Original article

Characteristics of respiratory syncytial virus-induced bronchiolitis co-infection with *Mycoplasma pneumoniae* and add-on therapy with montelukast

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Background: **The influence of *Mycoplasma pneumoniae* (MP) infection on bronchiolitis remains unclear. Additionally, reports on the efficacies of leukotriene receptor antagonists in the treatment of bronchiolitis have been inconclusive.**

Methods: **Children with respiratory syncytial virus (RSV)-induced bronchiolitis were divided into two groups: RSV+MP group and RSV group. Each group was randomly divided into two subgroups: one received routine and placebo treatment, while the other received routine and montelukast treatment for 9 months. The cumulative numbers of wheezing episodes and recurrent respiratory tract infections were recorded. Blood parameters were determined.**

Results: **Patients in the RSV+MP group exhibited an older average age, fever, more frequent flaky and patchy shadows in chest X-rays, more frequent extrapulmonary manifestations, and longer hospital stays compared with patients in the RSV group. Additionally, higher baseline blood eosinophil counts, eosinophil cationic protein (ECP), total immunoglobulin E (IgE), interleukin (IL)-4, IL-5, IL-4/interferon-γ ratios, leukotriene (LT) B_4, and LTC_4, and lower baseline lipoxin A_4 (LXA_4)/LTB_4 ratios were observed in the RSV+MP group compared with the RSV group. Montelukast treatment decreased the cumulative numbers of recurrent wheezing episodes and recurrent respiratory tract infections at 9 and 12 months. This efficacy may be related to the montelukast-induced reductions in peripheral eosinophil counts, ECP and total IgE, as well as the montelukast-dependent recovery in T helper (Th) 1/Th2 balance and LXA_4/LTB_4 ratios in children with bronchiolitis.**

Conclusions: **RSV bronchiolitis with MP infection was associated with clinical and laboratory features that differed from those of RSV bronchiolitis without MP infection. Add-on therapy with montelukast for 9 months was beneficial for children with bronchiolitis at 9 and 12 months after the initiation of treatment.**

World J Pediatr 2016;12(1):88-95

Key words: **bronchiolitis; leukotrienes; montelukast; *Mycoplasma pneumoniae*; respiratory syncytial virus**

Introduction

Acute bronchiolitis is predominantly a viral disease. Respiratory syncytial virus (RSV) is responsible for more than 50% of cases of acute bronchiolitis, and other agents including parainfluenza, adenovirus, *Mycoplasma*, and so on, also lead to this disease.[1] Severe RSV bronchiolitis with recurrent wheezing episodes (≥3 episodes) in early infancy is a strong risk factor for the occurrence of allergic asthma in early adolescence.[2] Recently, *Mycoplasma pneumoniae* (MP) infection has been recognized as a causative agent of asthma.[3] MP infection can precede the onset of asthma, promote exacerbation of asthmatic symptoms, and cause difficulties with asthma management.[3] Many cases of MP bronchiolitis in adults have been reported previously.[4] However, the influence of MP infection on bronchiolitis remains unclear. Therefore, this study was designed to investigate the clinical

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doi: 10.1007/s12519-015-0024-4
Online First April 2015

and laboratory characteristics of children with RSV bronchiolitis co-infection with MP. Additionally, severe RSV bronchiolitis may be associated with enhanced T helper (Th) 2 responses, such as overexpression of interleukin (IL)-4.[5] However, it is still unclear whether the increased Th2 responses occur in children with RSV bronchiolitis co-infection with MP. Thus, we also investigated Th1/Th2 cytokines production in children with RSV bronchiolitis co-infection with MP.

Leukotrienes (LTs) are important pro-inflammatory mediators of airway inflammation in asthma.[6] Our previous study suggested that children with severe asthma generated insufficient amounts of lipoxin A_4 (LXA_4), a native inhibitor of LTs, but overproduced LTs.[7] Diminished LXA_4 biosynthesis and enhanced generation of cysteinyl-LTs (CysLTs) have also been found in adults with severe asthma.[8] CysLTs are increased in respiratory secretions obtained from infants with acute RSV bronchiolitis, suggesting a possible role of CysLTs in the pathogenesis of the disease.[9] To the present, no studies have reported the production of LXA_4 in patients with bronchiolitis. Therefore, this study aimed to explore the generation of LXA_4 and LTs in children with RSV bronchiolitis with or without MP infection. In addition, the results of published randomized controlled trials (RCTs) describing the efficacy of LT receptor antagonists (LTRAs) in the treatment of RSV bronchiolitis have been inconclusive.[6] Thus, in this study, we explored the efficacy of add-on therapy with montelukast in the treatment of bronchiolitis.

