 37%.

The ROC curves for BAT and sIgE cow's milk show that the BAT is superior to sIgE (Figure 2).

4 | DISCUSSION

This is the first study which shows that the BAT is also a reliable test for the initial diagnosis of cow's milk allergy in infants/young children in addition to the potential of the test to accurately test for tolerance.^{7–10}

In our study, the BAT had a 100% sensitivity and specificity in IgE cow's milk-sensitized children and was superior to sIgE; as such a risky, time-consuming and expensive DBPCFC test is redundant.

Rubio et al,⁸ using the same BAT kit and allergen source for cow's milk extract, showed in contrast to our results false-negative and false-positive BAT results for patients with cow's milk sIgE ≥ 0.35 kU/L. The use of lower cow's milk concentrations in the BAT when compared to our protocol (i.e. up to 100 ng/mL and 400 ng/mL, respectively) may play a causal role. In our study, in 9 out of 24 patients, basophil activation was shown at a cow's milk concentration over 100 ng/mL. Furthermore, in the study by Rubio et al, the open provocation test was performed as reference test, which is in general considered less reliable than the DBPCFC. Remarkably, they did not observe dubious outcomes.¹⁵ In our study, we used the DBPCFC as reference test and found that 10 out of 89 DBPCFCs

TABLE 2 sIgE and Basophil Activation Test (BAT) results for cow's milk extract and the individual allergens for IgE cow's milk-sensitized patients

Patient	sIgE (kU/L)				BAT (maximum CD63 (%))			
	Cow's milk	Casein	α -lactalbumin	β -lactoglobulin	Cow's milk	Casein	α -lactalbumin	β -lactoglobulin
1	2.2	1.8	0.8	0.5	26	34	26	13
2	4.9	3.2	1.2	1.2	23	16	11	6
3	1.3	2.5	<0.35	<0.35	15	NT	NT	NT
4	13.3	6.6	3.6	7.2	42	36	21	21
5	6.3	5.3	1.4	2.4	42	37	36	35
6	3.8	1.6	1.2	0.6	21	4	18	2
7	0.6	<0.35	0.9	<0.35	14	7	9	0
8	5.2	4.5	<0.35	2.8	14	20	20	18
9	0.5	<0.35	0.6	<0.35	13	13	13	0
10	7.0	0.6	1.7	1.0	9	NT	NT	NT
11	58.7	52.9	12.9	10.0	38	31	11	15
12	59.4	74.6	0.4	4.5	17	38	3	7
13	2.8	0.4	3.2	0.7	23	6	10	3
14	64.1	55.0	12.0	9.7	16	21	11	16
15	32.9	24.5	12.7	1.9	24	25	12	3
16	4.4	3.6	2.9	<0.35	19	43	4	1
17	38.2	24.8	4.8	30.3	84	77	56	50
18	11.6	0.8	3.2	16.5	9	5	11	7
19	11.6	11.4	3.6	0.8	32	NT	NT	NT
20	7.0	4.8	4.7	1.1	40	48	36	30
21	19.1	17.5	8.3	1.7	20	NT	NT	NT
22	6.3	3.5	2.2	3.6	15	12	9	5
23	18.0	5.1	7.2	11.2	30	29	21	32
24	6.8	5.7	2.4	0.8	78	55	56	36
25	0.5	0.6	<0.35	<0.35	4	NT	NT	NT
26	1.1	<0.35	<0.35	<0.35	0	0	NT	NT
27	5.2	1.4	1.2	0.9	2	2	2	1
28	1.0	0.7	0.2	1.1	1	NT	NT	NT
29	2.8	0.8	<0.35	1.5	1	0	NT	1
30	0.5	0.8	<0.35	<0.35	9	0	0	0
31	1.4	<0.35	0.5	1.9	1	NT	0	0
32	1.6	0.8	0.6	0.9	0	NT	NT	NT

NT, not tested.

provided no clear result. In our opinion, the use of the open provocation test by Rubio et al may be linked to their false-positive/negative BAT outcomes.

One may wonder whether a BAT for non-IgE-sensitized patients was useful. The reason for the number of non-IgE-sensitized patients in our study was that in the Netherlands, the opinion prevailed that cow's milk sIgE in infants is low, due to a developing immune system. As such, sIgE test kits/methods were supposedly not sensitive enough to detect all IgE-sensitized children. However, recently, the EuroPrevall study showed that both in the Netherlands and in the United Kingdom, about half of the cow's milk allergic children have a non-IgE-mediated type (sIgE/

SPT negative but a positive DBPCFC test). This difference in pathophysiological mechanism is reflected in the fact that in IgE-sensitized children cutaneous symptoms, and in non-IgE-sensitized children, gastrointestinal tract symptoms were relatively more pronounced.¹⁶ In our study, five non-IgE-sensitized cow's milk allergic children out of a total of 31 patients with cow's milk allergy were observed. In line with the findings described above, these five children had mostly delayed and mild gastrointestinal symptoms in the DBPCFC test.

