Recurrent wheezing after bronchiolitis caused by respiratory syncytial virus in children younger than 3 years: A 1-year follow-up study

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Abstract
We analysis the frequency and risk factors of wheezing in infants. We chose children with initial wheezing before 3 years of age who were hospitalized for medical treatment. Wheezing frequency was determined by follow-up at 1 week, 1 month, 3 months, 6 months, and 1 year. Information such as birth status, age, sex, preterm, mode of delivery, birth order, eczema history, personal allergy history, family allergy history, passive smoking, and place of residence (urban/rural) was collected. Total serum IgE level, serum allergen testing, routine blood tests, C-reactive protein level, procalcitonin level, respiratory pathogens tests, sputum culture, chest radiography or computed tomography were performed in all patients. The correlation between each factor and wheezing recurrence was evaluated. A total of 259 children were included in the study. They were divided into single recurrence, multiple recurrences, and no recurrence groups. The recurrence rate of wheezing was 56.8% (30.5% had a single recurrence and 26.3% had 2 or more recurrences). The percentage of children with a personal allergy history in the multiple recurrences group was significantly higher than in the single recurrence and no recurrence groups ($P = 0.031$ and $0.008$, respectively). The age of the children in the multiple recurrences group was significantly lower than that in the single recurrence group ($P < 0.001$). Clinical severity scores were higher in the multiple recurrences group than in the single recurrence and no recurrence groups ($P = 0.002$ and $<0.001$, respectively). Most children did not experience multiple recurrent wheezing. Children with young age, serious condition, and allergic constitution were prone to recurrent wheezing.

Keywords
follow-up study, respiratory syncytial virus, wheezing

Introduction
About half of children experience at least one wheeze before school age, and some of the infants with acute bronchiolitis can develop into recurrent wheezing. Viral infection is the most common cause of wheezing in infants and young children, with human respiratory syncytial virus (RSV) being the most common cause. RSV infection can cause irreversible lung damage and increase the incidence of chronic lung disease in adulthood. Prospective epidemiologic studies of those with lower respiratory tract infections caused by RSV in early life have demonstrated subsequent asthma and airway hyper-responsiveness rates 25% to 80% greater than in uninfected controls up to 11 years later. But the incidence of wheezing...
may be related to multiple factors, such as maternal conditions, personal fitness, and environmental factors. An outbreak of unusual respiratory disease causing severe cases of pneumonia initially in Wuhan, was caused by the 2019 novel coronavirus disease (COVID-19) which rapidly spread throughout the world. However, there are few follow-up studies on recurrent wheezing in children during the COVID-19 pandemic. The frequency and risk factors of recurrent wheezing were analyzed in this study.

**Material and methods**

**Study population**

It is a study on course of RSV bronchiolitis by follow-up. We included children with an initial wheezing episode under 3 years old who were hospitalized for medical treatment and who were diagnosed with bronchiolitis caused by RSV in the Maternal and Child Health Hospital in Ganzhou from October 2019 to December 2019. The patients were followed up from December 2019 to December 2020. Qualified pediatricians identified bronchiolitis and its clinical severity, and they collected clinical information confidentially.

**Data collection**

Information pertaining to birth history, age, sex, prematurity, mode of delivery, birth order, eczema history, personal allergy history, family allergy history, passive smoking, and place of residence (urban/rural) was collected. Studies including total serum immunoglobulin E (IgE) level, serum allergen testing, routine blood testing, C-reactive protein level, procalcitonin level, testing for 11 antibodies against respiratory pathogens, nasopharyngeal aspirate testing, sputum culture, chest radiography or computed tomography, echocardiography, and color Doppler echocardiography were performed in all patients.

The patients were followed up at 1 week, 1 month, 3 months, 6 months, and 1 year. During the COVID-19 pandemic, information was collected mainly by telephone. The follow-up information involved the duration of additional wheezing episodes, what induced the wheezing, re-hospitalization, duration of re-hospitalization, prescriptions, comorbidities, recurrence of wheezing, cost of re-hospitalization, etc.

