

PEDIATRIC ASTHMA

A new index to identify risk of multi-trigger wheezing in infants with first episode of wheezingYuhe Zhang, MD¹, Chunju Zhou, MD², Jinrong Liu, MD³, Haiming Yang, PhD³, and Shunying Zhao, PhD³¹Department of Pediatrics, Beijing Renhe Hospital, Beijing, China, ²Department of Pathology, and ³Department of Respiratory Disease, Beijing Children's Hospital affiliated to the Capital Medical University, Beijing, China**Abstract**

Objective: Identification of young children who are likely to have multi-trigger wheezing is very important for early diagnosis and treatment of asthma. We investigate an index for predicting multi-trigger wheezing in infants with first episode of wheezing. **Methods:** One-hundred twenty-eight infants (2–20 months) with first episode of wheezing were followed for two years. Personal and family history of atopic diseases was recorded. Wheezing severity was evaluated using the Preschool Respiratory Assessment Measure. Sputum samples were collected from patients, stained with hematoxylin and eosin and studied by optical microscopy. The largest Creola body in sputum was located and the number of shed exfoliated airway epithelial cells (EAECs) counted. Recurrent wheezing was observed and classified as multi-trigger wheezing or non-multi-trigger wheezing. The predictive value of EAECs, family or personal history of atopic disease and the severity of wheezing for subsequent development of multi-trigger wheezing was analyzed. **Results:** Better predictive performance was achieved by considering the three measures together than by considering each separately. Receiver operator characteristic analysis showed that an index combining wheezing severity score of 9495 sputum EAECs and a family or personal history of atopic disease had a sensitivity of 95.1%, specificity of 74.2%, a positive predictive value of 58.6% and a negative predictive value of 93.6% for prediction of multi-trigger wheezing. **Conclusion:** For infants with first episode of wheezing, wheezing severity score, family or personal history of atopic disease and number of EAECs in sputum can predict future multi-trigger wheezing.

Keywords

Diagnosis, pediatrics, phenotypes, wheezing

History

Received 25 January 2014

Revised 5 June 2014

Accepted 15 June 2014

Published online 3 July 2014

Introduction

Identification of young children who are likely to have asthma is very important for the development of strategies for early intervention. The first episode of wheezing in infants is usually triggered by a viral infection. It may represent a single viral-induced event that does not recur or it may represent the start of an unremitting process that eventually results in asthma [1]. Therefore, in treating the first episode of wheezing in infants, it is critical to identify and treat children with unremitting wheezing or multi-trigger wheezing. Several studies have investigated the possible predictive factors for asthma. These include a parental history of asthma, history of atopic dermatitis, eosinophil granulocyte counts, rhinovirus infection, genetic polymorphisms, cytokine levels and elevated levels of exhaled nitric oxide [2–6]. However, these predictors do not target infants with first episode of wheezing. Previously, we focused on the relationship between cell types

in sputum and different prognoses in pediatric patients with bronchiolitis. We found that wheezing severity, personal and/or family history of atopic disease and the number of exfoliated airway epithelial cells (EAECs) in sputum were associated with the development of multi-trigger wheezing. To verify these findings, we determined the values of these indicators for predicting multi-trigger wheezing in 128 infants with first episode of wheezing.

Methods

The study protocol was approved by the ethics committee of Beijing Children's Hospital. Written informed consent was obtained from the parents of each patient.

Patients

One-hundred sixty-two patients (2–20 months) with first episode of wheezing were enrolled and evaluated during peak periods of respiratory syncytial virus (RSV) infection in Beijing, China. All patients were confirmed to have wheezing by auscultation. Thirty of 162 patients were confirmed to have RSV infection in a sentinel sampling/detection arranged by Centers for Disease Control and Prevention in China.

