Correlation of the Serum Levels of Leukotrienes with the Serum Levels of IgE and the Long-term Prognosis in Patients with Respiratory Syncytial Virus Infection

TWMUJ 5: 57-63, 2021

Original

Correlation of the Serum Levels of Leukotrienes with the Serum Levels of IgE and the Long-term Prognosis in Patients with Respiratory Syncytial Virus Infection

Nahoko Yasuda, Makiyo Ikutani, Yoko Shida, Kenichiro Takahashi, Tomoko Otani, Yoko Suzuki, and **Shigetaka Sugihara**

Department of Pediatrics, Tokyo Women's Medical University Medical Center East, Tokyo, Japan

(Accepted February 4, 2021)
(Advance Publication by J-STAGE March 30, 2021)

Objective: We analyzed the serum levels of leukotrienes (LTs) to obtain further insight into the pathogenesis of respiratory syncytial virus (RSV) infection in infants.

Methods: The subjects were 22 infants with RSV infection. The serum LT and immunoglobulin E (IgE) levels were measured at admission and at the end of the hospital stay. We investigated the correlations of the serum LT levels with the serum IgE levels and the long-term (3-6 years) prognosis of the infants.

Results: The mean serum LT level was significantly higher at the end of the hospital stay than at admission, particularly in the high-IgE group. The mean serum LT level at the end of the hospital stay was also significantly higher in the poor-prognosis group as compared to that in the good-prognosis group.

Conclusion: In infants with RSV infection, the serum LT levels were significantly elevated, particularly in the high-IgE group and the poor-prognosis group, suggesting a pathogenetic role of LTs of lower respiratory tract inflammation caused by RSV infection.

Keywords: respiratory syncytial virus (RSV), leukotrienes (LT), immunoglobulin E (IgE), long-term prognosis, lower respiratory tract infection

Introduction

Respiratory syncytial virus (RSV) is an RNA virus that belongs to the *Pneumoviridae* family of viruses. More than 50% of infants under one year of age suffer from RSV infection, and most infants have experienced RSV infection by the age of two years. About 30% of infants with RSV infection develop lower airway inflammation, including bronchiolitis and pneumonia, and a proportion of these infants goes on to develop severe infection necessitating hospitalization. However, at present, treatment of RSV infection still remains only symptomatic. It has been reported that children who suffer from RSV infection are at a higher risk of developing bronchial asthma and airway hyperresponsiveness later in life.[1]

Leukotrienes (LTs) are synthesized in the cells from arachidonic acid by arachidonate lipoxygenase. Among the LTs, LTC4, LTD4 and LTE4 are called cysteinyl leu-

Corresponding Author: Nahoko Yasuda, Department of Pediatrics, Tokyo Women's Medical University Medical Center East, 2-1-10 Nishiogu, Arakawa-ku, Tokyo 116-8567, Japan. yasuda.nahoko@twmu.ac.jp

doi: 10.24488/twmuj.2019106

kotrienes (CysLTs), which cause strong contraction of the airway smooth muscle, vascular hyperpermeability, and enhanced airway mucus secretion. Respiratory tract infections, such as RSV and parainfluenza virus infections, are known to be associated with increased upper airway secretions and/or elevated sputum levels of LTs.[2,3]

Recently, it has been suggested that LT receptor antagonists (LTRAs) may be effective for improving the long-term prognosis in patients with RSV infection, by preventing the development of bronchial asthma and airway hyperresponsiveness.[4] However, few studies have been conducted to investigate the serum levels of CysLTs (hereinafter simply LTs) in patients with lower respiratory tract infection caused by RSV.

The purpose of this study was to investigate the kinetics of the serum LT levels and their associations with the serum immunoglobulin E (IgE) levels and the prognosis as for the risk of development of bronchial asthma/airway hyperresponsiveness in later life in children with RSV infection.