**Disease severity scoring criteria**

We used scoring criteria to evaluate bronchiolitis severity according to the report by Luo et al. Infants who had clinical scores ≥9 were considered to have severe bronchiolitis. Details are shown in Table 1.

**Specimen collection and virus detection**

Using a standardized technique, nasopharyngeal aspirate samples were collected with disposable sterilized cotton swabs. Viral DNA and RNA were extracted from 200-μL aliquots of the nasopharyngeal aspirate samples using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). RNA was applied as the template for cDNA synthesis using the SuperScript III First-Strand Synthesis System (Invitrogen, CA, USA). The DNA and RNA extractions and cDNA products were used for the subsequent testing of 11 respiratory viruses. The 11 respiratory viruses detected included: RSV, human rhinovirus, enterovirus, influenza virus A, influenza virus B, influenza virus C, human coronaviruses, human metapneumovirus, parainfluenza virus, human bocavirus, and adenovirus. Sputum cultures were used to detect bacteria.

**Statistical analyses**

The data were analyzed using SPSS Statistics 17.0 for continuous variables (reported as mean ± standard
deviation). Patient age may be associated with certain laboratory indices, including white blood cell count and C-reactive protein level; thus, these quantitative data were transformed into categorical data (normal or abnormal). Statistical significance was assessed using the chi-square test or Fisher’s exact test for categorical variables and the t-test and ANOVA for continuous variables. A P-value <0.05 was considered statistically significant. GraphPadPrism 5 (GraphPad Software Inc.) was used to generate figures and graphs.

**Results**

**Demographic and clinical characteristics**

A total of 259 children diagnosed with bronchiolitis caused only by RSV were included in the study. These subjects were all tested negative for COVID-19. There were 188 males and 71 females. The average age at the time of the initial wheezing episode was 8.91 ± 7.71 months. There were 152 urban residents (58.7%). The average hospital stay duration was 6.31 ± 2.43 days. Among the 259 children, 147 experienced wheezing again within 1 year, and the recurrence rate was 56.8%. Among these 147 children, 79 (30.5%) had a single recurrence and 68 (26.3%) had 2 or more recurrences. The single recurrence and multiple recurrences groups were compared. There were no significant differences in terms of sex, place of residence, mode of delivery, prematurity, or family allergy history between the two groups (P > 0.05). There were significant differences between the two groups in terms of personal allergy history, passive smoking, total serum IgE level, age, hospital stay duration, and wheezing duration (P < 0.05). The percentage of children with a personal allergy history in the multiple recurrences group was significantly higher than in the single recurrence and no recurrence groups (P=0.031 and 0.008, respectively). The age of the children in the multiple recurrences group was significantly lower than that of the children in the single recurrence group (P < 0.001). The hospitalization duration for the children with initial wheezing in the multiple recurrences group was significantly longer than that for the children in the single recurrence and no recurrence groups (P=0.042 and 0.016, respectively). The cost of re-hospitalization in the multiple recurrences group was significantly higher than in the single recurrence and no recurrence groups (P<0.001 and <0.001, respectively). This information is presented in Table 2.

**Clinical severity and recurrent wheezing**

A total of 22 patients had severe bronchitis, and 18 of them had multiple wheezing recurrences. There were 116 cases of moderate bronchitis, among which 35 had multiple recurrences, 40 had a single recurrence, and 41 had no recurrence. There were 121 cases of mild bronchitis, and only 15 of these cases had multiple recurrences (Details are shown in Table 3). Clinical severity scores were higher in the multiple recurrences group than in the single recurrence and no recurrence groups (P=0.002 and <0.001, respectively), as shown in Figure 1.

**Discussion**

The strict social distancing measures implemented reduced people’s daily contacts seven-fold to eight-fold during the COVID-19 pandemic in China, with most interaction restricted to the household members.12 A reduction in probability of pathogen transfer via a potential link, that is, reduced physical contact between humans while they are active/mobile greatly reduce the number of infected as well as severity of virus. Thus, patients in this study did not experience frequent wheezing during the COVID-19 pandemic. But those patients with severe RSV infection or who had an allergic predisposition had a higher rate of recurrent wheezing.

