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WHAT'S KNOWN ON THIS SUBJECT: Induced sputum cytology is a routine test in diagnosing chronic cough. Although the reference range has been established in adults, inconclusive findings in children have been due to problematic study design, sample size, and limited publications in China or worldwide.

WHAT THIS STUDY ADDS: This is the first successful attempt to establish the reference range of induced sputum cytology in Chinese children. The results of cytology were not found to be influenced by gender, age, and passive smoking as these factors may do in adults.

abstract

OBJECTIVE: To establish normal reference values of induced sputum cytology in healthy children in southern China.

METHODS: During a period from January 2010 to December 2011, a total of 580 healthy children (5–16 years of age) were approached. A total of 266 children (137 boys and 129 girls) participated in the study. Sputum induction was carried out by using 5% hypertonic saline. Cell types in the sputum were examined by using routine methods.

RESULTS: Sputum induction was completed in 175 of the 266 subjects (65.79%), but 16 sputum samples were disqualified. The overall success rate was 59.77% (159/266). Macrophages and neutrophils were the predominant cell types: macrophages: median, 76.14%; interquartile range (IQR), 32.68%; and 2.5% to 97.5% percentile, 1.00% to 94.50%; neutrophils: median, 20.67%; IQR, 33.0%; and 2.5% to 97.5% percentile, 4.00% to 92.75%; eosinophils: median, 0.39%; IQR, 1.93%; and 2.5% to 97.5% percentile, 0.00% to 6.50%; and lymphocytes: median, 1.22%; IQR, 2.04%; and 2.5% to 97.5% percentile, 0.00% to 5.00%. The cell types did not differ among different age, gender, and passive smoking groups. Adverse events occurred in 4.4% (7/159) of the participants who completed the procedures but required no specific treatment to dissipate. Peak expiratory flow did not differ between those who completed the procedures compared with those who did not, suggesting that the procedure is safe and feasible in children.

CONCLUSIONS: The current study represents the first attempt to develop normal reference values of induced sputum cytology in Chinese children, and could be used as a control for future studies. *Pediatrics* 2013;131:e518–e524

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KEY WORDS
induced sputum, reference value, cough

ABBREVIATIONS
CI—confidence interval
FEV_1—forced expiratory volume in 1 second
FEV_1%—percentage of the forced expiratory volume in 1 second
IQR—interquartile range
PEF—peak expiratory flow
PEF%—peak expiratory flow in normal expected value

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Cough accounts for >35% of clinic visits in preschool-aged children, and ~10% of preschool-aged children have a medical history of chronic cough.[1–5] The incidence of chronic cough in Chinese children is estimated to be ~6.4%.[6] In the early 1990s, Pin et al[7] started using cytologic study of hypertonic saline-induced sputum to examine airway inflammation in children. Since then, such examination has been increasingly used in diagnostic investigation of chronic cough. In 2007, the Guangzhou Institute of Respiratory Disease conducted a large sample study and established normal reference values for induced sputum cytology in Chinese adults.[8] Partly based on that study, a recommendation was made to use induced sputum cytology as a routine part of diagnosis in Chinese adults with chronic cough.[8,9] The value of induced sputum cytology has been advocated by the *Interim Guideline for Diagnosis and Treatment of Pediatric Cough*.[10] However, there is limited information concerning the use of induced sputum cytology in children. Due to compliance issues, data on induced sputum cytology are difficult to obtain in this young patient population, and coupled with safety concerns from parents and social culture, data are even more difficult to obtain in healthy children. Consequently, the majority of studies using induced sputum in Chinese children over the past decade were designed with healthy controls much fewer in number compared with the patients (frequently 1:3 to 1:5)[11–16] or controls with other diseases.[17] So far there is a lack for normal reference values in healthy Chinese children. To some extent, this has prevented the use of induced-sputum cytologic study in pediatric practice.

In addition, Pignatti et al[18] found that neutrophil is the predominant cell type in induced sputum nonsmoking adults aged above 50 years. They also noted higher neutrophils and lower macrophages percentages with increasing age. It remains unknown whether the results of cytology in children differ with gender and age or passive smoking.