Correspondence: Shunying Zhao, Beijing Children's Hospital affiliated to the Capital Medical University, 56 Nan Li Shi Road, Xi Cheng District, Beijing 100045, China. Tel: +86-13661328133; +86-10-59612314. Fax: +86-10-59610836. E-mail: zhaoshunying2001@163.com

We investigated different causes of wheezing. For 21 of 162 children with biphasic wheezing or stridor, abnormally severe obstruction and prolonged recovery (more than two weeks), we did flexible bronchoscopy and found nine children had laryngomalacia or laryngotracheomalacia, two children had subglottic stenosis, one child had foreign body and three children had compression. Abnormal vascular ring were seen in these three children with compression through enhanced chest CT and echocardiography examination. Among the remaining 6 of these 21 children, two were diagnosed with persistent bacterial bronchiolitis, two with gastroesophageal reflux disease, one with primary immunodeficiency and one with bronchopulmonary dysplasia based on abnormal chest X-ray, positive sputum culture and response to antibiotics, immune examination or 24 h pH detection, barium swallowing and response to anti-reflux. Due to the conditions described above, these 21 patients were excluded after enrollment. The remaining 141 patients with first episode of wheezing were diagnosed with virus-induced wheezing or bronchiolitis and recruited in this study.

Personal and family (parents, grandparents and sibling) history of atopic diseases (asthma, rhinitis, allergic dermatitis (eczema) and food allergy) was recorded. The severity of wheezing was evaluated using the Preschool Respiratory Assessment Measure scoring scale was used. In this scale, a score of <5 represents mild wheezing, 5–8 represents moderate wheezing and 9–12 represents severe wheezing [7].

Of the 141 patients recruited, 13 were lost to follow-up and 128 were followed-up at outpatient and inpatient visits for two years after the initial wheezing episode. The following conditions were examined during follow-up: reoccurrence of wheezing, severity and frequency of wheezing, wheezing trigger, personal and family history of atopic diseases and related diagnoses and treatments. Recurrent wheezing was defined as ≥ 3 episodes of wheezing. Two general patterns of recurrent wheezing were considered: multi-trigger wheezing (also called unremitting wheezing) and episodic wheezing [1,8].

Multi-trigger wheezing was defined as recurrent wheezing, with intermittent symptoms between episodes such as coughing and wheezing at night, in response to exercise, crying, laughter, passive smoking or exposure to allergens, and with positive family history of atopic disease or personal atopic dermatitis or rhinitis, reversibility by bronchodilators and response to maintenance treatment with inhaled corticosteroids or leukotriene-receptor antagonists.

Episodic wheezing was defined as recurrent wheezing in response to viral infection, with no intermittent symptoms between episodes and with gradual decrease in wheezing.

Three children with episodic wheezing developed atopic rhinitis with positive allergen response and intermittent wheezing between episodes during follow-up. These three patients were re-classified as having multi-trigger wheezing.

Sputum collection

Patients were admitted to the inpatient department. Sputum samples were collected with sterile suction catheters (gauge number 6; Jingle Polymer Medical Devices, Suzhou, China) during coughing because coughing drives sputum up to the

trachea. The suction device comprised a two-way tube with a trap. One end of the tube was connected to an aspirator. Smears of sputum were spread onto glass slides and fixed with 95% ethanol. Samples were numbered. All sputum specimens were collected 1–2 days after the episode of wheezing.

Evaluation of Creola bodies and EAECs in sputum

Sputum samples were stained with hematoxylin and eosin and examined by a clinical pathologist under an optical microscope. The pathologist was blinded to the clinical information. If a sample was observed to contain >5% squamous epithelial cells without macrophages, it was believed to be collected from the upper respiratory tract and was excluded from the analyses. A sample containing macrophages and <5% squamous epithelial cells was considered to be collected from the lower respiratory tract and was analyzed.

Desquamated columnar epithelial cells are distributed in clusters called Creola bodies in the sputum [9]. Creola bodies were identified by their distinct shapes: spherical, S-, rectangular and striped (Figure 1A–E). Creola bodies are typically composed of densely arranged epithelial cells with some cytoplasmic vacuoles. Some epithelial cells also have surface cilia with curved tips that are arranged into “fence-like” structures. The largest Creola bodies in each sample were selected, and the number of shed epithelial cells counted manually and by automated image analyses.

