

Blood Eosinophilia Is Associated With Unfavorable Hospitalization Outcomes in Children With Bronchiolitis

Steven L. Shein, MD,^{1*} Hong Li, MS,² and Benjamin Gaston, MD³

Summary. Background: Bronchiolitis, the most common indication for hospitalization of young children, is associated with subsequent asthma. Blood eosinophilia is associated with increased severity of asthma, but it is unclear if eosinophilia is associated with severity of illness in bronchiolitis. We hypothesized that blood eosinophilia is associated with unfavorable short-term outcomes of bronchiolitis hospitalizations. Methods: Data from initial bronchiolitis admissions to our institution between 2010 and 2013 were extracted using Population Explorer software (Explorys, Cleveland, OH). Children were categorized as "CBC-none" (no complete blood count [CBC] data during the first 7 days of hospitalization), EOS-positive (at least one CBC with ≥ 300 eosinophils per microliter or $\geq 3\%$ of all leukocytes identified as eosinophils) or EOS-negative (at least one CBC and no eosinophilia). The association between hospitalization duration and maximum absolute eosinophil count (AEC) was analyzed using Spearman correlation. Variables independently associated with prolonged (≥ 72 hr) hospitalization were identified using stepwise multivariate logistic regression. Results: In 1356 inpatients <24 months with bronchiolitis, median hospitalization duration was 2.46 days and 38.0% had prolonged hospitalization. CBC data were available in 32.4% of subjects: 20.7% were EOS-positive and 79.3% were EOS-negative. Increased maximum AEC was significantly associated with longer duration of hospitalization. Prolonged hospitalization was independently associated with EOS-positive versus EOS-negative children (OR 1.88, 95%CI: 1.12–3.17, $P = 0.020$). Mechanical ventilation was most common in EOS-positive subjects (24.2% of cases), versus EOS-negative (7.2%) and CBC-none (0.7%) ($P < 0.001$). Conclusions: Blood eosinophilia is associated with unfavorable clinical outcomes in a large cohort of inpatients with bronchiolitis. **Pediatr Pulmonol.** © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

Bronchiolitis is the most common indication for young children to be hospitalized, accounting for $\approx 1/5$ of all post-birth admissions in children <2 years old in the US.^{1–3} It is a viral syndrome typically consisting of an upper respiratory prodrome followed by a lower respiratory tract infection with tachypnea and wheezing.⁴ The clinical course of bronchiolitis varies markedly between patients. Many children have mild disease and are cared for at home, while others require hospitalization and possibly intensive care unit (ICU) admission. In the US, there are $\approx 130,000$ – $170,000$ bronchiolitis admissions per year.^{2,3,5} Recent multicenter data show that 17% of bronchiolitis patients require ICU care and that approximately 2.3–4.6% of bronchiolitis inpatients undergo mechanical ventilation.^{3,6}

Similarities between bronchiolitis and asthma are well known, and bronchiolitis is associated with subsequent asthma during early childhood and possibly longer.^{7,8} More severe asthma phenotypes are associated with increased blood eosinophil count in children and

¹Division of Pediatric Critical Care Medicine, Rainbow Babies and Children's Hospital, Cleveland, Ohio.

²Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio.

³Division of Pediatric Pulmonology, Rainbow Babies and Children's Hospital, Cleveland, Ohio.

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*Correspondence to: Steven L. Shein, MD, Division of Critical Care, Rainbow Babies and Children's Hospital, 11100 Euclid Avenue, 3rd Floor, Cleveland, OH 44106. E-mail: steven.shein@uhhospitals.org

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adults.^{9,10} While prior studies of children with bronchiolitis have identified risk factors for unfavorable disease severity such as younger patient age, abnormal vital signs, and co-morbidities, associations between blood eosinophilia and disease severity have not been thoroughly investigated in large cohorts of bronchiolitis inpatients.^{6,11–14} We hypothesized that blood eosinophilia is associated with increased duration of hospitalization among children admitted to our hospital with bronchiolitis.