Methods

Patients

The clinical trial was registered with the Chinese Clinical Trial Registry (www.chictr.org; registration number: ChiCTR-TRC-13003735). A total of 270 children with acute bronchiolitis were recruited from the Departments of Pediatrics of the First Affiliated Hospital with Nanjing Medical University, Jiangsu Maternity and Children Healthcare Hospital, Nanjing First Hospital Affiliated to Nanjing Medical University, Central People's Hospital of Tengzhou, and Qidong People's Hospital from March 2012 to March 2013 (Fig. 1). The parents or guardians of the enrolled children provided informed consent for their children to participate in this study. The protocol was approved by the ethics committees of the above-mentioned hospitals. The diagnosis of bronchiolitis was based on clinical assessment of acute bronchiolitis as recommended by the American Academy of Pediatrics.[10] Patients were included in the study if they met the following inclusion criteria: age of 2 to 24 months, diagnosis of RSV bronchiolitis, and the first episode of RSV bronchiolitis with a respiratory symptom duration of less than 7 days. The RSV infection was confirmed by both identification of RSV-RNA in the nasopharyngeal aspirates and RSV-immunoglobulin (Ig) M in the serum, and MP infection was confirmed by both identification of MP-DNA in the nasopharyngeal aspirates and MP-IgM in the serum. Assessment of bronchiolitic episode severity was based on the clinical asthma score, as reported by Parkin et al.[11] Patients whose bronchiolitic symptoms persisted for more than 7 days from onset, those who experienced more than one episode of bronchiolitis, those with a history of intubation, underlying cardiopulmonary, laryngeal, tracheobronchial or other infectious disease, those with immunodeficiency, or those who had been on any steroid treatment within the previous 2 weeks were excluded from the study. Fifty-seven normal healthy,

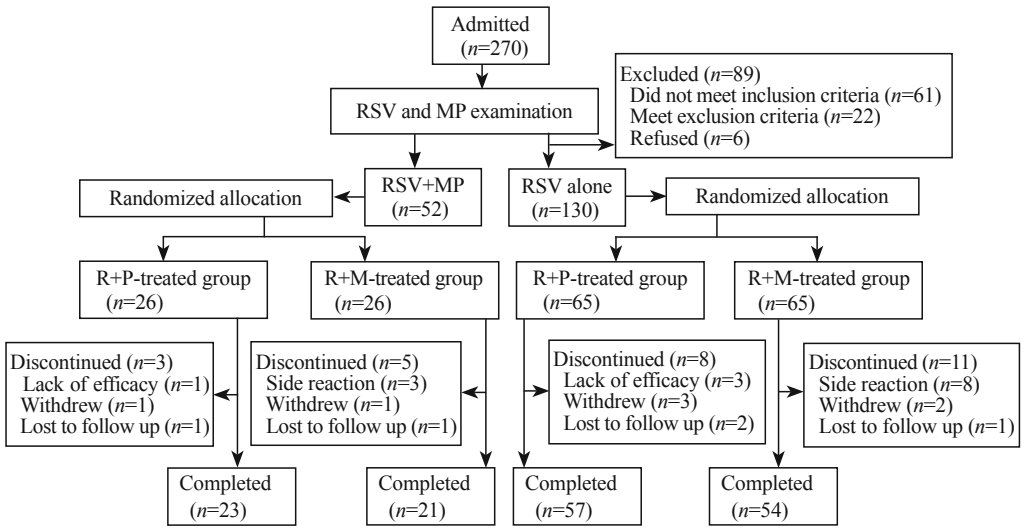

Fig. 1. Flow chart of study enrollment. RSV: respiratory syncytial virus; MP: *Mycoplasma pneumoniae*; R: routine treatment; P: placebo treatment; M: montelukast treatment.

age- and sex-matched children served as controls. The clinical data of all enrolled children are listed in Table.

