

ORIGINAL ARTICLE

Comparison of bronchial and nasal allergen provocation in children and adolescents with bronchial asthma and house dust mite sensitization

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Abstract

Background: Bronchial allergen provocation (BAP) is an established tool for the diagnosis of allergy in patients with asthma, but its use is limited by the potential risk of severe asthmatic reactions. Nasal provocation testing (NPT) may be an alternative safe method and does not require sophisticated equipment.

Objective: The aim of this prospective study was to evaluate the concordance of both methods in patients with asthma and house dust mite (HDM) sensitization.

Methods: A total of 112 patients with HDM sensitization underwent BAP and had the following parameters analysed: decrease in FEV1, exhaled NO, and total and specific IgE. Within 12 weeks, NPT with HDM was performed in 74 patients with a median age of 9 years (range, 5–16 years). The results were evaluated using the Lebel score which quantifies major symptoms like rhinorrhea, nasal obstruction, sneezes and minor symptoms, such as pruritus, conjunctivitis and pharyngitis.

Results: Fifty-seven of 74 patients had an early asthmatic reaction, of which 41 were identified using the Lebel score. The Lebel score had a sensitivity of 71.9% and a positive predictive value (PPV) of 89.1%. In addition, an eNO ≥ 10 ppb (AUC 0.78), a specific IgE *Dermatophagoïdes pteronyssinus* ≥ 25.6 kU/L (AUC 0.76) and a specific IgE *Dermatophagoïdes farinae* ≥ 6.6 kU/L (AUC 0.78) were good predictors of an early asthmatic reaction.

Conclusion: A sequential use of NPT prior to BAP is justified to establish the relevance of HDM allergy. In patients with a negative NPT, BAP is still recommended to rule out a HDM-induced asthmatic reaction.

KEYWORDS

asthma, bronchial allergen provocation, house dust allergy, Lebel score, nasal provocation test, specific IgE

Abbreviations: AR, allergic rhinitis; AU, allergen units; AUC, area under the curve; BAP, bronchial allergen provocation; CI, confidence interval; Dfa, *Dermatophagoïdes farinae*; Dpt, *Dermatophagoïdes pteronyssinus*; EAR, early asthmatic reaction; eNO, exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HDM, house dust mite; IgE, specific IgE; LAR, late asthmatic reaction; NPT, nasal provocation testing; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristics.

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1 | INTRODUCTION

The prevalence of asthma and allergic rhinitis (AR) is increasing worldwide. It is well known that the coexistence of AR and asthma is extremely common and detected in up to 90% of asthmatic subjects.¹⁻⁴ Many patients with asthma and comorbid AR are sensitized to house dust mite (HDM) allergens.⁵ However, the clinical relevance of HDM sensitization in asthma is often unclear.⁶ Therefore, current guidelines emphasize the use of allergen provocation tests to prove the relevance of a given HDM sensitization.^{7,8} Bronchial allergen provocation (BAP) has been established as an essential tool for the diagnosis of allergy in patients with asthma.⁹⁻¹² Furthermore, BAP is used for estimating the effectiveness of subcutaneous immunotherapy.^{13,14} Although great effort has been put into the tasks of standardizing the procedure¹⁵⁻¹⁷ and improving its safety, BAP is still rarely used by clinicians due to possible side effects and the fear of a severe asthmatic reaction.¹⁶

Nasal provocation testing (NPT) is another commonly used method in the field of diagnostics for atopic diseases and has been established as a safe and reproducible technique in diagnostics for atopic disease, especially AR.¹⁸⁻²⁰ NPT is relatively easy to perform, is safe and does not require sophisticated equipment. NPT with the Lebel score is a simpler testing procedure than BAP that not only reduces patient risk but is also cost-effective and has a more widespread availability.^{7,18,19}

Since the majority of asthmatic patients suffer from coexistent AR, NPT should have a high concordance with BAP in HDM allergic patients with asthma. However, the concordance of both methods is not well known. The aim of this prospective study was to evaluate the congruence of results obtained with both methods in children and adolescents with house HDM sensitization.