METHODS AND MATERIALS

The Institutional Review Board of University Hospitals of Cleveland approved this retrospective study. Cases were identified and data extracted using Population Explorer (Explorys, Cleveland, OH), automated data extraction software that is directly linked to our institution's electronic health record. Data for children with a diagnosis of "bronchiolitis" and age less than 4 years old were extracted into a limited data set. Only the first admission for bronchiolitis for each subject was analyzed. Available data included race, gender, co-morbidities, inpatient laboratory results, and inpatient medications prescribed. Importantly, to protect patient privacy, only birth year (not exact birth date) was available. Analysis was restricted to admissions with a diagnosis of bronchiolitis occurring during the same year as a child's birth or the following year, ensuring that all included subjects were <2 years old. Age was dichotomized as admission occurring during the same year as the child's birth (i.e., definitively <12 months old) or admission occurring in the year following the child's birth. Premature birth was defined as <37 weeks gestation. Analyzed medications were grouped as bronchodilators (albuterol, inhaled epinephrine, or levalbuterol) or corticosteroids (methylprednisolone, prednisolone, or prednisone). Subjects prescribed corticosteroids were subdivided based on route of administration (enteral only vs. parenteral $\pm$ enteral).

Complete blood count (CBC) data from the first 7 days of hospitalization were analyzed. Children were categorized as either "CBC-none" (no CBC data during the first 7 days of hospitalization), EOS-positive (at least one CBC in the first 7 days with ≥ 300 eosinophils per microliter or $\geq 3\%$ of all leukocytes identified as eosinophils) or EOS-negative (at least one CBC and no instances of ≥ 300 eosinophils per microliter or $\geq 3\%$ of all leukocytes identified as eosinophils in the first 7 days).^{15,16} Pre-admission CBC data were not included. Primary outcome was duration of hospitalization and prolonged hospitalization was defined as duration ≥ 72 hr, based on national length of stay data.¹⁷ The secondary outcome was mechanical ventilation. Exact indications for mechanical ventilation (hypoxia, hypercarbia, apnea, etc.) were not available.

The association between hospital length of stay and each patient's maximum absolute eosinophil count (AEC) was analyzed using Spearman correlation. Length of stay was log transformed due to a skewed distribution. Associations between prolonged hospitalization and patient variables were analyzed using χ^2 , *t*-test, and univariate logistic regression analysis. Variables with $P < 0.20$ in univariate analysis were included in a stepwise multivariate logistic regression analysis to identify independently associated variables. Analysis was performed using SAS 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Data for 1,356 children <24 months old admitted with bronchiolitis between 2010 and 2013 were analyzed. The majority of subjects were male (59.3%) and respiratory syncytial virus (RSV) infection was common (57.2% of subjects). More than one-third of admissions occurred during the child's birth year (39.0%). Most subjects were identified in the electronic health record as either African-American (46.7%) or Caucasian (44.5%). Co-morbidities included prematurity (11.1%), congenital heart disease (9.4%), and bronchopulmonary dysplasia (3.2%). Median duration of hospitalization was 2.46 days (interquartile range: 1.67–4.11 days), and 515 (38.0%) subjects had prolonged hospitalization. Fifty-three subjects (3.9%) required mechanical ventilation, all of whom also had prolonged hospitalization. Among the 32.4% of the cohort that had CBC data available, 91 (20.7%) were EOS-positive and 348 (79.3%) were EOS-negative. Maximum AEC ranged from 0 to 2980 cells/ μ l, with a median of 90 (mean = 193.1, standard deviation = 309.6). Increased maximum AEC in these children was significantly associated with longer duration of hospitalization (Fig. 1).

As shown in Table 1, univariate analysis revealed several factors significantly associated with prolonged hospitalization, including younger age, race, premature birth, congenital heart disease, RSV infection, and prescribed medications. First week CBC data were also associated with prolonged hospitalization, which occurred in 67.0% of EOS-positive subjects, 54.9% of EOS-negative subjects, and 28.7% of CBC-none subjects ($P < 0.001$). In our multivariate logistic regression model (Table 2), several variables were independently associated with prolonged hospitalization. EOS-positive children were nearly twice as likely to have prolonged hospitalization as EOS-negative children (OR 1.88, 95%CI: 1.12–3.17, $P = 0.020$) and five times more likely to have prolonged hospitalization than CBC-none children. RSV infection, apnea, congenital heart disease, and medication prescription were also independently associated with prolonged hospitalization.