RSV and MP assessment

Upon admission, a nasopharyngeal aspirate sample was collected from the nostrils of each child using a standardized protocol. The obtained secretions were immediately placed into tubes containing viral transport media and were then sent to a laboratory for processing. Aliquots of samples were stored at -70°C until use. RSV and MP infections were confirmed by the identification of RSV-RNA and MP-DNA, respectively, in the nasopharyngeal aspirates using fluorescein-based real-time reverse transcription-polymerase chain reaction and real-time PCR kits (Daangene Co., Guangzhou, China), respectively. Additionally, on admission, serological tests for IgM antibodies against RSV and MP were performed in all patients using commercial enzyme-linked immunosorbent assay (ELISA) kits (IBL international, Hamburg, Germany) following the manufacturer's instructions.

Treatment protocol

In this prospective, randomized, placebo-controlled, double-blind, parallel-group study conducted in five medical centers, every pair of patients with comparable age distributions and clinical asthma scores in the RSV group or RSV+MP group were randomly divided into two subgroups according to a computer-generated randomized allocation schedule, i.e., for each pair of patients, one patient was allocated to one subgroup, and the other patient was allocated to another subgroup. One subgroup received routine treatment plus treatment with placebo granules in sachet (P-treated) for 9 months, while the other subgroup received routine treatment plus treatment with montelukast sodium granules in sachet (Singulair, Merck Sharp & Dohme, Hangzhou, China; M-treated) for 9 months. The excipients of montelukast granules (mannitol, hydroxypropyl cellulose, and magnesium stearate) were the same as those of placebo granules. The white color, smell and external appearance of the placebo and montelukast granules were identical. The placebo granules were prepared by the hospital pharmacy, and both montelukast and placebo granules were packed and sealed by the hospital pharmacy in identical sachets. As indicated in the manufacturer's directions on the package insert, the dose of montelukast for children aged 1-5 years was 4 mg daily. Accordingly, the patients aged 12-24 months received 1 sachet (4 mg montelukast sodium or placebo granules) daily, those aged 6-12 months initially received three-quarters of a sachet daily, and those aged 2-6 months initially received one-half of a sachet daily. The treatments were initiated in patients with a first

Table. Clinical data of patients with bronchiolits before treatment and those of controls

	RSV+MP group (n=52)		RSV group (n=130)		Controls (n=57)
	P-treated (n=26)	M-treated (n=26)	P-treated (n=65)	M-treated (n=65)	
Male/female	18/8	16/10	47/18	45/20	39/18
Age (mon)	11.8±4.5*	10.3±4.7*	5.5±3.2	5.7±3.6	8.9±5.8
Fever (>37.5°C) (%)	42.3*	46.1*	13.8	12.3	0
Flaky shadow in X-ray (%)	38.4*	42.3*	12.3	13.8	0
Extrapulmonary findings (%)	69.2*	61.5*	16.9	20.0	0
Atopic dermatitis (%)	15.3†	11.5†	16.9†	18.4†	5.2
Asthma in family (%)	3.8	3.8	3.1	1.5	1.7
Clinical asthma score (0-10)	6.8±1.6	6.5±1.7	6.4±1.8	6.6±1.7	0
Inhaled oxygen (%)	23.1	19.2	21.5	24.6	0
3-drugs inhalation (%)	100	100	100	100	0
Systemic corticosteroids (%)	30.7	26.9	24.6	23.0	0
Antibiotics (%)	100*	100*	10.7	16.9	0
Length of hospital stay (d)	14.6±3.5*	15.8±4.2*	6.9±3.2	7.5±3.9	0
Blood eosinophil (cells/μL)	249±84*†	259±92*†	166±49†	158±56†	94±56
Serum IL-4 (pg/mL)	88.4±26.7*†	91.6±34.7*†	58.3±22.6†	46.7±23.2†	12.3±6.8
Serum IL-5 (pg/mL)	126.1±42.8*†	132.2±45.4*†	84.9±31.3†	91.8±36.6†	15.2±6.2
Serum IFN-γ (IU/mL)	39.8±18.2†	41.9±19.2†	42.9±22.6†	43.6±21.7†	27.3±14.9
Serum IL-4/IFN-γ	2.2±0.8*†	2.5±0.8*†	1.2±0.8†	1.1±0.7†	0.5±0.3
Serum total IgE (IU/mL)	143.2±32.1*†	152.6±35.5*†	83.0±38.2†	88.6±22.9†	20.6±13.6
Serum ECP (ng/mL)	86.9±18.4*†	92.4±17.8*†	39.7±16.2†	38.9±15.9†	7.5±4.2
Blood LXA_4 (ng/mL)	4.4±2.3†	4.5±2.5†	4.9±2.2†	5.1±3.7†	1.4±0.6
Blood LTB_4 (pg/mL)	85.8±35.2*†	84.9±32.3*†	63.2±24.1†	68.9±22.1†	12.4±5.2
Blood LTC_4 (pg/mL)	123.6±43.1*†	127.4±42.8*†	72.9±23.9†	82.7±26.1†	12.7±5.3
Blood $LXA_4/LTB_4\times1000$	51.4±19.7*†	53.1±20.1*†	77.8±27.5†	74.0±26.9†	112.9±45.1