Materials and Methods

The subjects of this study were 22 children, consisting of 15 male and 7 female children, who were between 2 and 23 months old (median age, 7.5 months). They were diagnosed as having RSV lower respiratory tract infection and hospitalized between November 2007 and September 2010 at the Department of Pediatrics, Tokyo Women's Medical University Medical Center East. The diagnosis was based on the presence of lower respiratory tract symptoms, including cough and wheezing, and a positive result of the nasal swab test for RSV antigen. Children were excluded from this study if they a) were born after less than 37 weeks of gestation, b) suffered from any chronic respiratory or chronic cardiac disease, c) were already diagnosed as having bronchial asthma, d) had received systemic steroid treatment within two weeks prior to the start of the study, e) had received oral LTRA agents prior to hospitalization, or f) needed mechanical ventilation.

Nine of the 22 infants were administered LTRAs during their hospital stay and during six months of discharge from hospital, while the remaining 13 infants did not receive any LTRAs. To prevent the placebo effect and observer bias on the results, whether or not to prescribe a LTRA was determined randomly by a double-blind control method.

The serum levels of LTs were measured using the CysLT EIA Kit (Cayman Chemical, USA) at admission and at the end of the hospital stay. The peripheral blood eosinophil counts and serum total IgE levels were also measured at admission and at the end of the hospital stay. The normal serum total IgE level was set as < 10 IU/mL for infants less than 6 months of age, 10-20 IU/mL for infants between 6 and 12 months of age, and < 50 IU/mL for children between 12 and 24 months of age.

The respiratory rate and percutaneous oxygen saturation (SpO_2) in the acute phase, and the length (days) of hospital stay were measured and recorded for each of the subjects.

We investigated the long-term prognosis of the subjects until April 2014, that is, between 3 and 6 years after the start of the study, by a medical interview via a letter or over the telephone, and a review of the medical records. Children who had been diagnosed/suspected as having bronchial asthma by a pediatrician were classified into the poor-prognosis group, and those who had not been diagnosed/suspected as having bronchial asthma by a pediatrician were classified into the good-prognosis group.

The protocol of this study conformed to the ethical guidelines of the 2008 Declaration of Helsinki and was approved by the ethics committee of Tokyo Women's Medical University (Tokyo, Japan; approval #1084). Written informed consent was obtained from the parents of each of the subjects.

The results are expressed as the means ± SD or median (range). The statistical significances of the results were evaluated by Student's t-test, with the significance level set at $p < 0.05$.

Results

Serum LT levels were measured in 20 subjects at hospital admission, and in 22 subjects at the end of the hospital stay. Specimens were obtained on day 1 (day 1 or day 2) and day 6 (day 4 to day 13) of the hospital stay. These time-points corresponded to day 5 (day 3 to day 15) and day 10 (day 7 to day 22) from disease onset. The serum

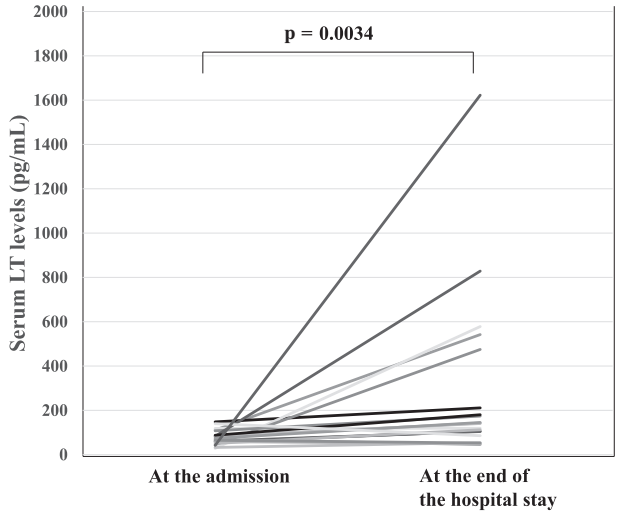

Figure 1. Serum LT levels at admission and at the end of the hospital stay.
Serum LT levels were measured in 20 subjects at hospital admission, and in 22 subjects at the end of the hospital stay.
LT, leukotriene.