Key Message

Bronchial allergen provocation (BAP) cannot be used in multi-centre trials due to possible side effects and a lack of experienced investigators. Nasal provocation testing (NPT) is an alternative method that is safe and does not require sophisticated equipment. However, the concordance of both methods is not well known. This is the first report showing that the NPT can substitute a BAP in most cases. In patients with a negative NPT, BAP is still recommended to rule out an HDM-induced asthmatic reaction.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients were selected from the outpatient Department of Pediatric Allergy, Pulmonology and Cystic Fibrosis at the Children's Hospital of the Goethe University in Frankfurt/Main, Germany, from February 2015 to March 2016. The study was approved by the local institutional ethics committee and performed in accordance with the Declarations of Helsinki (1975) and Edinburgh (2000).

2.2 | Study design

This study was a non-randomized prospective cross-sectional study that encompassed one visit. The population consisted of 157 patients >6 years suffering from allergic asthma with or without AR. The routine investigation of these patients included skin

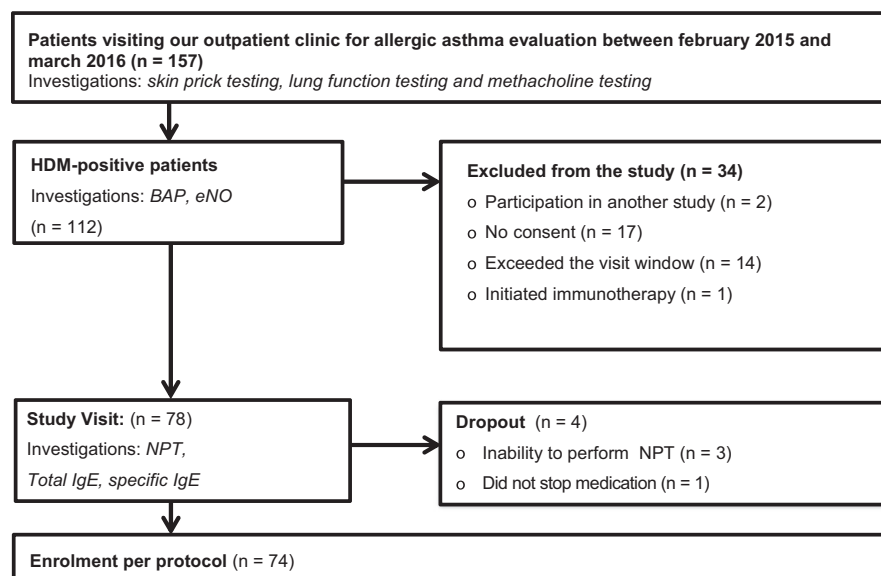


FIGURE 1 Study flow chart

prick testing (SPT), spirometry, reversibility testing with 400 µg salbutamol or methacholine testing. All patients with a positive SPT for HDM, 112 of 157, underwent measurement of eNO and BAP with HDM routinely. Seventy-eight patients provided written informed consent and participated in the study visit to undergo NPT (Figure 1). Three patients showed no sufficient cooperation to complete NPT, and one patient did not stop medication; these patients were dropouts.

The inclusion criteria were as follows: positive SPT for HDM, signs of allergic asthma with/without AR, BAP within the last 12 weeks, no asthma exacerbation within the last four weeks and signed informed consent. The reasons for exclusion included the following: age < 6 years and >18 years, and asthma exacerbation within the last 28 days prior to screening. Furthermore, patients with serious underlying conditions were excluded from the study.

2.3 | Skin prick test

A SPT using standard allergens (Allergopharma) was performed. The mean of the largest diameter of the wheal was recorded for each allergen as the response. A response of at least 3 mm was deemed positive.

2.4 | Measurement of exhaled NO

Measurements of eNO were collected using the NIOX Vero® (NIOX Nitric Oxide Monitoring System). NIOX Vero® measures the eNO in exhaled air according to the American Thoracic Society (ATS) guidelines.²¹

2.5 | Pulmonary function testing

Baseline pulmonary function tests were performed using the MasterScreen spirometer (CareFusion, Germany). The following parameters were recorded: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and the FEV1/FVC.²²

2.6 | Laboratory measurements

Serum was analysed for total IgE and specific IgE *Dermatophagoides pteronyssinus* (Dpt) and *Dermatophagoides farinae* (Dfa) by chemiluminescence immunoassay (Siemens).