RSV: respiratory syncytial virus; MP: *Mycoplasma pneumoniae*; P: placebo treatment; M: montelukast treatment; 3-drugs inhalation: budesonide, salbutamol sulfate and ipratropium bronude; IL: interleukin; IFN-γ: interferon-γ; ECP: eosinophil cationic protein; LXA_4: lipoxin A_4; LT: leukotriene. *: P<0.05, compared with that of RSV group; †: P<0.05, compared with that of controls.

episode and the respiratory symptom duration of less than 7 days. Investigators, parents, and caregivers of the enrolled patients remained blinded to the treatment throughout the study period.

The routine treatment of the patients included the following: 1) Inhaled oxygen, nutrition, and intravenous fluids were used according to standard clinical practice; 2) Budesonide,[1,10,12] terbutaline sulfate,[1,10,12,13] and ipratropium bronude[12] were administered by aerosol during wheezing episodes; 3) Intravenous corticosteroids[1,10,12] were administered for 3-5 days during hospitalization when the clinical asthma score was greater than 7; 4) The patients received inhaled and intravenous ribavilin daily during hospitalization;[1,10] 5) The patients in the RSV+MP group received intravenous erythromycin daily for 3-5 days during hospitalization, and then oral azithromycin daily for 3 days per week for 2 weeks.

Experimental study

Serum interleukin (IL)-4, IL-5, and interferon-γ (IFN-γ) levels were determined using ELISA kits (IL-4 and IL-5: Jingmei Biotech, Shenzhen, China; IFN-γ: R&D Systems, Minneapolis, MN, USA). The IL-4/IFN-γ ratio was calculated as a surrogate of the Th2/Th1 ratio.[14] Serum eosinophil cationic protein (ECP) was measured using a fluoro-enzyme immunoassay kit (Pharmacia CAP System, Kabipharmacia, Stockholm, Sweden). Serum total IgE was detected using an ELISA kit (Yanjin Biotech, Shanghai, China). The above experiments were performed according to the manufacturer's instructions. Whole blood LXA_4, LTB_4, and LTC_4 levels were also measured by ELISA (Neogen, Lexington, KY, USA) following the manufacturer's instructions.[7] The LXA_4/LTB_4 ratio was calculated as a surrogate of the anti-LT/LT bio-activity balance.

Follow-up

Follow-up observations were made every month until the end of the 12-month study period (clinical endpoints), beginning at the first day of treatment. On the day of follow-up observation, the cumulative numbers of wheezing episodes and recurrent respiratory tract infections without wheezing were recorded, the treatment safety and tolerability were evaluated, and blood and urine samples were collected for additional investigations.

Statistical analysis

Results were expressed as mean±standard deviation. The cumulative numbers of wheezing episodes and recurrent respiratory tract infections were analyzed using the Kruskal-Wallis test. Clinical data including patient age, length of hospital stay, and results of blood examinations were analyzed using one-way analysis of variance followed by *f* and *q* tests. The clinical data were analyzed using the Chi-square test (Table). The asthma scores were analyzed using Wilcoxon's rank-sum test at a 95% confidence level. All analyses were performed using Statistical Package for Social Sciences version 16.0 (Chicago, IL, USA). Differences were considered to be statistically significant when *P* values were less than 0.05.

Results

RSV and MP determination

Of the 270 patients, 182 (67.4%) were tested positive for both RSV-RNA in the nasopharyngeal aspirates and RSV-IgM in the serum (Fig. 1). Of the 182 patients with RSV bronchiolitis, 52 (28.5%) were tested positive for both MP-DNA in the nasopharyngeal aspirates and MP-IgM in the serum.