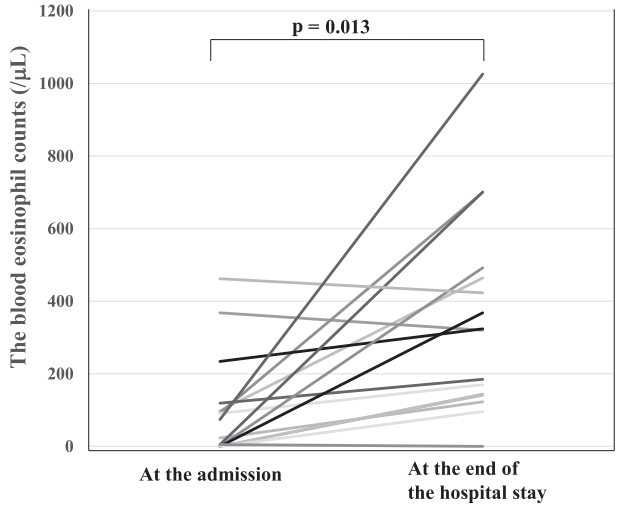

Figure 2. Peripheral blood eosinophil counts at admission and at the end of the hospital stay.
Peripheral blood eosinophil counts were measured in 20 subjects at hospital admission, and in 16 subjects at the end of the hospital stay.

level of LT was 77.4 ± 33.7 pg/mL at admission and 317.6 ± 374.0 pg/mL at the end of the hospital stay; the level was significantly higher at the end of the hospital stay than at admission (p = 0.0034) (**Figure 1**). The serum LT level at admission was 61.9 ± 49.8 pg/mL in the LTRA-treated group (n = 8) and 78.5 ± 35.6 pg/mL in the non-LTRA-treated group (n = 12); the difference between the two groups was not significant (p = 0.22). The serum LT level at the end of the hospital stay was 211.3 ± 241.7 pg/mL in the LTRA-treated group (n = 9) and 140.9 ± 453.6 pg/mL in the non-LTRA-treated group (n = 13); the difference between the two groups was not significant (p = 0.32).

The blood eosinophil count was 97.9 ± 129.7/μL at admission and 354.8 ± 274.1/μL at the end of the hospital stay (**Figure 2**). The blood eosinophil count showed a significant increase (p = 0.013) during the hospital stay, similar to the serum LT levels. The blood eosinophil count at hospital admission was 96.5 ± 117.3/μL in the LTRA-treated group and 5.0 ± 135.0/μL in the non-LTRA-treated group; the difference between the two groups was not significant (p = 0.64). The blood eosino-