RSV is not only the main cause of early respiratory tract infection in infants, but it is also a main risk factor for infant wheezing.13 Severe RSV infection has repeatedly been associated with long-term complications, including impaired lung function, recurrent wheezing, and asthma.14 Because infant respiratory system development is not perfect, neither is immune system maturation, and early pathogen exposure can easily cause wheezing. The immunity acquired by RSV infection is not lasting. Bertrand et al.15 suggested that interleukin (IL)-3 and IL-12p40 could be molecular predictors for recurrent wheezing due to RSV infection. He presented a comprehensive cytokine and chemokine profile analysis in the upper and lower airways of infants with RSV bronchiolitis and identified key mediators directly associated with clinical outcomes.
within 3 years of patient follow-up. Bronchiolitis in infancy is considered a risk factor for recurrent wheezing in childhood. The present study assessed the prevalence of wheezing, the clinical manifestations and risk factors for recurrent wheezing during the first 3 years of life, and persistent wheezing beyond this age in children hospitalized as young infants for bronchiolitis. Hospitalization for bronchiolitis within the first 6 months of life is an independent risk factor for recurrent wheezing during the first 3 years of life.16 We found that when infants experienced mild bronchiolitis, it was generally self-limiting, and there was little wheezing again. In a multicenter cohort study of infants hospitalized with bronchiolitis, intensive care treatment was associated with respiratory morbidity during early childhood recurrent wheezing of childhood by age 3 years.17

This study showed that infants with an allergic constitution had a higher probability of frequent

Table 2. Comparison of each factor among three groups.

| Factor                                | Single recurrence group\(^a\) (n=79) | Multiple recurrences group\(^b\) (n=68) | No recurrence group\(^c\) (n=112) | P value   |
|---------------------------------------|-------------------------------------|---------------------------------------|----------------------------------|-----------|
| With personal allergy history         | 3                                   | 9                                     | 3                                | \(P=0.031\)\(^{ab}\) \(P=0.008\)\(^{ab}\) |
| With family allergy history           | 11                                  | 8                                     | 12                               | \(P=0.796\) |
| Birth order                           | 1.87 ± 0.79 (1–5)                   | 2.18 ± 0.98 (1–6)                     | 1.96 ± 1.03 (1–8)                | \(P=0.05\)\(^{ab}\) \(P=0.043\)\(^{bc}\) |
| Age (months)                          | 12.04 ± 8.25 (2–36)                 | 7.71 ± 7.03 (1–34)                    | 7.86 ± 5.97 (1–36)               | \(P<0.001\)\(^{ab}\) \(P<0.001\)\(^{bc}\) |
| Place of residence (urban/rural)      | 45                                  | 43                                    | 64                               | \(P=0.675\) |
| Preterm                               | 10                                  | 9                                     | 14                               | \(P=0.084\) |
| Sex (man)                             | 53                                  | 55                                    | 80                               | \(P=0.163\) |
| Mode of delivery (spontaneous labor) | 54                                  | 45                                    | 74                               | \(P=0.94\) |
| Passive smoking                       | 3                                   | 12                                    | 4                                | \(P=0.023\)\(^{ab}\) \(P=0.008\)\(^{bc}\) |
| Total serum IgE level (IU/ml)         | 123.13 ± 257.61 (0–1372)            | 103.75 ± 180.17 (0–990)               | 58.76 ± 100.34 (0–470)           | \(P=0.012\)\(^{bc}\) |
| Serum allergen testing (positive)     | 9                                   | 10                                    | 7                                | \(P=0.833\) |
| Clinical severity (sever)             | 3                                   | 18                                    | 1                                | \(P=0.002\)\(^{ab}\) \(P<0.001\)\(^{bc}\) |
| Hospital stay duration (days)         | 6.42 ± 2.2 (2–12)                   | 6.71 ± 2.39 (2–18)                    | 598 ± 2.56 (1–16)                | \(P=0.042\)\(^{ab}\) \(P=0.016\)\(^{bc}\) |
| Wheezing duration when hospitalization (days) | 5.95 ± 8.05 (1–37) | 6.44 ± 6.19 (1–31) | 5.1 ± 8.33 (1–66) | \(P=0.097\)\(^{ab}\) \(P=0.286\)\(^{bc}\) \(P=0.003\)\(^{bc}\) |
| Cost of re-hospitalization (yuan)     | 2938.65 ± 2009.887 (0–8790)         | 6987.14 ± 4074.981 (0–21,680)         | 0                                | \(P<0.001\)\(^{ab}\) \(P<0.001\)\(^{ac}\) \(P<0.001\)\(^{bc}\) |