Patients with bronchiolitis versus healthy controls

At baseline, peripheral eosinophil counts, ECP, total IgE, IL-4, IL-5, IFN-γ, IL-4/IFN-γ ratios, LXA_4, LTB_4 and LTC_4 levels in children with bronchiolitis were above the standard levels obtained from healthy children (Table), whereas the LXA_4/LTB_4 ratios in children with bronchiolitis were below the standard levels obtained from healthy children (Table).

RSV+MP bronchiolitis versus RSV bronchiolitis

In contrast to the patients in the RSV group, the patients in the RSV+MP group exhibited relatively older age at disease onset, fever, increased occurrence of flaky and patchy shadows on chest X-rays, frequent extrapulmonary manifestations, and longer hospital stays (Table). Extrapulmonary manifestations included abnormal liver function, abnormal urinary examination, enhanced creatine kinase-muscle brain and troponin, myocarditis, convulsion, diarrhea, *de novo* skin rash, hapatomegaly, splenomegaly, anemia, and thrombocytopenia. The baseline levels of blood eosinophil counts, ECP, total IgE, IL-4, IL-5, IL-4/IFN-γ ratios, LTB_4, and LTC_4 in children in the RSV+MP group were significantly higher than those in children in the RSV group, and the LXA_4/LTB_4 ratios in children in the RSV+MP group were lower than those in children in the RSV group. Furthermore, there were no significant differences in peripheral white blood cell counts, C-reactive protein, IgA, IgG, IgM (data not shown), IFN-γ, and LXA_4 levels between patients in the RSV+MP group and those in the RSV group before the treatment (Table).

Effects of montelukast

The temporal relationship was observed between the

treatment and cumulative recurrent wheezing episodes (Fig. 2). Cumulative recurrent wheezing episodes were more frequent in patients receiving placebo than in those receiving montelukast at the 9- and 12-month follow-up appointments. In addition, the cumulative number of recurrent respiratory tract infections was reduced more significantly in patients receiving montelukast than in those receiving placebo at the 9- and 12-month follow-up appointments (Fig. 3). There were no significant differences in the length of hospital stay between the montelukast-treated group and the placebo-treated group (Table).

Nine-month treatment with montelukast significantly decreased the peripheral eosinophil counts, ECP, total IgE, IL-4, IL-5, IL-4/IFN-γ ratios, LTB_4, and LTC_4 in both RSV+MP group and RSV group at 9 (data not shown) and 12 months (Fig. 4) compared with those in the placebo-treated controls at 9 and 12 months. However, blood levels of LXA_4, IFN-γ, C-reactive protein, IgA, IgG, IgM, and white blood cell count were not altered in patients receiving placebo and patients receiving montelukast during the 9- and 12-month study periods (data not shown). In addition, the LXA_4/LTB_4 ratios in montelukast-treated patients were enhanced at 9 (data not shown) and 12 months (Fig. 4) compared to those in the placebo-treated controls.

Adverse events related to the use of montelukast

Of the 27 patients who discontinued the study prematurely, 11 patients were discontinued because of adverse events related to the use of montelukast (Fig. 1). Common adverse reactions to montelukast included dysphoria (2 patients), excitatory state (3 patients), night cry (1 patient), hyperactivity (3 patients), and nocturnal fretfulness (2 patients). These reactions disappeared after withdrawal of montelukast.

Discussion

In the current study, the prevalence of MP infection in children with RSV bronchiolitis who were under 2 years of age was 28.5%. MP infections may be more frequent in China than in other countries. An investigation conducted in China found that 25.3% of children under the age of 8.9 years and 23.1% of

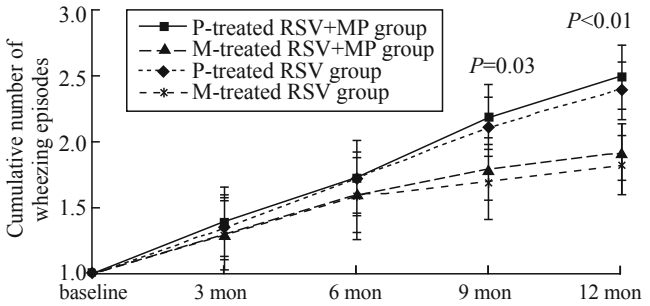

Fig. 2. Cumulative number of wheezing episodes at baseline and 3, 6, 9 and 12 months in children with bronchiolitis. P: placebo treatment; M: montelukast treatment; RSV: respiratory syncytial virus; MP: *Mycoplasma pneumoniae*. Results are expressed as mean±standard deviation. Statistical significance (P=0.03 and P<0.01) is shown between P-treated groups versus M-treated groups.