2.7 | Bronchial allergen provocation

BAP was performed using the APS dosimeter technique (CareFusion, Germany) as recently described.^{15,16} Before BAP, lyophilized HDM allergen (*Dermatophagoides pteronyssinus*) (Allergopharma KG, Reinbek, Germany) was dissolved in 5 mL of

0.9% saline. The entire protocol delivered cumulative doses of 10, 30, 70, 150 and 310 AU.¹⁵ Ten minutes after each BAP, spirometry was performed. A decrease of 20% in FEV1 was defined as an early asthmatic response (EAR). After EAR, patients received two to four puffs of salbutamol (0.1 mg) until the FEV1 value returned to at least 80% of the baseline value. FEV1 was measured for up to ten hours following BAP, and a late asthmatic reaction (LAR) was defined as a decrease of FEV1 ≥ 15%, 6-9 hours after BAP. We classified our study group according to the dosage resulting in a positive BAP as follows: <40 AU as highly allergic, >40 AU as allergic and >310 AU as negative.

2.8 | Nasal allergen provocation

NPT was performed using the same lyophilized HDM extract that was used for BAP (5000 AU/mL). The solution containing 5000 AU/mL was diluted 1/10 two times with 0.9% saline to obtain two further solutions with concentrations of 500 AU/mL and 50 AU/mL allowing a four-step provocation with NaCl (0.9%), 4, 40 and 400 AU, respectively, and one puff was administered to each nostril. The baseline Lebel score was required to be below three points to continue with NPT. To exclude non-specific nasal reactions, a baseline measurement with 0.9% saline was conducted by spraying one puff in each nostril. After ten minutes, a second Lebel score was recorded. Then, NPT with HDM was conducted starting with the 50 AU/mL solution until the Lebel score was positive ≥6 points.²³ NPT results (Lebel score ≥ 6 points) were divided into three groups: 4-40 AU as highly allergic, >40-400 AU as allergic and a Lebel score < 6 at the third dose as negative.

2.9 | Statistical analysis

Data were analysed using the statistical programme GraphPad Prism 5.01 (GraphPad Software). The distribution of age, sex, lung function parameters, total and specific IgE was compared. The parameters were tested for normal and log-normal distributions using the Kolmogorov-Smirnov test. The correlation between NPT and BAP was calculated using Spearman's rank correlation test. To define the sensitivity and specificity of the Lebel score to detect an

TABLE 1 Characteristics of screened patients

Parameters	Patients per protocol	Patients not included
Patients (n)	n = 74	n = 38
Sex (m/f; %)	54/46	66/34
Age (y; mean ± SD)	9.1 ± 3.1	9.2 ± 2.7
FEV1 (%Pred; mean ± SD)	96.6 ± 13.8	96.9 ± 12.7
FVC (%Pred; mean ± SD)	89.2 ± 13.2	90.5 ± 10
Methacholine (mg; mean ± SD)	0.5 ± 0.5	0.6 ± 0.5

Note: No statistical difference was found.

Patients	Total	BAP positive	BAP negative	P-value
Number (n)	74	57	17	
FEV1 (%)	96.6% ± 13.8	97.1% ± 14.4	94.6% ± 11.8	n.s
FVC (%)	89.2% ± 13.2	89.5% ± 13.1	88.2 ± 14.2	n.s
FEV1/VC (%)	89.7 % ± 13.6	89.6% ± 12.7	89.9 ± 16.5	n.s
Age (y)	9.1 ± 3.1	9.1 ± 2.9	9.2 ± 3.7	n.s
Total IgE (kU/L)	632.9 ± 803.6	683.2 ± 878.6	349.3 ± 250.4	n.s
sIgE Dpt. (kU/L)	56.5 ± 38.1	63.4 ± 35.6	30.9 ± 37.4	<0.05
sIgE Dfa. (kU/L)	51.8 ± 36.7	42.6 ± 25.9	20.2 ± 34.0	<0.05

TABLE 2 Patient characteristics with positive and negative BAP results (mean ± SD)

EAR, a receiver operating characteristic (ROC) curve was plotted. Cohen's Kappa statistic was used to test the reliability of BAP and NPT for negative, allergic and highly allergic patients. The accuracy was measured by area under the curve (AUC) analysis. A probability of $P < .05$ was regarded as statistically significant.