In 2011, we completed an induced sputum cytology study in a relatively large sample of healthy children in southern China.

METHODS

Subjects

During a period from January 2010 to December 2010, a total of 580 elementary and middle school students attended regular health check-ups at our institution, 1 of designated health care service centers for children and teenagers in Guangzhou City. These children were subsequently approached by telephone, letter, or home visits for their intention to be recruited for an induced sputum test. Informed consent was obtained from 352 students (60.7%); the remaining 228 (39.3%) rejected participation in the study. The age of the participants ranged from 5 to 16 years, with ~32 subjects in each age. The boy: girl ratio was ~1:1. The participants' residence addresses came from the 4 large administrative districts of Guangzhou proper (Dongshan, Yuexiu, Haizhu, and Baiyun Districts), representing a wide range of metropolitan coverage.

A routine physical examination and a pulmonary function test (using Masterscreen IOS Pulmonary Function Analyzer; Erich Jaeger, Hoechberg, Germany) were conducted to verify the health status. Predicted values of forced expiratory volume in 1 second (FEV_1) were based on a published prediction equation for southern Chinese children by Zheng et al[19] to minimize variations from ethnic differences. A 14-item questionnaire[19] with regards to respiratory/digestive diseases, allergic diseases, and harmful gaseous substance/dust in residence was completed by the participants as well as their legal guardians. Exclusion criteria were as follows: (1) history of bronchial asthma and/or allergic rhinitis; (2) percentage of the FEV_1 (FEV_1%) <85% of the normal expected value, or FEV_1/forced vital capacity ratio ≤85%, or proportion of peak expiratory flow (PEF) in normal expected value (PEF %) <85%[20]; (3) family history of allergy (allergic rhinitis, bronchial asthma, or urticaria); (4) history of respiratory infections during the previous 6 weeks; (5) use of any prescribed or over-the-counter drugs in the previous 4 weeks; (6) medical history or family history of chronic respiratory diseases such as chronic bronchitis and chronic cough; (7) history of severe disorders in digestive systems or other systems; and (8) any abnormality in the nasopharynx and chest upon physical examination. FEV_1% was measured in all children before the induction. PEF was measured before and after sputum induction with a peak flowmeter (GlaxoSmithKline, China), and PEF% was calculated.

This study was approved by the Institutional Ethics Review Committee of First Affiliated Hospital, Guangzhou Medical College (GYFYY-2010-12-05). Written informed consent was obtained from the legal guardians of all participants after a detailed description of the purpose and potential benefits of the study.

Sputum Induction

Hypertonic saline (5%) was nebulized and inhaled for sputum induction. Because of potentially less compliance and weak tolerance in children as compared with adults, the duration of induction time was not strictly standardized; instead, each subject was encouraged to hold on for at least 15 minutes or preferably throughout the procedure (30 minutes). At every 10 minutes apart during the induction, the participants were instructed to spit saliva into a container labeled "saliva" before coughing sputum into a container labeled "sputum." The coughed-up sputum of each individual subject was mixed before sample processing.

No expectoration within 30 minutes was defined as a failure, and the procedure was terminated. The sputum was diluted fourfold with 0.1% dithiothreitol, incubated at 37°C for 10 minutes, and then sifted through a 300-μm mesh sieve before trypan blue staining. Cell smears were prepared, fixed in neutral formalin, and stained with hematoxylin-eosin. Two independent investigators (Drs Luo and Chen) counted the cells manually in a blind manner by using an optical microscope. The proportion of eosinophils, lymphocytes, neutrophils, and macrophages was calculated according to the cell morphology of a total of 400 cells on each slide of cell smears. Sample slides with >20% epithelial cells and <40% viable cells were considered disqualified and were not included in the data analysis.

The two investigators were well-trained and experienced with cytology and differential cell counting. The interobserver variability for cell counts was <5% for neutrophils and macrophages and <1% for eosinophils and lymphocytes.