The number of EAECs in sputum samples from the 128 subjects ranged from 51 to 1456. 39 samples had less than 100 EAECs; 14 had 100–200 EAECs; 38 had 201–500 EAECs; 24 samples had 501–1000 EAECs; and 13 samples had more than 1001 EAECs.

Statistical analyses

Data were processed using SPSS v17.0 (SPSS, Chicago, IL). Age, day of illness, wheezing severity and number of sputum EACAs are presented as the mean $\pm$ SD. Measurement data were compared using the independent samples *t*-test. Count data were compared using the chi-square test. Multivariable logistic regression analysis was performed using a stepwise procedure to determine factors most predictive of multi-trigger wheezing. The receiver operator characteristic (ROC) curve was analyzed with the wheezing severity score, history of allergy and number of EAECs as well as the predictive index (based on the combination of these three indicators). The area under the curve (AUC) was compared with MedCalc software. $p < 0.05$ in the two-sided test was considered significant. The values of predictive index for prediction of multi-trigger wheezing were calculated in terms of sensitivity, specificity, positive predictive value and negative predictive value with 95% confidence intervals.

Results

Clinical characteristics and follow-up data

Of the 128 patients in this study, 20 had only a personal history of atopic-related disease, 29 had only a family history of atopic disease and 10 had both family and personal history of atopic disease. 11 cases of wheezing were mild,