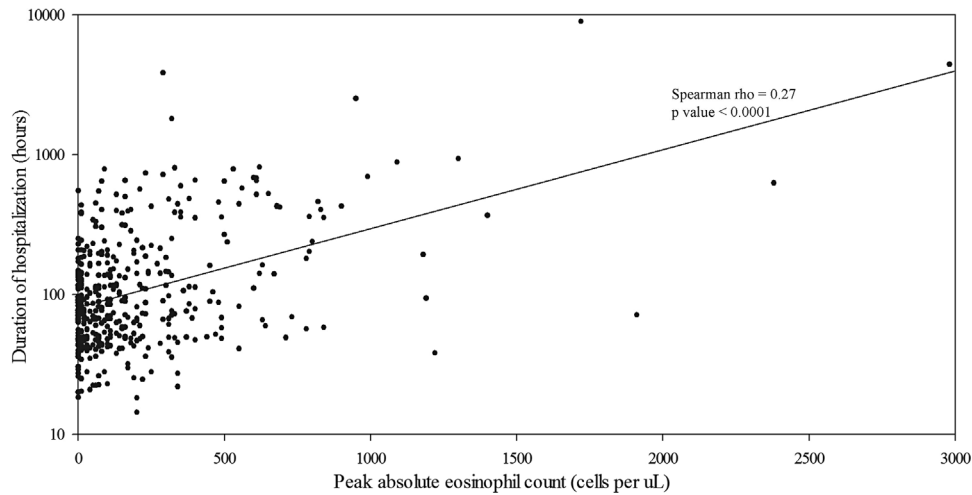


Fig. 1. The maximum measured absolute eosinophil count correlated significantly with increasing duration of hospitalization ($P < 0.0001$).

EOS-positive children were more likely to require mechanical ventilation. As seen in Figure 2, nearly one-quarter of all EOS-positive subjects (24.2%) underwent mechanical ventilation, which was significant among EOS-negative subjects (7.2% underwent mechanical ventilation) and CBC-none (0.7%) (P for trend < 0.001).

DISCUSSION

In this large cohort of bronchiolitis inpatients, blood eosinophilia (≥ 300 cells per microliter or $\geq 3\%$ of all leukocytes) was associated independently with

prolonged hospitalization and also with increased mechanical ventilation prevalence. Our data confirm prior studies of smaller cohorts of bronchiolitis patients that show that eosinophil counts in bronchiolitis vary markedly, with a standard deviation greater than the mean.^{18–20} Reported prevalence of eosinophilia ranges from 4.3 to 68%, though definitions of eosinophilia are inconsistent.^{21–25} In our cohort, 20.7% of children with CBC data had eosinophilia (6.7% of the entire cohort). Associations between eosinophil level and illness severity have been inconsistent in prior studies. Garofalo reported increased mean AEC in RSV

TABLE 1—Patient Characteristics and Univariate Analysis

	Hospitalization <3 days (n = 841)	Prolonged hospitalization (n = 515)	P-value
Age <12months	307 (37%)	222 (43%)	0.016
Male	514 (61%)	290 (56%)	0.081
African-American	409 (52%)	224 (46%)	0.047
White	353 (45%)	251 (52%)	
Premature birth (<37 weeks)	75 (9%)	76 (15%)	<0.001
Congenital heart disease	50 (6%)	78 (15%)	<0.001
Bronchopulmonary dysplasia	18 (2%)	26 (5%)	0.003
RSV positive	415 (49%)	361 (70%)	<0.001
Apnea	4 (<1%)	16 (3%)	<0.001
CBC-none	654 (78%)	263 (51%)	<0.001
EOS-negative	157 (19%)	191 (37%)	
EOS-positive	30 (4%)	61 (12%)	
Bronchodilator prescription	318 (38%)	236 (46%)	0.004
No steroid prescription	680 (81%)	387 (75%)	<0.001
Steroids—enteral only	138 (16%)	73 (14%)	
Steroids—parenteral $\pm$ enteral	23 (3%)	55 (11%)	

RSV, respiratory syncytial virus; CBC-none, patient with no complete blood count (CBC) data during the first week of hospitalization; EOS-negative, patient that had CBC data in the first week of hospitalization, but did not meet criteria for eosinophilia; EOS-positive, patient that had CBC data in the first week of hospitalization, and did meet criteria for eosinophilia (absolute eosinophil count ≥ 300 per microliter or $\geq 3\%$ leukocytes that are eosinophils).