3 | RESULTS

3.1 | Patient characteristics

We compared the patients who were enrolled per protocol with those patients who were not included (Table 1). There was no difference between patients enrolled per protocol and those who were not included in the study in terms of age, sex, FVC or mean FEV1 or methacholine testing. The clinical characteristics of the patients included per protocol are shown in Table 2. The median age of the 74 patients (34 females and 40 males) was 9 years (range, 5-16 years). All 74 patients suffered from asthma, and 53 (71.6%) had concomitant AR. In addition, 16 (21.6%) of 74 patients were mono-sensitized. Interestingly, negative NPTs were equally distributed in patients with concomitant AR and without AR, 29% vs 23.8%, respectively. Fifty of the 78 (64.1%) included patients were taking long-term medication (fluticasone = n=16; montelukast: n = 1, budesonide: n = 6; budesonide + formoterol: n = 4; fluticasone + salmeterol: n = 17; budesonide + montelukast: n = 1; fluticasone + salmeterol + montelukast: n = 6), which was paused 48 hours before the study visit.

3.2 | Bronchial allergen provocation

BAP overall was well tolerated but one patient had to be admitted to the hospital, due to prolonged asthmatic symptoms that improved with systemic steroids and inhaled salbutamol. Overall, 57 of 74 patients had positive BAP results and showed significant EARs (mean decrease in FEV1 (%), 29.2 ± 7.4). The clinical characteristics of patients with positive and negative BAP are shown in Table 2. There was no difference between patients with positive and negative BAP results in terms of age, FVC, FEV1 or total IgE. In contrast, patients

with positive BAP results had significantly higher values for sIgE to HDM than BAP-negative patients.

3.3 | Nasal provocation test

Forty-six patients had positive NPT results with a Lebel score ≥ 6 points. According to this analysis, 41 out of 57 patients had positive BAP and NPT results (Figure 2), revealing a sensitivity of 71.9% (95% confidence interval (CI) 58.5%-83.0%) and a specificity of 70.6% (95% CI 44.0%-89.7%). The positive predictive value (PPV) was good (89%), and the negative predictive value (NPV) was not as strong (42.8%; Table 3). A cut-off value of 4.5 points was set as the best predictor with an AUC of 0.7, a sensitivity of 78.9% and a specificity of 64.7% (Figure 3; ROC curves). Patients with positive BAP results had a mean Lebel score of 7 points, whereas patients with negative BAP results had a mean Lebel score of 3 points ($P < .05$). Then, we investigated whether there was a better correlation between BAP and NPT results in highly allergic patients than in low allergic patients. However, no concordance between the results was found ($r = .13$; $P = .21$), using the Cohen's Kappa test. In addition, the Spearman test showed only a weak correlation ($r = .26$ 'poor', $P = .02$). However, we found a correlation between a decrease in FEV1 and the Lebel score ($r = .37$; $P < .005$).

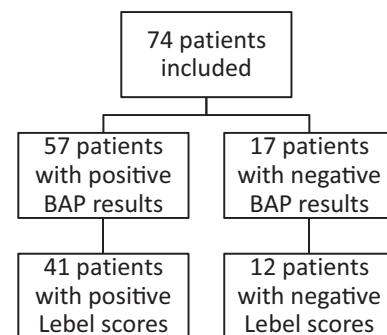
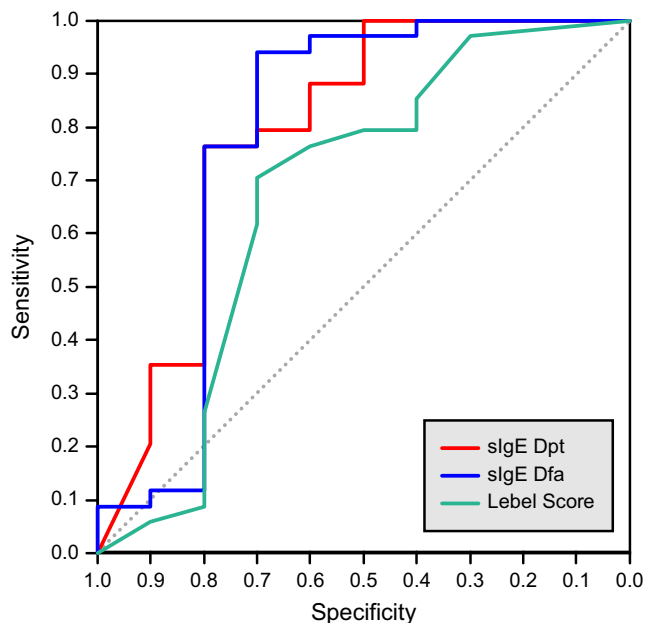