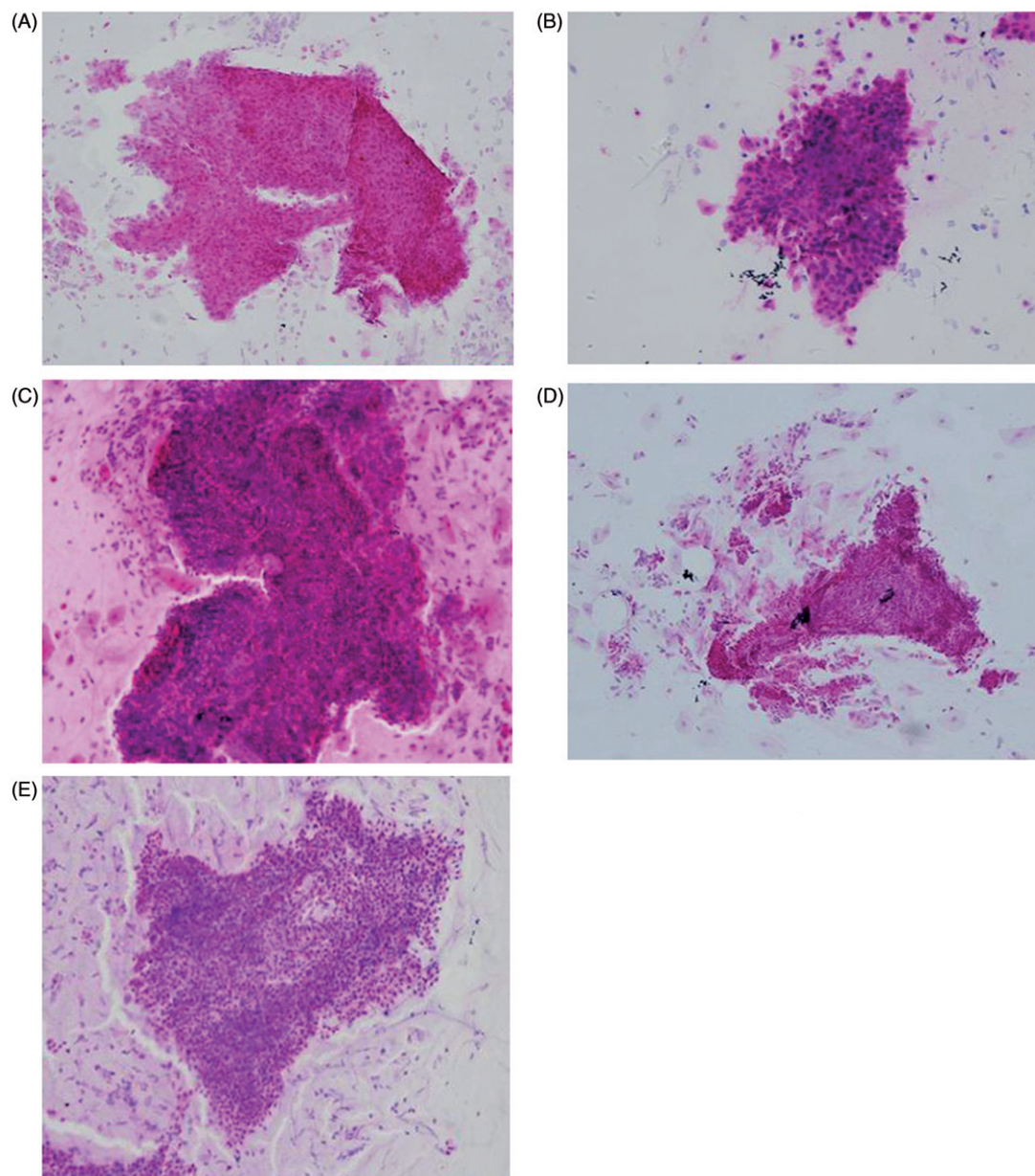


Figure 1. (A–E) Different shapes and sizes of Creola bodies and different number of shed epithelial cells were seen.

64 moderate and 53 severe. Multi-trigger wheezing was noted in 35 patients and episodic wheezing in 43 patients. Fifty patients did not experience recurrent wheezing. Since subjects with episodic wheezing and subjects with no recurrent wheezing had similar baseline characteristics with respect to factors studied (age, gender, wheezing severity, number of EAECs, family or personal history of atopic disease), these two groups of subjects were combined to form the control, non-multi-trigger wheezing group.

Correlation of wheezing severity score, family or personal history of atopic disease and number of EAECs with multi-trigger wheezing

The gender distribution, age and duration of illness showed no significant differences between the multi-trigger and non-multi-trigger groups (all $p > 0.05$). However, the wheezing severity score, family or personal history of atopic disease and

number of EAECs in sputum were significantly different ($p < 0.05$) (Table 1).

Logistic regression analysis was conducted with age, gender, wheezing severity score, history of atopic disease and number of EAECs as independent variable and multi-trigger wheezing as dependent variable. As no significant difference was found in age and gender, these indexes were excluded from the logistic regression equation. Statistical difference was detected in the wheezing severity score, history of atopic disease and number of EAECs. Therefore, these factors were included and were used for establishment of the logistic regression model (Table 2). A likelihood ratio test was undertaken for this logistic regression model. $p < 0.05$ was regarded as the regression model that elicited a better fit to the data.

The results showed that the combination of three indicators produced a significantly larger AUC than the wheezing severity score ($Z = 2.584$, $p = 0.0098$), personal or family

Table 1. Wheezing severity, history of atopic disease, and the number of EAECs in subjects with and without multi-trigger wheezing.

Group	Multi-trigger (n = 35)	Non multi-trigger (n = 93)	χ^2 or z	p
Positive family or personal history of atopic disease	80%	33.33%	22.288	0.000
Gender				
Male	69.69%	68.57%	0.070	0.791
Female	30.30%	31.42%		
Age (months)	7.62 ± 3.85	7.87 ± 4.04	0.306	0.760
Duration of illness (d)	4.02 ± 1.93	4.00 ± 1.65	−0.083	0.934
Severity score	9.28 ± 1.40	6.96 ± 2.03	−5.713	0.000
EAEC	802.62 ± 428.05	274.52 ± 230.5	−5.957	0.000

* $p < 0.05$

EAEC: exfoliated airway epithelial cells.

Positive family history of atopic diseases is defined as parents, grandparents or siblings having one of the following conditions: asthma, rhinitis, allergic dermatitis or food allergy. Positive personal history of atopic diseases is defined as the patient having one of the following conditions: rhinitis, allergic dermatitis or food allergy.