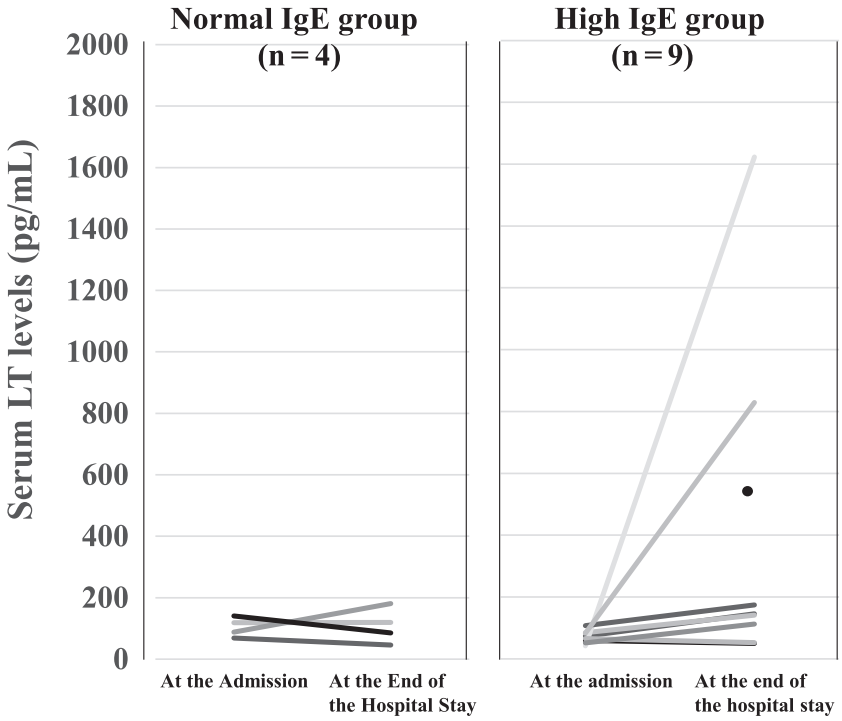

Figure 3. Comparison of the serum LT levels at admission and at the end of the hospital stay between the normal IgE group and high IgE group.
The 13 children with RSV lower respiratory tract infection without receiving LTRA-treatment were divided into two groups, namely, the normal IgE group and the high IgE group, according to the criteria described below. The normal serum total IgE level was set as <10 IU/mL for infants less than 6 months of age, 10-20 IU/mL for infants between 6 and 12 months of age, and <50 IU/mL for children between 12 and 24 months of age. The serum LT levels increased significantly only in the high IgE group from admission to the end of the hospital stay ($p = 0.045$).
LT, leukotriene; IgE, immunoglobulin E; RSV, respiratory syncytial virus; LTRA, LT receptor antagonist.

phil count at the end of the hospital stay was 252.5 ± 241.8/μL in the LTRA-treated group and 346 ± 314.5/μL in the non-LTRA-treated group; the difference between the two groups was not significant ($p = 0.25$).

The relationships between changes of the serum LT levels during the hospital stay and the serum total IgE levels at admission were analyzed in the 13 subjects who did not receive LTRA agents (**Figure 3**). The 13 subjects were divided into two groups, namely, the normal IgE group ($n = 4$) and the high IgE group ($n = 9$), according to the criteria described in the Subjects and Methods. In the normal IgE group, the serum LT level was 103.8 ± 32.0 pg/mL at admission and 108.0 ± 57.1 pg/mL at the end of the hospital stay, with no significant difference ($p = 0.452$). However, in the high IgE group, the serum LT level was 70.9 ± 21.0 pg/mL at admission and significantly higher, 408.2 ± 525.6 pg/mL, at the end of the hospital stay. Thus, the serum LT levels increased significantly only in the high IgE group from admission to the end of the hospital stay ($p = 0.045$) (**Figure 3**).

We also examined association between the increased serum LT levels and the length of the hospital stay, SpO_2, and respiratory rate in the acute phase in the subjects with RSV lower respiratory tract infection, however, no associations were observed (data not shown).

We investigated the long-term prognosis of the subjects, as for the development of bronchial asthma and airway hyperresponsiveness in later life, as described in the Subjects and Methods. Among the 13 subjects who did not receive LTRAs, 7 subjects had a good prognosis and 6 had a poor prognosis. Four of the 6 subjects in the poor-prognosis group were diagnosed as having bronchial asthma, while the remaining 2 of the 6 were diagnosed as "suspected bronchial asthma." The serum LT level at the end of the hospital stay was 87.3 ± 39.6 pg/mL in the good-prognosis group and 582.4 ± 577.6 pg/