TABLE 2—Patient Characteristics Associated With Prolonged Hospitalization in Multivariate Analysis

	Adjusted OR (95%CI)	P-value
CBC-none	0.37 (0.27–0.50)	<0.001
EOS-negative	1	
EOS-positive	1.88 (1.12–3.17)	0.020
Bronchodilator prescription	1.58 (1.15–2.16)	0.004
No steroid prescription	1	
Steroids—enteral only	0.92 (0.60–1.40)	0.690
Steroids—parenteral ± enteral	3.36 (1.90–5.91)	<0.001
Confirmed RSV	2.76 (2.04–3.73)	<0.001
Apnea	7.88 (2.37–26.22)	<0.001
Congenital heart disease	3.11 (2.02–4.77)	<0.001

RSV, respiratory syncytial virus; CBC-none, patient with no complete blood count (CBC) data during the first week of hospitalization; EOS-negative, patient that had CBC data in the first week of hospitalization, but did not meet criteria for eosinophilia; EOS-positive, patient that had CBC data in the first week of hospitalization, and did meet criteria for eosinophilia (absolute eosinophil count ≥ 300 per microliter or $\geq 3\%$ leukocytes that are eosinophils).

bronchiolitis versus RSV upper respiratory tract infections.¹⁹ Autopsy reports of children who died from bronchiolitis after receiving formalin-inactivated vaccine showed marked tissue eosinophilia, though eosinophils are rare in bronchoalveolar lavage.^{26,27} However, Papoff did not find differences in mean AEC when patients were grouped based on the magnitude of medical interventions (intravenous fluids, oxygen, etc.) required.²⁰ That cohort may have been less ill than ours, as only 2 of 310 children required

mechanical ventilation and 62% required neither intravenous fluids nor supplemental oxygen. Our data strongly support that eosinophilia is associated with more severe disease in a large cohort of bronchiolitis patients.

Eosinophilia may reflect increased secretion of interleukin-5 and interleukin-13 in a subset of bronchiolitis patients, suggesting an altered immune response that predisposes to more severe acute infections and/or the subsequent development of asthma.^{28–30} There are multiple mechanisms by which eosinophils may increase illness severity in bronchiolitis. Eosinophils may cause airway obstruction in bronchiolitis subjects by mucus secretion, cellular desquamation, or bronchospasm. Eosinophilic cationic protein (ECP), which is released from activated eosinophils, causes a dose-dependent increase in the release of respiratory glycoproteins from feline and human airway explants.³¹ Another eosinophilic granule, major basic protein (MBP), causes desquamation of respiratory epithelium and destruction of ciliated cells, as do ECP and eosinophil peroxidase (EPO).^{32,33} Eosinophil granules stimulate mast cell degranulation and histamine release, and cause ciliostasis.^{33,34} Eosinophils are also necessary for inducing airway hyper-responsiveness in a murine model of RSV infection.³⁵ This may be related to the ability of EPO and MBP to antagonize the muscarinic M2 receptor, thereby allowing unopposed M3 receptor-induced bronchoconstriction.³⁶

Eosinophilia may also identify a unique cohort of “pre-asthmatic” bronchiolitis patients, a group perhaps more

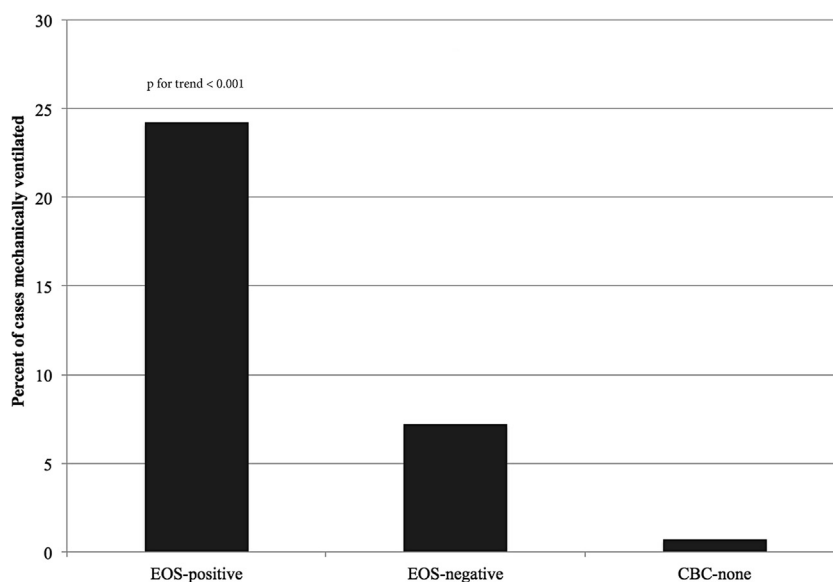


Fig. 2. Rates of mechanical ventilation for each group of subjects. Nearly one-quarter of all EOS-positive subjects (24.2%) underwent mechanical ventilation, which was significant among EOS-negative subjects (7.2%) and CBC-none (0.7%) ($P < 0.001$).

susceptible to certain pharmacologic agents, as eosinophilia during wellness or during bronchiolitis is associated with subsequent asthma.^{10,23,24} In asthmatic adults, increased eosinophil counts are associated both with unfavorable pulmonary function and symptoms, and with improved response to certain treatments.^{9,15,16,37} Corticosteroids, commonly used to treat asthma, cause eosinophil apoptosis, reduce eosinophil chemotaxis and are associated with decrements in eosinophil counts in asthmatic adults.^{37,38} Utility of eosinophilia as a marker of corticosteroid susceptibility in children with bronchiolitis requires investigation.