We propose the re-evaluation of the protocol for children suspected of cow's milk allergy and sIgE < 0.35 kU/L. An in-hospital DBPCFC seems less appropriate to diagnose non-IgE-mediated cow's

TABLE 3 Results of the Basophil Activation Test (BAT) and sIgE in patients with and without cow's milk allergy (based on the outcome of the DBPCFC/open provocation)

	<i>DBPCFC/open provocation positive</i>	<i>DBPCFC/open provocation negative</i>	<i>DBPCFC doubtful</i>
BAT positive, sIgE ≥ 0.35 kU/L	24	0	0
BAT negative, sIgE ≥ 0.35 kU/L	0	8	0
BAT negative, sIgE < 0.35 kU/L	5	40	7
BAT inconclusive (non-responder)	2 ^a	8 ^a	3 ^a
Total	31	56	10

^aTwo patients are IgE-sensitized for cow's milk.

^bOne patient is IgE-sensitized for cow's milk.

The bold printed edge of the contingency table concerns the IgE-sensitized patients from which the sensitivity and specificity of the BAT for IgE-sensitized children were calculated.

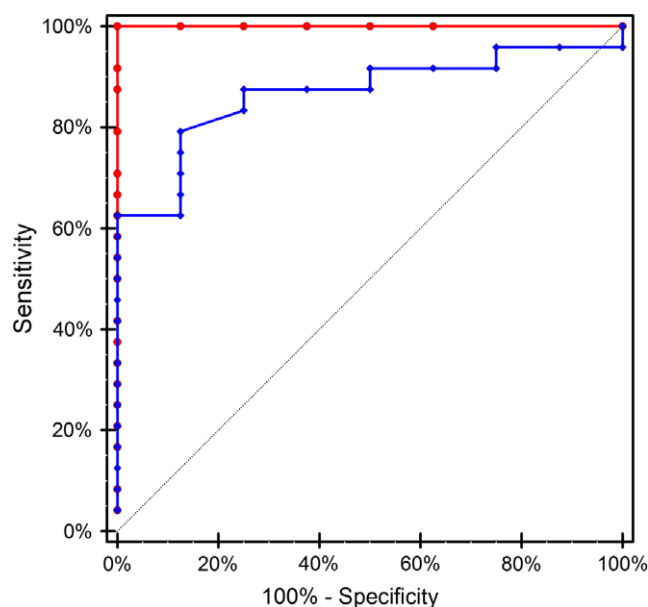


FIGURE 2 ROC curves for Basophil Activation Test (red, AUC: 1.0) and sIgE cow's milk (blue, AUC: 0.87) for IgE-sensitized patients

milk allergy presenting with mild and delayed symptoms. A double-blind extended (time) challenge test at home (directed by a paediatrician or a physician-supervised primary care centre) may be more appropriate, safe and less expensive.¹² In case of serious presenting symptoms, a food challenge is required at the hospital.

Drawback of our study is the quite limited number of IgE-sensitized patients with a negative BAT. However, since introduction of

the BAT test in our daily clinical practise, we observed another six cow's milk IgE-sensitized children with a negative BAT. They did not show any adverse effects upon introduction of cow's milk in the diet. Nevertheless, the promising results in this study should be re-evaluated in another independent study population.

Upon introduction of the BAT in clinical practise, we noticed a reduction of approximately 30%-40% in the number of DBPCFC tests (which was compensated by a similar number of BAT orders). The reduction of DBPCFCs is in line with the theoretical reduction of 37% as determined in this study. Future research is required to determine whether this reduction is only attributable to the group of IgE-sensitized children (or whether the low prevalence of non-IgE-mediated cow's milk allergy in our population has affected the clinical indications for a DBPCFC in non-IgE-sensitized children).

Further optimization of the diagnostic protocol might be possible by combining both in vitro tests. In our patient population a cow's milk sIgE of >5.2 kU/L corresponds to a positive DBPCFC outcome. Using a 2-step diagnostic approach starting with a sIgE test, which is only followed by a BAT when sIgE is ≥ 0.35 and ≤ 5.2 kU/L, could result in a theoretical 50% reduction in the number of BATs. Further research with more patient data is needed to optimize this 2-step diagnostic approach.

In conclusion, the BAT seems safe, reliable and cost-effective to diagnose and follow-up patients with an IgE-mediated cow's milk allergy. A potentially risky, time-consuming and expensive DBPCFC is redundant in these patients. For non-IgE-sensitized patients reporting mild symptoms to cow's milk an expensive in-hospital DBPCFC is not necessary; in these patients, we propose to consider a (double-blind) extended (time) challenge test at home.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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