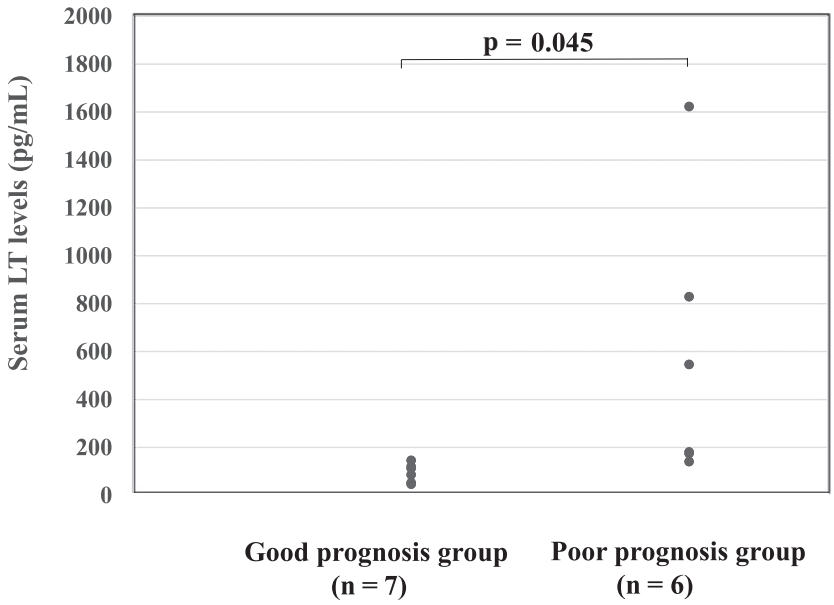

Figure 4. Comparison of the serum LT levels measured at the end of the hospital stay between the good-prognosis group and the poor-prognosis group.
The 13 children with RSV lower respiratory tract infection without receiving LTRA-treatment were divided into two groups, namely, the good-prognosis group and the poor-prognosis group, judged until April 2014, that is, between 3 and 6 years after the start of the study. Six subjects who were diagnosed/suspected as having bronchial asthma by a pediatrician were classified into the poor-prognosis group, and 7 subjects without bronchial asthma were classified into the good-prognosis group.
LT, leukotriene; RSV, respiratory syncytial virus; LTRA, LT receptor antagonist.

mL in the poor-prognosis group, being significantly higher in the poor prognosis group (p = 0.045) (**Figure 4**).

Discussion

While the immune mechanisms involved in lower respiratory infection (bronchiolitis) caused by RSV are not yet completely understood, LTs and IgE have been suggested as being closely involved in the pathogenesis of RSV infection and bronchial asthma.[5-7] LTs are known to cause contraction of the bronchial smooth muscle, being many times more potent than histamine or acetylcholine. LTs are produced in inflammatory cells such as eosinophils following stimulation with IgE, cytokines, etc.[5] Kim et al. reported elevated levels of LTs in the bronchoalveolar lavage fluid of children with RSV bronchiolitis.[6] Volovitz et al. investigated the levels of LTs in the nasopharyngeal secretions in children with RSV respiratory tract infection,[7] and reported greater elevation of the nasopharyngeal fluid LT levels in children with lower respiratory tract infection than in those with upper respiratory tract infection. The LT levels remained elevated even on the 28th day after the onset of symptoms. In our study we did not obtain bronchoalveolar lavage fluid or nasopharyngeal fluid specimens, because of technical difficulties. Instead, we used serum specimens and showed significant elevation of the serum LT levels in the children subjects with RSV lower respiratory tract infection. Since the types of specimens examined were different, a simple comparison of the results would not be possible, but our study added to the results suggesting the potential critical role played by LTs in the pathogenesis of RSV lower respiratory tract infection.

The standard values for serum LT levels have not yet been determined, and there are no previous reports indicating differences in the standard values depending on the age and/or sex. Although we did not compare our results with those in age-matched controls in this study, the average ± SD serum LT levels in 6 healthy adults volunteers measured by the same method was 58.26 ± 32.4 pg/mL. It means that the average+2SD was 123.06 pg/mL. According to Endo, the average serum LT level in 1 month old babies was 32.3 pg/mL.[8] Iwasaki reported that

the average serum LT level in children aged 4-12 years old was 19.40 pg/mL.[9] Our results were slightly higher than in other studies, but in any case, based on these results, we presume that the baseline serum LT level may be 100 pg/mL or lower.

Volovitz et al. detected LTs in the nasopharyngeal secretions of RSV-specific IgE-positive children with RSV infection, and suggested that the severe bronchiolitis caused by RSV results from IgE-mediated hypersensitivity reactions to viral antigens and the release of chemical mediators of airway obstruction.[7] We demonstrated that the serum LT levels at the end of the hospital stay were significantly elevated in children with RSV infection who had elevated serum total IgE levels at admission. This finding may suggest the association of the serum IgE levels with LT production in children with RSV lower respiratory tract infection.