\(^a\)Single recurrence group.
\(^b\)Multiple recurrences group.
\(^c\)No recurrence group.

Table 3. Clinical severity in single recurrence group, multiple recurrences group, and no recurrence group.

| Severity Category | Single recurrence group \((n=79)\) | Multiple recurrences group \((n=68)\) | No recurrence group \((n=112)\) |
|-------------------|------------------------------------|--------------------------------------|-------------------------------|
| Mild (score 0–4.9 points) | 36                                 | 15                                   | 70                            |
| Moderate (score 0–4.9 points) | 40                                 | 35                                   | 41                            |
| Sever (score 0–4.9 points)    | 3                                  | 18                                   | 1                             |
wheezing than those without an allergic constitution. This part of children with recurrent wheezing was not affected by the epidemic. An atopic constitution and infant wheezing have certain correlation. Allergic sensitization is a main cause of wheezing.18 RSV infection prevalence, eosinophil percentage, and serum eosinophil-derived neurotoxin levels are the predominant risk factors of wheezing and could therefore be used to predict recurrent wheezing in infants.1 The risk factors for developing asthma by 6 years of age in children hospitalized for wheezing before 2 years of age are eosinophils in the blood, atopic dermatitis, elevated serum IgE levels, and wheezing onset in early life. It has been suggested that the defining factor of childhood asthma is atopy.8 A viral infection may aggravate an allergic reaction in some cases.19 Martinez20 suggested that transient wheezing in young children is not associated with an atopic predisposition.

This study also found that wheezing recurrence was related to age which was consistent with some literature reports. Matricardi et al.21 showed that the incidence of wheezing declines with age. Guo et al.22 found that the number of wheezing episodes decreases significantly during the follow-up stage and that the age at wheezing onset is earlier in those with transient wheezing than in those with persistent wheezing. The respiratory system gradually undergoes development with age, which may be an important reason for the decrease in the number of wheezing episodes.23

Conclusions
It found that personal allergy history and age were predictive of recurrent wheezing in children within an initial RSV infection. The younger the age at the time of the onset of wheezing, the more wheezing recurrences will occur in the following year. As the study was conducted during the pandemic, it was limited by a small sample size and was limited to patients who were all tested negative for COVID-19. It lacked the follow-up data before the pandemic, so it did not compare the wheezing episodes before and after the epidemic. In all, this study would provide more sufficient clinical evidence for the diagnosis, treatment, and prevention of recurrent wheezing in infants.

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Authors’ contributions
L Z and J R contributed to the concept and design of the study, analyzed and interpreted the data, and assisted in the critical writing. J L and F H helped to analysis statistic. Z X contributed to the collection of clinical information. All authors read and approved the final manuscript.

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Ethics approval
This study was approved by the Ethics and Research Council of Women and Children’s hospital of Ganzhou. Approval number: Gzfy20207420.

Informed consent
Signed consent was obtained from each child’s parents or foster parents. Written informed consent was obtained from all subjects before the study.
Consent for publication

All authors have read and approved the content, and agree to submit it for consideration for publication.

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