Table 2. Logistic regression analysis of association between multi-trigger wheezing and the disease severity, history of atopic disease and the number of EAECs (adjusted age and gender).

variable	Severity score	History of atopic disease	EAECs	Intercept
Regression coefficient	0.512	1.624	0.002	−6.972
Standard error	0.231	0.551	0.001	1.851
Wald	4.936	8.697	4.166	14.193
p	0.026	0.003	0.041	0.000
OR	1.669	5.073	1.002	0.001
95% CI	1.062–2.623	1.724–14.926	1.000–1.004	

Positive family history of atopic diseases is defined as parents, grandparents or siblings having one of the following conditions: asthma, rhinitis, allergic dermatitis or food allergy. Positive personal history of atopic diseases is defined as the patient having one of the following conditions: rhinitis, allergic dermatitis or food allergy.

history of atopic disease ($Z = 3.630$, $p = 0.0003$) and number of EAECs ($Z = 1.983$, $p = 0.0474$) (Figure 2). Therefore, the combination of these three indicators was used as a predictive index.

Determining the optimal predictive index for multi-trigger wheezing

The sensitivities and specificities of the ROC curves of the indicative index (pre-1) at different cutoff points were analyzed. A point with the largest value of the Youden index was chosen as the optimal cutoff point. The optimal cutoff value of the predictive variable (pre-1) was 0.1857 (Table 3), which represents a wheezing severity score of 9, 495 EAECs and positive personal or family history of atopic-related disease.

Discussion

In this study, we showed that wheezing severity score, family or personal history of atopic disease and number of EAECs in

sputum can be used to predict whether a child who experiences a first episode of wheezing by two years of age will have multi-trigger wheezing in the future.

It has been reported that the severity of obstructive airway disease at two years of age predicts asthma at 10 years of age [10]. Kusel et al. demonstrated that infections with human rhinovirus and RSV were associated with an increased risk of subsequent asthma [11]. This effect was observed in children with an onset of allergic sensitization before two years of age. Our findings further demonstrate a correlation between the degree of airway obstruction in children younger than two years of age and multi-trigger wheezing later in childhood, and a correlation between family or personal history of atopic disease and future multi-trigger wheezing.

Castro-Rodriguez et al. proposed an approximate index for predicting the probability of infants to exhibit asthma at school age [2] based on two major criteria: patient eczema and parental asthma. In this study, among subjects who exhibited multi-trigger wheezing, only four had parental history of asthma and only six had eczema. However, almost half of these subjects had parental history of rhinitis or eczema, or grandparental history of asthma, rhinitis or eczema. This suggests that not only parental but also grandparental history of rhinitis and eczema as well as history of asthma can influence the development of multi-trigger wheezing in children. Asthma, rhinitis and eczema arise through similar mechanisms. Epigenetic regulation, including gene methylation, plays an important role in the development of asthma. Since gene methylation can be passed down over several generations, the value of multigenerational family history in predicting multi-trigger wheezing may be due, in part, to epigenetic regulation of asthma and related diseases.

Exfoliation of airway epithelial cells is a pathologic feature of asthma. Yamada et al. [12] were the first to report that the number of EAECs in sputum can predict asthma. They observed that sputum samples from 65% of infants (age, 1–10 months) with more than one episode of wheezing had Creola bodies, and that 80% of infants with Creola bodies developed asthma during the subsequent two-year follow-up. In contrast, no infant without Creola bodies developed asthma in the same period. Recently, Yamada and Yoshihara [13] reported that Creola bodies were detected in 23 of 33 subjects with RSV (69.7%). The prevalence of recurrent wheezing in subjects aged ≤ 2 years was 92.8% for the RSV-Creola bodies group and 30.0% for the RSV-non-Creola bodies group. Furthermore, the development of asthma in subjects aged ≤ 5 years was 70.0% for the RSV-Creola bodies group and 10.0% for the RSV-non Creola bodies group.