FIGURE 2 Concordance between the bronchial allergen provocation (BAP) results and the nasal provocation testing (NPT) results using the Lebel score

TABLE 3 Predictive power of the Lebel score, sIgE and eNO to detect an EAR in BAP

Parameter	Cut-off	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]	AUC	P
NPT Lebel score	4.5	71.9	70.6	89.1	42.8	0.7	<0.001
sIgE Dpt	25.6 KU/L	82.2	66.6	88.2	50.4	0.76	<0.01
sIgE Dfa	6.6 KU/L	94.1	70.0	84.3	44.1	0.78	<0.05
eNO	10 ppb	91.84	53.8	88.24	63.64	0.78	<0.01

**FIGURE 3** ROC analysis for the Lebel score, sIgE Dpt and Dfa to detect an early asthmatic reaction (EAR) to house dust mite (HDM) investigated by a bronchial allergen provocation (BAP)

3.4 | IgE values

Total IgE (mean $623 \text{ kU/L} \pm 803$), sIgE Dpt ($56.2 \text{ kU/L} \pm 37.1$) and sIgE Dfa (51.8 ± 36.7) were measured in 57 patients. In patients with positive BAP results, sIgE Dpt ($P < .01$) and sIgE Dfa ($P < .05$) were significantly higher than in patients with a negative BAP. There was a correlation of the maximum decrease in FEV1 with sIgE Dpt ($r = .44$; $P < .005$) and sIgE Dfa ($r = .34$; $P < .05$). sIgE Dpt and sIgE Dfa were shown to be significant predictors for an EAR with an AUC of 0.76 and 0.78, respectively (Table 3). With a cut-off point of 25.6 kU/L, sIgE Dpt showed a sensitivity of 82.2% and a specificity of 66.6%. sIgE Dfa had a lower cut-off point of 6.6 kU/L, with a sensitivity of 94.1% and specificity of 70.0% (Figure 3; ROC curves). There was no correlation of the Lebel score with the total IgE and specific IgE.

3.5 | Exhaled NO

The eNO was a good predictor of an EAR. Patients with positive BAP results had a mean eNO of 32 ppb compared to 9 ppb in

patients with negative BAP results. ROC curve analyses evaluating sensitivity and specificity of the eNO for predicting an EAR showed an optimal cut-off point of 10 ppb (AUC = 0.78), which had a sensitivity of 91.8% but a low specificity of 53.8% (Table 3). No correlation and no cut-off point were found for the eNO and Lebel score.

4 | DISCUSSION

BAP is well established and is considered the gold standard to prove the clinical relevance of an allergen, but its use is limited by the potential risk of severe asthmatic reactions.^{24,25} The purpose of this study was to investigate whether NPT could be a simple alternative to BAP for determining the clinical relevance of HDM sensitization in patients with bronchial asthma. We found that the Lebel score had a sensitivity of 71.9% and a PPV of 89.1%. Unfortunately, the NPV was not as strong (42.8%). For a clinician, this result is of great value, since a positive NPT may be a substitute for BAP in most of the cases. So far, only two studies investigated the relationship between the allergen responses at nose and bronchus.^{26,27} The study of Mosbech et al concluded that BAP cannot be replaced by NPT, since the sensitivity of the mucosa in different organs of the same patient seems unrelated,²⁶ but our findings are in line with a recent study by Choi et al.²⁷ The authors showed that the NPT was specific and accurate for the prediction of an EAR in patients with asthma. The sensitivity of the NPT was even higher than in our study (82.4% vs 71.9%) since almost all patients with an EAR had a positive NPT. This difference is most likely explained by the fact that our study was done in children and adolescents with well-characterized asthma, whereas Choi et al investigated young adults to obtain a military certification for asthma.²⁷