Statistical Analysis

All statistical analyses were performed by using SPSS version 13.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY). Normally distributed data were described as mean ± SD. Nonnormally distributed data were expressed by using median and interquartile range (IQR), and the reference range was described as 2.5% to 97.5% percentile. To provide important information for reference, the mean and mean's 95% confidence interval (CI) were also calculated for the cell counts despite skewed data. Categorical data were expressed by frequency (percentage). Student's *t* test (2-sided) was used to compare the normally distributed results, and the differences of nonnormally distributed results were tested for statistical significance by using the Mann–Whitney *U* test and the Kruskal–Wallis test. A $P < .05$ was considered significant. The data were stratified into the following 3 age groups: 5 to 6 years old; 7 to 12 years old; and ≥12 years old.

RESULTS

General Information of the Included Subjects

Of 580 students approached, 352 students (60.7%) volunteered to participate in the study. Eighty-six students met the exclusion criteria, and finally, 266 subjects (137 boys and 129 girls) were included in the current study. Of these included subjects, sputum induction was successful in 175 (175/266, 65.8%) and failed in 91 (91/266, 34.2%). Of the 175 children who succeeded for sputum induction, 159 (92 boys and 67 girls) produced qualified sputum samples, which were eligible for data analysis (rate of success: 59.8% [159/266]; Fig 1). The mean age of the included subjects was 10.19 ± 2.68 years; height, 138.65 ± 13.47 cm; and body weight, 34.05 ± 12.32 kg. FEV_1% and PEF% before sputum induction were 106.3% ± 11.0% and 99.6% ± 11.5%, respectively.

Cytologic Findings According to Age, Gender, and Passive Smoking

The frequency of eosinophils, lymphocytes, neutrophils, and macrophages is shown in Table 1. The cell counts appeared skewed and, therefore, are presented as median, IQR, and 2.5% to 97.5% percentile. To provide important information for reference, the mean and mean's 95% CI were also calculated for the cell counts despite skewed data. Macrophages and neutrophils were apparently the predominant cells, accounting for nearly 76% and 21% by medians, respectively, of total cells in induced sputum from the healthy children.

The cytologic results did not differ across age groups (5–6, 7–11, and ≥12 years of age; Table 2). Pulmonary function, as measured by FEV_1% and PEF% before sputum induction, also did not differ among the 3 age groups.

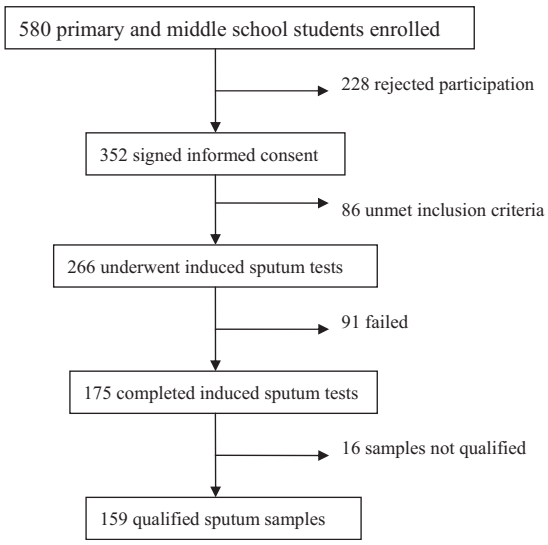

FIGURE 1
Subject enrollment. Of 558 primary and middle school students enrolled in the study, 228 rejected participation and 352 signed informed consent. There were a total of 266 subjects who underwent induced sputum test; of these subjects, 175 completed and 91 failed. Of the 175 subjects, 159 were included in the final statistical analysis, and 16 samples were discarded due to unmet qualifications of test results.

TABLE 1 Cell Types in Induced Sputum

Cell Type	Mean	Mean's 95% CI[a]	Median	IQR	2.5%–97.5% Percentile
Eos, %	1.73	1.25–2.20	0.39	1.93	0.00–6.50
Lym, %	1.86	1.54–2.18	1.22	2.04	0.00–5.00
Neu, %	31.09	26.61–35.56	20.67	33.03	4.00–92.75
Mac, %	65.31	60.71–69.92	76.14	32.68	1.00–94.50

Eos, eosinophils; lym, lymphocytes; mac, macrophages; neu, neutrophils.