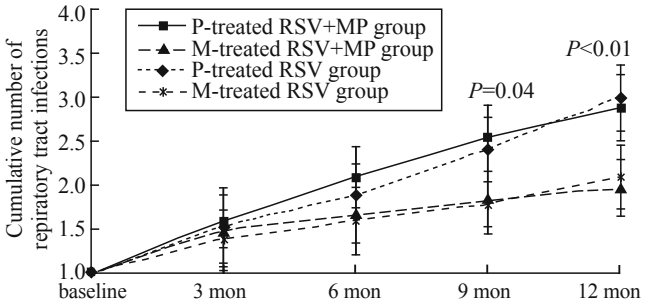

Fig. 3. Cumulative number of respiratory tract infections at baseline and 3, 6, 9 and 12 months in children with bronchiolitis. P: placebo treatment; M: montelukast treatment; RSV: respiratory syncytial virus; MP: *Mycoplasma pneumoniae*. Results are expressed as mean±standard deviation. Statistical significance (P=0.04 and P<0.01) is observed between P-treated groups versus M-treated groups.

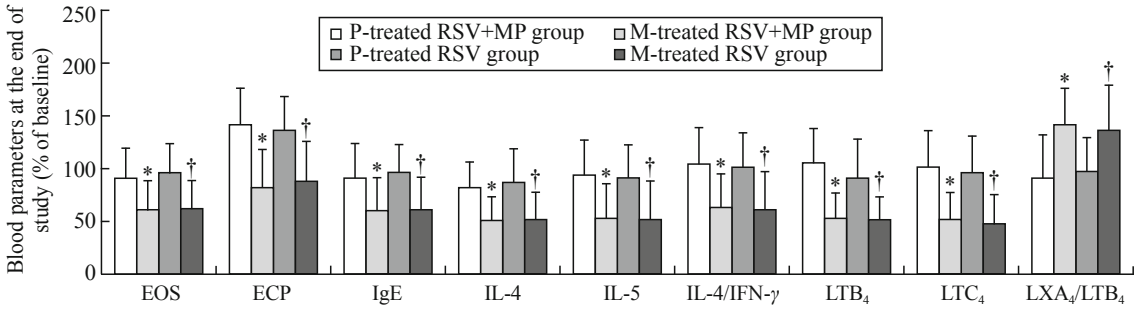

Fig. 4. Peripheral blood parameters at the end of the 12-month study in children with bronchiolitis. EOS: eosinophil count; ECP: eosinophil cationic protein; IgE: total immunoglobulin E; IL: interleukin; IFN-γ: interferon-γ; LXA_4: lipoxin A_4; LT: leukotriene; P: placebo treatment; M: montelukast treatment; MP: *Mycoplasma pneumoniae*; RSV: respiratory syncytial virus. Results are expressed as mean±standard deviation. *: P<0.05, compared to the same parameter in the P-treated RSV+MP group; †: P<0.05, compared to the same parameter in the P-treated RSV group.

children aged 1-6 months who had asthmatic diseases also suffered from MP infections.[15] Similarly, another larger study conducted in China reported that the MP infection rate in 10 243 hospitalized children with acute respiratory tract infections was 25.74%, comprising 8.25% of patients aged 0-6 months, 19.46% of patients aged 7 months to 1 year, and 33.0% of patients aged 2-3 years.[16] The current study also revealed the clinical and laboratory features of RSV+MP bronchiolitis. As compared with patients without MP infection, patients co-infected with MP exhibited a relatively older mean age at disease onset, fever, increased flaky opacities on chest X-rays, frequent extrapulmonary manifestations and longer hospital stays (Table). Since there were no significant differences in baseline clinical asthma scores and routine symptomatic treatment between the RSV+MP and RSV groups, the longer hospital stay in the RSV+MP group was attributed to the more frequent occurrence of extrapulmonary manifestations, which required additional treatment.