There are multiple potential applications of this finding. Clinically, measuring eosinophils is readily available and relatively inexpensive, and may allow for identification of children who would benefit from closer observation for clinical deterioration. It is known that children with pre-existing airway hyper responsiveness are prone to more severe bronchiolitis, but these data are rarely clinically available.³⁹ Acute eosinophilia may identify a similar cohort in a much more feasible manner. Our data support that bronchiolitis inpatients found to have eosinophilia should be monitored closely, but we do not recommend phlebotomizing all bronchiolitis inpatients solely to diagnose eosinophilia until confirmatory data are available. Additionally, eosinophilia could be used in research purposes to identify children that are sufficiently ill to warrant inclusion in trials, especially if duration of hospitalization and/or development of respiratory failure are outcome variables.

Several other factors were independently associated with prolonged hospitalization in our data. RSV, apnea and congenital heart disease have all been previously described as risk factors for severe bronchiolitis.^{6,11–14} Bronchodilator and parenteral corticosteroid prescription were also associated with prolonged hospitalization. Expert guidelines published after these data were collected now provide “strong recommendations” that corticosteroids, albuterol, and inhaled epinephrine not be prescribed to non-ICU bronchiolitis patients as the aggregate data suggest no benefit to these medications.⁴ However, recent data show that bronchodilators and steroids are prescribed in 58% and 16% of bronchiolitis admissions, respectively.⁴⁰ Our data suggest that these medications may actually be detrimental, though our retrospective study design precludes proof of causality. Additionally, enteral corticosteroid prescription was not associated with prolonged hospitalization, suggesting that corticosteroids themselves are not detrimental; parenteral corticosteroid prescription may simply be a marker of children deemed too ill to take medications by mouth. Similarly, the decreased odds ratio of prolonged hospitalization in “CBC-none” patients is likely because these children were deemed well enough to not require inpatient phlebotomy. The strengths of this study include data reflecting a very large cohort of subjects

obtained in a narrow and contemporary time frame. The large sample size allowed us to control for multiple variables and identify risk factors independently associated with our primary outcome. Use of automated data extraction software eliminates transcription errors that may occur with manually extracted data. Our median length of stay and mechanical ventilation rate are similar to large, multicenter reports, suggesting generalizability of our data.^{3,12,17} Weaknesses, beyond those inherent to all retrospective studies, include lack of exact age data and duration of symptoms prior to admission. Because we cannot standardize our data to each child’s “day of illness,” we chose to combine all data from the first 7 days rather than assume that all children are admitted at the same point during their illness. Prospective data collection is needed to evaluate how eosinophil counts evolve over the course of bronchiolitis, as our findings may be influenced by recovery from viral-induced eosinopenia over the initial days of hospitalization. Non-universal availability of CBC data may bias our findings towards a more severely ill cohort, as those without CBC data were likely “well enough” to not require phlebotomy, so our findings may not apply to less ill subjects. Though all included subjects were diagnosed with bronchiolitis by a clinician, diagnostic criteria were not standardized and symptoms of bronchiolitis overlap with other conditions (e.g., asthma, pneumonia, etc.). However, as no universal single definition of bronchiolitis exists, this limitation is inherent to all bronchiolitis research. Not all data are extractable with Explorys software, including radiographic results and many microbiology test results. Pre-hospital data are unavailable, and medications (e.g., steroids) administered prior to admission may influence eosinophil levels. Also, the small number of children requiring mechanical ventilation precluded multivariate analysis of that outcome.

In conclusion, in a large cohort of children hospitalized with bronchiolitis, blood eosinophilia, RSV infection, apnea, congenital heart disease, bronchodilators, and parenteral corticosteroids were independently associated with prolonged hospitalization. Eosinophilia was also associated with mechanical ventilation. Further study is needed to confirm the utility of eosinophilia as a clinical marker of bronchiolitis severity and as a research tool.

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