[a] Given that the data are skewed, the mean and mean's 95% CI are provided for reference only. The mean's 95% CI was calculated by mean ± 1.96 × SD/square root(*n*).

TABLE 2 Induced Sputum Cytology According to Age

Characteristics	5–6 y of Age (35 Cases)	7–11 y of Age (90 Cases)	>12 y of Age (34 Cases)	F/χ^2	*P*
Age, y	6.67 ± 0.48	10.12 ± 1.06	14.00 ± 1.74	349.82	.000
Height, cm	122.19 ± 5.40	138.48 ± 7.92	156.03 ± 8.43	172.78	.000
Body weight, kg	21.85 ± 3.01	33.62 ± 8.60	47.73 ± 12.93	72.90	.000
FEV_1%	108.8 ± 10.6	106.2 ± 10.8	103.9 ± 11.8	1.722	.182
PEF% before induction	99.2 ± 10.6	99.0 ± 11.1	101.6 ± 13.3	0.672	.512
PEF% after induction	96.9 ± 14.4	94.7 ± 13.1	98.7 ± 16.3	1.083	.341
Eos, %					
Mean[a]	1.42	1.80	1.85	—	—
Median (IQR)	0.31 (0.84)	0.50 (1.71)	0.13 (2.09)	1.182	.554
Mean's 95% CI[a]	0.24–2.60	1.21–2.39	0.76–2.93	—	—
2.5%–97.5% percentiles	0.00–5.63	0.00–6.73	0.00–7.50	—	—
Lym, %					
Mean[a]	1.44	2.09	1.69	—	—
Median (IQR)	1.28 (1.23)	1.38 (2.43)	1.00 (2.07)	1.314	.518
Mean's 95% CI[a]	1.08–1.79	1.58–2.60	1.09–2.29	—	—
2.5%–97.5% percentiles	0.00–3.40	0.14–5.11	0.00–5.81	—	—
Neu, %					
Mean[a]	23.82	32.30	35.36	—	—
Median (IQR)	13.75 (18.53)	22.13 (33.5)	23.50 (58.50)	3.559	.169
Mean's 95% CI[a]	15.62–32.02	26.18–38.41	24.65–46.07	—	—
2.5%–97.5% percentiles	2.50–91.50	2.50–94.06	4.00–93.50	—	—
Mac, %					
Mean[a]	73.26	63.81	61.10	—	—
Median (IQR)	82.25 (17.16)	74.00 (31.58)	73.00 (60.50)	4.136	.126
Mean's 95% CI[a]	65.03–81.50	57.54–70.08	49.89–72.31	—	—
2.5%–97.5% percentiles	3.00–94.50	0.00–94.23	0.00–95.00	—	—

Eos, eosinophils; lym, lymphocytes; mac, macrophages; neu, neutrophils.

[a] Given that the data are skewed, the mean and mean's 95% CI are provided for reference only. The mean's 95% CI was calculated by mean ± 1.96 × SD/square root(*n*).

Neither cytologic results nor pulmonary function (FEV_1% and PEF% before sputum induction) differed significantly between both genders (Table 3). PEF% was statistically higher after successful sputum induction in boys than in girls ($P < .05$).

No participants in the study had a history of active smoking. The participants who had exposure to tobacco smoke at home ($n = 65$) were significantly younger than those who did not ($n = 94$). However, neither FEV_1% before sputum induction nor PEF% before and after sputum induction differed between the 2 groups ($P > .05$). Also, cytologic results did not differ between the 2 groups ($P > .05$ for all cell types; Table 4).