In contrast with the study reported by Yamada, in this study we found that the sputum samples of every subject with first episode of wheezing contained Creola bodies, and that subsequent multi-trigger wheezing was correlated with the size of Creola bodies (i.e. the number of EAECs within them), but not the presence or absence of Creola bodies. These conflicting results may be due, in part, to differences in timing of sputum collection. We have observed that the shedding of epithelial cells in sputum occurs mainly in the first five days of illness, particularly within three days of initial wheezing (data not shown), and decreases or disappears

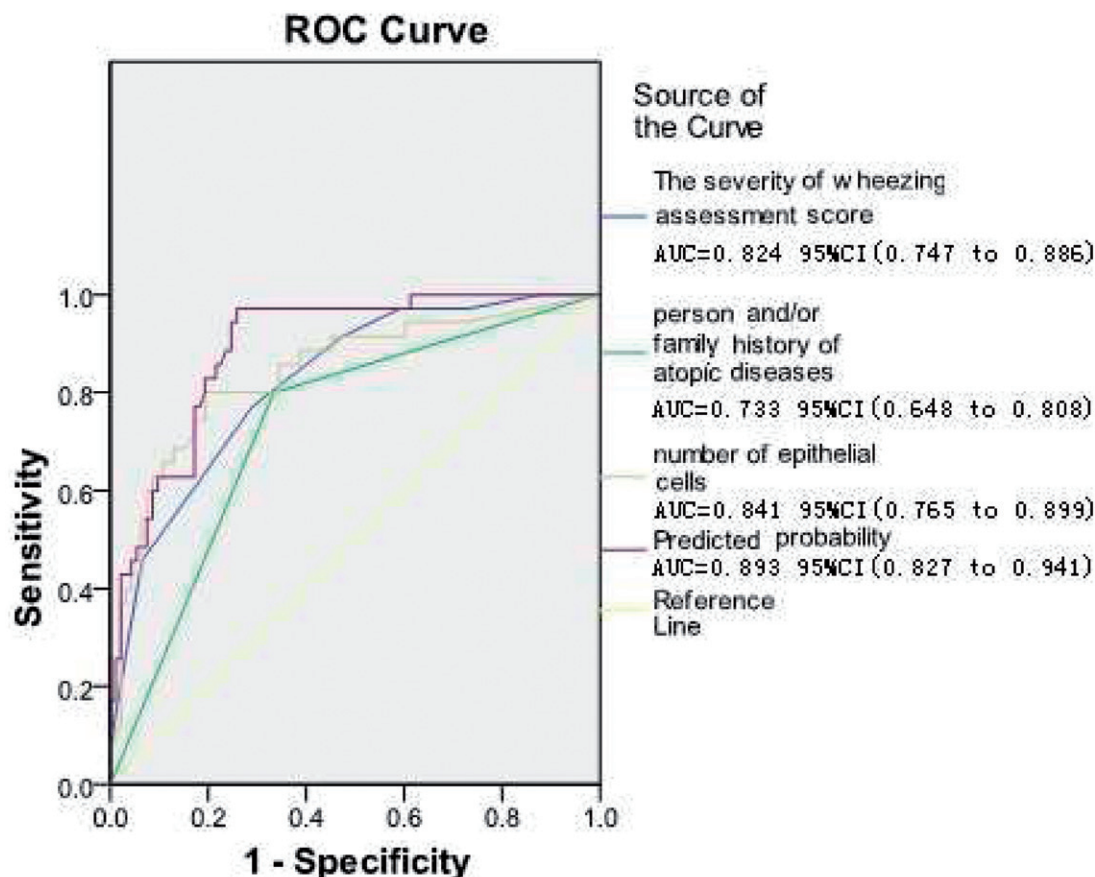


Figure 2. ROC curve analysis. Sensitivity and specificity values were plotted for three potential predictors of multi-trigger wheezing (severity of wheezing, person and/or family history of atopic disease and number of epithelial cells) separately and together (predicted probability). Area under the curve (AUC) is shown with 95% confidence intervals.

Table 3. Optimal cutoff points of the predictive variable (pre-1).