The baseline levels of blood IL-4, IL-5, and IL-4/IFN-γ ratios in the RSV+MP group were higher than those in the RSV group, suggesting that shifts in the Th1/Th2 balance towards Th2 predominance were more frequent in the RSV+MP group (Table). Our results are supported by previous studies. For example, Jeong et al[17] reported that the serum level of IL-5 increased in atopic children with MP pneumonia as compared with that in atopic children with viral pneumonia. Choi et al[18] reported that children with MP pneumonia and wheezing have significantly higher serum levels of IL-5, which stimulates eosinophil generation and activation. A study found that the IL-4 levels and IL-4/IFN-γ ratios in bronchoalveolar lavage fluid are significantly elevated in patients with MP pneumonia, but not in patients with pneumococcal pneumonia, suggesting a tendency toward increased IgE production since IL-4 stimulates the production of IgE by B lymphocytes.[19] Indeed, serum IgE increases in MP-positive children,[20] and both serum IgE and peripheral eosinophil counts increase in children with MP pneumonia.[21] Consistent with this, the present study also found enhanced serum total IgE, peripheral eosinophil counts, and ECP in patients in the RSV+MP group compared with those in patients in the RSV group (Table).

LXA_4 is an endogenously produced eicosanoid that acts as an anti-LTs factor and a "braking signal" during the inflammatory process.[8,22,23] LXA_4 blocks airway hyper-responsiveness and pulmonary inflammation, and inhibits the secretion of CysLTs in bronchoalveolar lavage fluids obtained from asthmatic mice.[22] Our previous study demonstrated that LXA_4 inhibits the LTB_4 synthesis by regulating neutrophils.[23] Additionally, other studies[7,8] revealed that reduced production of LXA_4 and overproduction of LTs may cause the onset of more severe disease in children and adults with asthma, suggesting that the shift in the anti-LT/LT bio-activity balance towards LT predominance participates in the pathogenesis of asthma. In the present study, the baseline levels of blood LTB_4 and LTC_4 in children with bronchiolitis were higher than those in healthy controls, and the LXA_4/LTB_4 ratios in children with bronchiolitis were lower than those in the healthy controls, despite the observation that LXA_4 levels were higher in children with bronchiolitis than in the healthy controls (Table). In addition, baseline levels of blood LTB_4 and LTC_4 in the RSV+MP group were higher than those in the RSV group, and the LXA_4/LTB_4 ratios were lower in the RSV+MP group than in the RSV group (Table). These data suggested that there was a greater shift in the anti-LT/LT bio-activity balance towards LT predominance in children with RSV+MP bronchiolitis than in those with RSV bronchiolitis. Our results were supported by previous studies demonstrating that the serum LTD_4 and urinary LTE_4 are higher in children with MP pneumonia than in those with non-MP pneumonia.[24]

Since CysLTs contribute to the inflammation in the pathogenesis of bronchiolitis,[9] it is reasonable to use LTRAs in the treatment of bronchiolitis. Unfortunately, LTRA treatment of RSV bronchiolitis and post-RSV bronchiolitic symptoms has yielded conflicting results.[6] Zedan et al[25] reported that montelukast administration to infants with bronchiolitis from the time of hospital admission to discharge reduces the clinical severity scores and the length of hospital stay. Conversely, Amirav et al[14] demonstrated that montelukast administration to infants with bronchiolitis from the time of hospital admission to discharge (4.65±1.97 days) does not reduce the length of hospital stay or improve the clinical severity scores. In a study of montelukast as a 3-month add-on therapy, Kim et al[13] found that montelukast reduces eosinophilic degranulation at 3 months and decreases the cumulative numbers of recurrent wheezing episodes at 12 months in children with post-RSV bronchiolitis. Conversely, a small RCT showed that montelukast administration for 3 months after hospital admission to treat RSV bronchiolitis does not reduce the occurrence of respiratory symptoms both in the treatment and follow-up periods.[26] In 2003, Bisgaard and colleagues[27] reported that montelukast administration for 28 days results in significant improvement of symptoms scores in children with RSV bronchiolitis. In contrast to this conclusion, Bisgaard demonstrated in 2008 in his second study that montelukast administration for 4 and 20 weeks had no clinical significant benefits in infants with post-RSV bronchiolitis in a 24-week follow-up period.[12] Despite this overall negative