Safety Issues and Factors for Procedure Failure

FEV_1% was 106.3% ± 11.0% before sputum induction in the 159 participants who completed the eligible study. PEF% was 99.6% ± 11.5% and 96.0% ± 14.1% before and after induction, respectively. PEF reduction after sputum induction was noted in 84 (52.8%) out of the 159 subjects. In 4 cases, the reduction was >20%. A comparison of pulmonary function measures did not reveal significant difference between subjects who completed the test and those who did not. Also, there was no significant difference in the magnitude of PEF% reduction after sputum induction between these 2 groups ($P > .05$; Table 5). Adverse events were noted in 4.4% (7/159) of the participants who completed the eligible test and included severe cough ($n = 2$), dizziness ($n = 2$), flush ($n = 2$), and vomiting ($n = 1$). All these events dissipated within 30 minutes and required no specific treatments. In the 91 participants who did not complete the test, the procedural failure was due to severe cough in 58 cases (63.74%) and no sputum expectoration in 33 cases (36.26%). In 16 cases (6.02%, 16/266), sufficient amount of sputum was produced, but the sputum cell counts did not meet the criteria for data analysis. The overall failure rate was 40.23% (107/266). The failure rate was higher in girls than in boys ($P < .05$) but did not correlate with pulmonary function ($P > .05$).

DISCUSSION

Induced sputum cytology has been used in the diagnosis of pulmonary tuberculosis and lung cancers for over half a century. In 1992, Pin et al[7] employed cytologic study of induced sputum to investigate the airway inflammation in patients with asthma. Since then, induced sputum cytology has been widely used in clinical settings worldwide.[21,22] However, most of the studies on this topic in children used a limited sample of healthy controls.[12,23–27] Lack of normal reference values in children has, to some extent, prevented the use of cytologic examination of induced sputum in pediatric practice.

In a study of nonsmoking adults aged above 50 years, Pignatti et al[18] found that neutrophil is the predominant cell type in induced sputum, with a median of 58% and IQR of 26%, followed by

TABLE 3 Induced Sputum Cytology According to Gender

Characteristics	Boy (92 Cases)	Girl (67 Cases)	*t*/*Z*	*P*
Age, y	10.22 ± 2.86	10.15 ± 2.96	.158	.875
Height, cm	139.58 ± 13.82	137.36 ± 12.96	1.026	.307
Body weight, kg	35.18 ± 13.09	32.50 ± 11.09	1.357	.177
FEV_1%	105.6 ± 10.7	107.1 ± 11.5	.845	.399
PEF% before induction	98.2 ± 10.3	101.5 ± 12.8	1.806	.073
PEF% after induction	93.7 ± 13.6	99.3 ± 14.2	2.537	.012
Eos, %				
Mean[a]	1.99	1.36	—	—
Median (IQR)	0.38 (2.44)	0.50 (2.36)	.82	.412
Mean's 95% CI[a]	0.68–2.05	1.34–2.64	—	—
2.5%–97.5% percentiles	0.00–8.14	0.00–5.56	—	—
Lym, %				
Mean[a]	1.99	1.68	—	—
Median (IQR)	1.50 (1.99)	1.00 (2.12)	1.170	.242
Mean's 95% CI[a]	1.51–2.47	1.28–2.08	—	—
2.5%–97.5% percentiles	0.00–6.09	0.00–6.45	—	—
Neu, %				
Mean[a]	30.21	32.29	—	—
Median (IQR)	20.38 (25.37)	21.00 (37.57)	.262	.794
Mean's 95% CI[a]	24.59–35.83	24.83–39.75	—	—
2.5%–97.5% percentiles	3.33–95.03	2.20–96.03	—	—
Mac, %				
Mean[a]	65.79	64.65	—	—
Median (IQR)	76.80 (27.00)	77.00 (38.38)	.534	.594
Mean's 95% CI[a]	60.03–71.56	56.94–72.36	—	—
2.5%–97.5% percentiles	2.48–92.59	0.00–94.00	—	—

Eos, eosinophils; lym, lymphocytes; mac, macrophages; neu, neutrophils.

[a] Given that the data are skewed, the mean and mean's 95% CI are provided for reference only. The mean's 95% CI was calculated by mean ± 1.96 × SD/square root(*n*).

macrophages, with a median of 37% and IQR of 24%. They also noted higher neutrophils and lower macrophages percentages with increasing age. Gibson et al[23] carried out cytologic examination of the sputum induced by hypertonic saline in 72 healthy children but did not correlate the findings with age.

Results from the current study demonstrated that macrophages accounted for ~75% of the cells. The next highest cell type was neutrophils accounting for ~20%. The cell type did not correlate with gender, age, or passive smoking.