Variable	Optimal cutoff	OR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Pre-1	0.1857	97.75 (12.68–753.7)	95.1% (85.08–97.93)	74.2% (64.08–82.71)	58.56% (44.74–71.46)	93.55% (92.27–96.96)

The optimal cutoff point of the predictive variable (pre-1) represents those infants with a wheezing severity score of 9, exfoliated airway epithelial cells in sputum of 495 and a family or personal history of atopic related disease.

afterwards. In this study, sputum specimens were collected two days after the onset of wheezing, a time point at which a large number of EAECs are present.

Our finding that sputum samples from all subjects in this study contained Creola bodies suggests that epithelial shedding is common in virus-induced wheezing. Airway epithelial cells are the first line of defense against invading microbes. Apoptosis or death can be elicited in the epithelium as a response to viral infection, leading to injury and shedding. However, this does not explain why subjects who later exhibited multi-trigger wheezing had significantly higher numbers of EAECs in their sputum. The work of Holgate et al. [14] may provide an answer. They found that the airway epithelium is defective in patients with asthma, and is unable to produce adequate interferon- γ to suppress virus replication. As a consequence, host sensitivity to viruses and virus cytotoxicity increases, leading to more extensive cell death.

While sputum EAEC number adds to the predictive value of the index proposed in this study, sputum collection and determination of EAEC number in each patient with first episode of wheezing increases the medical expense and complexity of the prediction. In addition, these procedures may be difficult to perform in outpatient clinic. On the other hand, since severity of wheezing is correlated to multi-trigger wheezing later, children with severe wheezing often require hospitalization, earlier identification and treatment of children likely to have unremitting wheezing may reduce the frequency and severity of exacerbation and hospitalization. Given that determination of sputum EAEC number added to the predictive value for multi-trigger wheezing, and that the procedure is relatively inexpensive and noninvasive, the effect-cost ratio of determining EAEC number in these hospitalized children has obvious advantage. We recommend that sputum EAEC number be determined in children hospitalized due to severe wheezing.

Conclusions

Our results demonstrate that a new index combining wheezing severity, personal and family history of atopic diseases and number of EAECs in sputum can predict the risk of having multi-trigger wheezing in infants with first episode of wheezing with good sensitivity, specificity and positive and negative predictive value. The use of this index may allow earlier identification and treatment of pediatric patients likely to have unremitting wheezing.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

The funding for research was from Beijing Children's Hospital.

References

1. Frey U, von Mutius E. The challenge of managing wheezing in infants. *N Engl J* 2009;360:2130–2133.
2. Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000;162:1403–1406.
3. Walton RP, Johnston SL. Role of respiratory viral infections in the development of atopic conditions. *Curr Opin Allergy Clin Immunol* 2008;8:150–153.
4. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev* 2008;8:169–182.
5. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004;170:175–180.
6. Latzin P, Kuehni CE, Baldwin DN, Roiha HL, Casaulta C, Frey U. Elevated exhaled nitric oxide in newborns of atopic mothers precedes respiratory symptoms. *Am J Respir Crit Care Med* 2006;174:1292–1298.
7. Ducharme FM, Chalut D, Plotnick L, Savdie C, Kudirka D, Zhang X, Meng L, McGillivray D. The Pediatric Respiratory Assessment Measure: a valid clinical score for assessing acute asthma severity from toddlers to teenagers. *J Pediatr* 2008;152:476–480.
8. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, de Blic J, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008;32:1096–1110.
9. Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962;17:69–73.
10. Devulapalli CS, Carlsen KC, Haland G, Munthe-Kaas MC, Pettersen M, Mowinkel P, Carlsen KH. Severity of obstructive airways disease by two years predicts asthma at 10 years of age. *Thorax* 2008;63:8–13.
11. Kusel MM, de Klerk NH, Keadze T, Vohma V, Holt PG, Johnston SL, Sly PD. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;119:1105–1110.
12. Yamada Y, Yoshihara S, Arisaka O. Creola bodies in wheezing infants predict the development of asthma. *Pediatr Allergy Immunol* 2004;15:159–162.
13. Yamada Y, Yoshihara S. Creola bodies in infancy with respiratory syncytial virus bronchiolitis predict the development of asthma. *Allergol Int* 2010;59:375–380.
14. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005;201:937–947.