Sastre et al. reported a statistically significant declines of multiple pro-inflammatory parameters and cytokines in the nasopharyngeal aspirates in the recovery period after bronchiolitis.[10] The LTC4 levels in the nasopharyngeal aspirates decreased to a less significant degree in children who suffered from wheezing within 12 months of a bronchiolitis episode than in those who did not suffer from wheezing.[10] These results suggest the role of elevated LT and IgE levels in deteriorating the prognosis in the poor-prognosis group of children with RSV infection. We expected that our findings might provide further insight into the pathogenesis of RSV infection.

This study had several limitations. The sample size was relatively small and unified diagnostic criteria were not used for the diagnosis of bronchial asthma by the attending pediatrician. We measured the serum LT levels at the end of the hospital stay, but did not follow up the further course of changes of the serum LT levels; evaluation of the course of changes of the serum LT levels over the long term is needed to clarify the roles of LTs and IgE in the poor-prognosis group of children with RSV infection.

Conclusions

The serum LT levels at the end of the hospital stay were clearly elevated in cases with elevated serum total IgE levels at admission. The serum LT levels at the end of the hospital stay were clearly elevated in cases with elevated serum total IgE levels at admission. In addition, the serum LT levels at the end of the hospital stay were significantly elevated in the poor-prognosis group as compared to the good-prognosis group, suggesting that the risk of development of bronchial asthma after RSV infection may be related to increase of the serum total IgE levels in the acute phase and increase of the serum LT levels during the remission phase. However, more studies including a larger number of subjects and specimens and the longer survey period are necessary to clarify the exact mechanism(s).

Conflicts of Interest: None of the authors has any conflicts of interests to declare.

Author Contributions: Nahoko Yasuda, Makiyo Ikutani, Yoko Suzuki, Tomoko Otani, and Shigetaka Sugihara were involved in study conception or design. Nahoko Yasuda, Makiyo Ikutani, Yoko Shida, Kenichiro Takahashi, Yoko Suzuki, and Shigetaka Sugihara were involved in acquisition and analysis of data. All authors critically revised the report, commented on drafts of he manuscript, and approved the final report, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integnity of any part of the work appropriately investigated and resolved.

References

1. Tsutumi H. Pediatric infection science. Tokyo (Japan): Shindan to Chiryo-sha; 2011. RS virus; p. 367−71.
2. Dimova-Yaneva D, Russell D, Main M, et al. Eosinophil activation and cysteinyl leukotriene production in infants with respiratory syncytial virus bronchiolitis. Clin Exp Allergy. 2004;34:555−8.
3. Matsuse H, Kondo Y, Saeki S, et al. Naturally occurring parainfluenza virus 3 infection in adults induces mild exacerbation of asthma associated with increased sputum concentrations of cysteinyl leukotrienes. Int Arch Allergy Immunol. 2005;138:267−72.
4. Kim CK, Choi J, Kim HB, et al. A randomized intervention of montelukast for post-bronchiolitis: effect on eosinophil degranulation. J Pediatr. 2010;156(5):749−54.
5. Morita Y. Complete guide of cysteinyl leukotriene receptor antagonist. Tokyo (Japan): Sentan Igaku-Sha; 2003. Pathogenesis of bronchial asthma and chemical mediators; p. 22−9.
6. Kim CK, Koh JY, Han TH, et al. Increased levels of BAL cysteinyl leukotrienes in acute RSV bronchiolitis. Acta Paediatr. 2006;95:479−85.
7. Volovitz B, Welliver RC, Castro G De, et al. The release of leukotrienes in the respiratory tract during infection

with respiratory syncytial virus: role in obstructive airway disease. Pediatr Res. 1988;24(4):504−7.
8. Endo A. Blood leukotrienes in infants with chronic lung disease. Acta Neonatologica Japonica. 1998;34(1):19−25. Japanese.
9. Iwasaki E. Leukotriene C4 in children with atopic asthma. I. Plasma levels in acute asthma. Acta Paediatr Jpn. 1989;31(3):286−94.
10. Sastre B, García-García ML, Calvo C, et al. Immune recovery following bronchiolitis is linked to a drop in cytokine and LTC4 levels. Pediatr Res. 2020;87(3):581−7.