Increased eosinophils in induced sputum is one of the most relevant indicators for airway inflammation and an important clue toward diagnosis of asthma and/or eosinophilic bronchitis. The Chinese Guidelines for Diagnosis and Treatment of Childhood Asthma listed increased eosinophils (≥4% in peripheral blood or induced sputum) as one of the secondary risk factors for asthma in young children (<5 years of age) with repeated episodes of coughing.[10] The European Respiratory Society set the upper limit of the normal value of eosinophils in the induced sputum in adults at 2.5%. The upper limit of eosinophils in induced sputum in adults was similarly set at 2% by the Guangzhou Institute of Respiratory Diseases.[7–10,14,24–29] In the current study, the median of eosinophils in induced sputum was 0.39%; IQR, 1.93%; 2.5% to 97.5% percentile between 0.00% and 6.50%; and the upper limit of mean's 95% CI, 2.20%. Such findings were generally consistent with the results in the adult population.[10] However, the cell count of eosinophils in induced sputum was ≥4% in 18 (11.32%) participants in the current study. The critically high level of eosinophils in this small percentage of healthy children, when pooled in the final calculation, may have conceivably increased the 97.5% percentile and upper limit of mean's 95% CI. This may arise from the potential enrollment of children with subclinical atopy due to absence of laboratory allergen tests and skin prick tests in screening for healthy subjects in our study.

Airway hyperresponsiveness may be induced with inhalation of nebulized hypertonic saline. In a study of 304 adult patients with asthma by Vlachos-Mayer et al,[30] FEV_1 was reduced by ≥20% in 8% of patients, and recovered to the baseline level within 30 minutes after salbutamol treatment. In a study of 93 patients with severe asthma by Brinke et al,[31] FEV_1 reduction by >20% occurred in 22% of the patients. The authors of previous studies have indicated that hypertonic saline sputum induction is safe in children aged 6 years or older, even in those with moderate/severe asthma and acute asthma attack.[21,32] Data with regards to children at <6 years of age are limited[23,24,33,34] but seem to indicate that sputum induction is safe and could achieve a reasonable success rate. The overall success rate in the current study was significantly lower than that obtained in an adult population and in children with asthma. The lower rate of success may reflect lack of inflammation in the airway and the resulting secretion in the healthy children. Lack of will (due to the healthy status) may have also contributed to the relatively low success rate. Our results indicated that the success rate was higher in boys than in girls. Also, the success rate increased with age. Interestingly, the success rate did not correlate with pulmonary function.

Sputum induction in the adult population generally starts with 3% saline in a stepwise concentration increment manner (up to 5%). Such a conventional protocol requires good compliance from the study subjects. Given the difficulty to obtain an equivalent level of

TABLE 4 Induced Sputum Cytology According to Passive Smoking Status

Characteristics	Nonpassive Smoking Group (94 Cases)	Passive Smoking Group (65 Cases)	*t*/*Z*	*P*
Age, y	10.62 ± 3.07	9.57 ± 1.85	2.458	.015
Height, cm	141.0 ± 15.0	135.3 ± 10.2	2.698	.008
Body weight, kg	36.33 ± 14.0	30.8 ± 8.6	2.821	.005
FEV_1%	105.2 ± 10.6	107.9 ± 11.5	1.535	.127
PEF% before induction	100.5 ± 12.3	98.2 ± 10.2	1.233	.220
PEF% after induction	97.2 ± 14.3	94.5 ± 13.9	1.185	.238
Eos, %				
Mean[a]	1.74	1.70	—	—
Median (IQR)	0.25 (1.83)	0.50 (1.97)	.336	.737
Mean's 95% CI[a]	1.12–2.37	0.97–2.43	—	—
2.5%–97.5% percentiles	0.00–6.63	0.00–6.05	—	—
Lym, %				
Mean[a]	1.94	1.74	—	—
Median (IQR)	1.25 (1.95)	1.00 (2.12)	.320	.749
Mean's 95% CI[a]	1.46–2.43	1.36–2.13	—	—
2.5%–97.5% percentiles	0.00–6.156	0.00–5.88	—	—
Neu, %				
Mean[a]	30.06	32.57	—	—
Median (IQR)	19.88 (28.87)	21.75 (49.19)	.212	.832
Mean's 95% CI[a]	24.34–35.78	25.23–39.92	—	—
2.5%–97.5% percentiles	4.00–93.13	2.15–94.38	—	—
Mac, %				
Mean[a]	66.24	63.97	—	—
Median (IQR)	76.5 0 (29.00)	75.00 (47.75)	.363	.717
Mean's 95% CI[a]	60.28–72.20	56.54–71.40	—	—
2.5%–97.5% percentiles	0.00–94.00	0.50–96.35	—	—