It is important to note that one of the children had a serious medical adverse event due to asthma and had to be monitored overnight. Depending on the patient population, level of asthma control and allergen used for BAP, different information on the occurrence of LARs has been reported in the literature, with an incidence ranging from 20% to 91%.^{8,24,25,28} On average, at least 50% of the patients tested with HDM BAP had an LAR.^{8,24,25,28} Warner²⁶ challenged 69 children with HDMs, and 73% had a significant LAR. Interestingly, in one of our studies, using FEV1 instead of peak flow measurements, 91% of patients had an LAR.²⁹ However, severe bronchial symptoms have only been described in isolated

cases and are usually well controlled by symptomatic therapy. For this reason, the use of BAP in asthmatic patients with negative NPT results is still justified.¹⁶

Recently, measurement of sIgE Dpt and sIgE Dfa was considered to be a good and safe predictor of an EAR after BAP.^{16,29-31} We found optimal cut-off values of 25.6 and 6.6 kU/L for sIgE Dpt and sIgE Dfa, respectively, with good sensitivities of 82.2% and 94.1%, respectively, for detecting an EAR. In addition, there was a fair correlation between sIgE Dpt/Dfa and the provocation dose of HDM causing a 20% decrease in FEV₁ ($r = .38$; $P < .005$ and $r = .37$; $P < .005$) in our patients. Thus, the level of specific serum IgE seems to be important for predicting significant bronchial obstruction after BAP. This strong relationship was also found in patients with grass pollen AR.³² In contrast, no correlation was found for the Lebel score and the specific IgE in our cohort. Similar results were found by two other studies. In both studies, specific IgE values did not correlate with nasal symptoms as measured by NPT.^{33,34} This finding was confirmed by Riechelmann et al, who found that SPT and specific IgE were unreliable predictors of the outcomes of NPT with HDMs.³⁵ This conclusion is difficult to explain but may be in part related to the fact that serum levels of specific IgE do not necessarily reflect local levels of IgE at the site of inflammation in the nose. In contrast, many studies have shown that IgE levels increase, as the severity of asthma increases.³⁶⁻³⁸ In addition, we were unable to find a correlation between Lebel score and eNO, although eNO has been described as an useful predictor of an EAR after BAP by many authors.^{16,32,39}

This study has some limitations:

As shown in the results, our study has a selection bias due to a cohort of children with well-characterized asthma of which 71.2% had concomitant AR. This might be a reason why the sensitivity of the NPT in our study was lower as previously reported in unselected adults.²⁷ Furthermore, 64.1% of our asthma patients were treated with inhaled corticosteroids before the study. This fact might explain why the cut-off levels of eNO were rather low. In addition, the NPT was solely done by the Lebel test and not confirmed by rhinomanometry, which might be a more objective method as recently discussed.⁴⁰ However, the availability and practicability of the Lebel score in everyday practice was the reason for choosing Lebel score.

It has to be acknowledged that measurement of total IgE is not helpful in predicting BAP or NPT results and should not be used in clinical practice. The fact that higher total IgEs may be predictive for asthma does not mean that once asthma is established such parameter should be assessed, unless in specific settings, that are not considered in the present paper.

In conclusion, a sequential use of NPT prior to BAP is justified to establish the relevance of HDM allergy in most cases with asthma. In patients with a negative NPT, BAP is still recommended to rule out a HDM-induced asthmatic reaction.

CONFLICT OF INTEREST

Stefan Zielen received fees for lectures and advisory boards from the following companies: bene-Arzneimittel GmbH; Vifor Pharma Deutschland GmbH; AstraZeneca; Novartis AG; Sanofi/Aventis,

GlaxoSmithKline GmbH; HAL Allergy, Stallergenes-Greer; ALK-Abelló Arzneimittel GmbH; Allergy Therapeutics; Boehringer Ingelheim, Lofarma GmbH, Engelhard Arzneimittel GmbH, IMS Health GmbH & Co. and Biotest Pharma GmbH. All other authors have no conflicts of interest.

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