result, however, post hoc analyses suggested that patients with more persistent symptoms may show significant improvement at the primary end point when treated with montelukast.[12] Since Bisgaard reported that montelukast treatment did not yield significant benefit after a 6-month treatment and follow-up,[12] and since a 1-year treatment with montelukast was shown to safely reduce asthma exacerbation in children with asthma,[28] and the reported data supported a good safety profile of montelukast used in preschool children,[6] we investigated the effects of a 9-month add-on therapy with montelukast in the present study. Montelukast administration for 9 months decreased the cumulative numbers of recurrent wheezing episodes and cumulative recurrent respiratory tract infections at 9 and 12 months in children with bronchiolitis compared with those receiving placebo (Figs. 2 and 3). Similarly, montelukast administration for a couple of weeks did not decrease the length of hospital stay (Table), and that for a period of less than 9 months did not reduce the cumulative numbers of wheezing episodes or cumulative recurrent respiratory tract infections (Figs. 2 and 3).

The mechanisms mediating the efficacy of add-on therapy with montelukast in the present study were also investigated. Peripheral eosinophil counts, ECP, and total IgE levels decreased at 9 and 12 months in children with bronchiolitis who received montelukast as compared with those who received placebo (Fig. 4). Our results were supported by previous findings that montelukast treatment reduced eosinophil degranulation and recurrent wheezing episodes in patients with post-RSV bronchiolitis,[13] and that treatment of food allergy with pranlukast, another LTRA, reduced peripheral eosinophil counts, ECP, and total IgE.[29] In addition, we also found that compared with placebo treatment, treatment of bronchiolitis with montelukast reduced the serum IL-4 and IL-5 levels and decreased the IL-4/IFN-γ ratios at 9 and 12 months, suggesting that the efficacy of montelukast may be related to the recovery of Th1/Th2 balance in children with bronchiolitis. Similarly, treatment of asthmatic children with montelukast reduces the serum IL-4 levels,[30] and treatment of children with food allergy with pranlukast abrogates the enhancement in serum IL-4 and IL-5.[29] Montelukast also increases IFN-γ production and promotes apoptosis in the allergen-specific T-cell population in the treatment of asthma.[31] In the current study, the levels of LTB_4 and LTC_4 in blood decreased, and the LXA_4/LTB_4 ratios increased in the montelukast-treated patients compared with placebo-treated controls at 9 and 12 months. This finding indicates that the efficacy of montelukast may be also related to the recovery of anti-LT/LT bio-activity balance in children with bronchiolitis. Indeed, a recent study[32] also revealed that the efficacy of montelukast in the treatment of children with exercise-induced bronchoconstriction is associated with the inhibition of sputum and urine LTE_4.

In summary, we found that compared with those with RSV bronchiolitis without MP infection, children with RSV+MP bronchiolitis exhibited a relatively older age at disease onset, fever, increased flaky opacities on chest X-rays, more frequent extrapulmonary manifestations, and longer hospital stays. Additionally, baseline levels of blood eosinophil counts, IL-4/IFN-γ ratios, and ECP, total IgE, IL-4, IL-5, LTB_4 and LTC_4 levels were higher, whereas the LXA_4/LTB_4 ratios were lower in patients with RSV+MP bronchiolitis compared with those in patients with RSV bronchiolitis. We also found that compared with placebo treatment, montelukast administration for 9 months decreased the cumulative numbers of recurrent wheezing episodes and recurrent respiratory tract infections at 9 and 12 months, and that the efficacy of montelukast may be related to the montelukast-induced reduction in peripheral eosinophil counts, ECP, and total IgE, and recovery of the Th1/Th2 balance and anti-LT/LT bio-activity balance in children with bronchiolitis. Since bronchiolitis with recurrent wheezing episodes in early infancy is a strong risk factor for the occurrence of allergic asthma in early adolescence,[2] the present study suggested that long-term add-on therapy with montelukast in the treatment of bronchiolitis may reduce the risk of allergic asthma in early adolescence. Further follow-up observations are needed to study this possibility.

Funding: This study was supported by a grant from the Priority Academic Program Development of Jiangsu Higher Education Institution (JX10231801).

Ethical approval: The protocol was approved by the ethics committees of the above mentioned five hospitals.

Competing interest: The authors declared no conflicts of interest relevant to this study.

Contributors: Wu SH wrote the first draft of this paper. All authors contributed to the intellectual content and approved the final version. Chen XQ is the guarantor.

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Received April 29, 2014
Accepted after revision August 26, 2014