Eos, eosinophils; lym, lymphocytes; mac, macrophages; neu, neutrophils.

[a] Given that the data are skewed, the mean and mean's 95% CI are provided for reference only. The mean's 95% CI was calculated by mean ± 1.96 × SD/square root(*n*).

TABLE 5 Factors That Influence the Success Rate

Characteristics	Success (159; 59.77%)	Failure (91; 34.21%)	Disqualified Sputum Sample (16; 6.02%)	F/χ^2	*P*
Age, y	10.19 ± 2.68	9.20 ± 2.77	9.38 ± 2.90	4.031	.019
Height, cm	138.7 ± 13.5	133.0 ± 14.3	133.9 ± 16.5	5.021	.007
Body weight, kg	34.05 ± 12.32	30.12 ± 11.07	31.21 ± 11.91	3.228	.041
Boy, %	92 (57.9)	36 (39.6)	9 (56.3)	7.914	.019
Girl, %	67 (42.1)	55 (60.4)	7 (43.8)	—	—
FEV_1%	106.3 ± 11.0	107.9 ± 14.3	107.1 ± 16.7	.507	.603
PEF% before induction	99.6 ± 11.5	101.44 ± 16.1	101.1 ± 19.4	.569	.567
PEF% after induction	96.0 ± 14.1	100.5 ± 17.5	97.5 ± 18.2	2.385	.094
Percentage PEF reduction	84 (52.8)	36 (39.6)	7 (43.8)	4.194	.123
Number (%) of participants with >20% PEF reduction	4 (2.5)	4 (4.4)	0 (0)	1.084	.581

compliance in healthy children, we adopted a different protocol and used 5% saline throughout the test in an attempt to increase the chance of success upon first pass. Under this modified protocol, the PEF% was seemingly reduced after sputum induction compared with baseline, but the difference was not significant. Adverse events occurred at a rate of 4.4% (7/159) but did not require specific treatments. These results indicated that sputum induction starting from 5% saline can be safe and feasible in children.

The duration of inhalation is an important variable in sputum induction. Studies have revealed the changing cytology along the course of sputum induction.[35,36] These studies revealed that neutrophils and eosinophils are prominent in samples collected early during sputum induction, whereas lymphocyte and macrophage counts are increased in samples collected later. Owing to potentially less compliance and weak tolerance in children, we did not strictly standardize the induction time. Instead, the sputum was collected every 10 minutes during the 30-minute induction and was mixed to obtain a relatively balanced sample from each subject. Although this was not an ideal solution, it may help reduce the significant variation in cell counts arising from nonstandardized induction time among the children.

We acknowledge limitations of this study in several aspects. Firstly, the postinduction FEV_1 (but not PEF) data in these children were missing because of difficulties in completing an additional spirometry after PEF measurement after sputum induction in the children. Secondly, the lack of the allergen test and the bronchial provocation test in screening for healthy subjects may result in potential enrollment of children with allergic diseases and lead to a bias in statistics. Thirdly, because only healthy Guangzhou children aged between 5 and 16 years were enrolled in this study, the profiles of those aged below 5 years remain to be explored in future studies with multicenter collaboration, larger sample size, and wider age distribution. Nevertheless, this study represents the first successful attempt to establish the normative reference range of induced sputum cytology in Chinese children. As such, the findings are valuable for future studies.

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Reference Values of Induced Sputum Cytology in Healthy Children in Guangzhou, Southern China

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