Published in final edited form as:

Pediatrics. 2010 February ; 125(2): e225–e233. doi:10.1542/peds.2009-0411.

LDH Concentration in Nasal-Wash Fluid as a Biochemical Predictor of Bronchiolitis Severity

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Abstract

Objective—Because the decision to hospitalize an infant with bronchiolitis is often supported by subjective criteria and objective indicators of bronchiolitis severity are lacking, we tested the hypothesis that lactate dehydrogenase (LDH), which is released from injured cells, is a useful biochemical indicator of bronchiolitis severity.

Patients and Methods—We retrospectively analyzed a study of children <24 months old presenting to the emergency department with bronchiolitis. Demographic, clinical information, nasal-wash (NW) and serum specimens were obtained. NW samples were analyzed for respiratory viruses, caspase 3/7 activity and a panel of cytokines and chemokines. Total LDH activity was tested in NW samples and sera.

Results—Of 101 enrolled children (median age, 5.6 months), 98 had NW specimens available. A viral etiology was found in 82 patients (83.6%), with respiratory syncytial virus (RSV) (66%) and rhinovirus (19%) being the most common viruses detected. Concentrations of LDH in NW specimens were independent from those in sera, and were higher in children with RSV infection or with dual infection. Significant correlations were found between NW LDH and NW cytokines/chemokines. Similarly, NW LDH correlated with NW-caspase 3/7 activity ($r=0.75$; $P<.001$). In a multivariate analysis, NW LDH concentration in the upper quartile was significantly associated with a reduced risk of hospitalization (odds ratio: 0.19; 95% confidence interval: 0.05–0.68; $P=0.011$).

Conclusions—NW LDH levels in young children with bronchiolitis varied according to viral etiology and disease severity. Values in the upper quartile were associated with ~80% risk reduction in hospitalization, likely reflecting a robust antiviral response. NW LDH may be a useful biomarker to assist the clinician in the decision to hospitalize a child with bronchiolitis.

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Conflict of interest statements: the authors report no conflict of interest.

INTRODUCTION

Bronchiolitis is an acute lower respiratory tract illness that affects the bronchioles of infants and young children.¹ It is predominantly a viral disease, and the number of cases where no agent is identified has decreased over the years with the widespread use of more sensitive diagnostic techniques² and the recognition of new viruses.^{3–7} Respiratory syncytial virus (RSV) is the chief cause of hospitalization for respiratory tract illness in young children^{8, 9} and the most common agent recovered from children with bronchiolitis.¹⁰

There is a need to have a biochemical marker of disease severity to assist the clinician in determining the need for hospitalization in children with bronchiolitis. Lactate dehydrogenase (LDH) may be such a biochemical marker because of its relation to tissue injury. LDH is a membrane-associated protein involved in the oxidation of various organic substrates.¹¹ It is present in all major organ systems, and when found extracellularly, indicates cell damage and inflammation.¹² Measurement of LDH in bronchoalveolar fluid is commonly done as a surrogate for the presence of leukocytes and overall inflammation. In children with RSV bronchiolitis, neutrophils account for over 90% of total cells in nasal washes (NWs) and more than 70% in bronchoalveolar fluid.^{13, 14} LDH activity in NW and serum has not been reported for children with bronchiolitis.

We postulated that LDH would directly correlate with the proinflammatory immune response observed in bronchiolitis, and that it would be a useful biochemical marker of disease severity. We measured LDH in NW and serum specimens from a cohort of children with bronchiolitis that we have reported previously.¹⁵ Our findings indicate that NW LDH levels are greater in children with less severe bronchiolitis; are independently associated with disease outcome, and may constitute a new clinical tool allowing physicians to better establish risk for disease progression. These findings are in direct agreement with our prior observations that reported on the benefits of a robust pro-inflammatory innate response in ameliorating bronchiolitis disease.¹⁵

PATIENTS AND METHODS

Study Design

Aspects of the study have been detailed previously¹⁵, and consisted of a prospective investigation of children <24 months old presenting to the emergency department (ED) with bronchiolitis from November 1, 2004 to February 28, 2005. Children presenting with respiratory distress unrelated to a viral-like respiratory illness were excluded. The study was approved by the institutional review board of Baylor College of Medicine and Texas Children's Hospital. Bronchiolitis was defined as an acute lower airway infection (presence of tachypnea, crackles, wheezing, and/or retractions) and a history of a previous upper-airway infection (defined as nasal congestion and/or rhinorrhea) diagnosed by experienced pediatric ED physicians. The decision to hospitalize a patient was under the discretion of the attending physician following standard clinical pathways. Parameters of disease severity included the need for hospitalization, oxygen supplementation, intravenous fluids and requirement for intubation.

Sample Collection

On arrival to the ED, and after parental consent was obtained, study personnel recorded patient demographic and clinical information and conducted a physical examination. A nasal-wash sample was obtained using a standardize procedure.¹⁵ Viral transport medium was added to a final 1:1 dilution. Aliquots were made, snapped-frozen in a dry ice/alcohol bath, and stored at -70°C .

Detection of Viruses

Viral identification was done in our original report by tissue culture and reverse-transcriptase polymerase chain reaction (RT-PCR), allowing for viral detection in 80 of 98 enrolled children¹⁵ with a 5% prevalence of viral co-infection. In this report, we improved our detection of viruses by testing all specimens with real-time RT-PCR using specific primers and fluorescent probes for RSV, human metapneumovirus (hMPV) and human rhinovirus.^{16–18} As a result, 17 new diagnoses of rhinovirus and two of RSV infection (1 for each subtype A and B) were made. No new case of HMPV infection was detected.

RSV Quantification

RSV concentration was quantified by using 2 methods. Firstly, all RSV-positive samples were titrated by plaque assay in duplicates, and reported as plaque forming units per milliliter (pfu/mL), as previously described.^{15, 19, 20} Secondly, all samples were analyzed by real-time RT-PCR blinded to quantitative culture results. Calibrated standards for each target were assayed in parallel to the samples. The number of cycles required for the fluorescent signal to reach a threshold level was recorded as the crossing threshold. Therefore, the lower the crossing threshold, the higher the amount of genomic material and vice versa.

Cytokine/Chemokine Determination

NW samples were tested for cytokines and chemokines with the Bioplex Human Cytokine 17-plex panel (Bio-Rad Laboratories [Hercules, CA]) following manufacturer instructions. The lower limit of detection for all cytokines and chemokines was 1 pg/mL. These values have been previously reported.¹⁵

Total Protein Measurement

Total protein was quantified in nasal secretions by the bicinchoninic acid method (Pierce BCA Protein Assay Kit, Thermo Scientific, Rockford IL) following the manufacturer instructions and expressed as micrograms per milliliter.

Lactic Dehydrogenase Determination

Total LDH activity was tested in NW samples and sera. NW and serum specimens were diluted 1:1 and 1:20 in phosphate-buffered saline, respectively, and assayed in duplicates following protocol instructions (Cytotoxic Detection Kit, Roche Applied Science [Indianapolis, IN]). To calculate absolute values, L-LDH (Roche Applied Science) was used to construct a standard curve that demonstrated an ample linear dynamic range ($r=.998$) at the dilutions tested from 0.8 to 110 U/mL. The concentration of LDH in NW samples were adjusted fourfold for the dilution resulting from specimen collection.

Apoptosis Quantification

LDH release may be a marker of cell destruction caused by virus or the host cellular immune response, or may be due to apoptosis induced by virus or the innate immune response. To measure apoptosis in nasal secretions, the Caspase-Glo-3/7 kit (Promega [Madison, WI]) was used. The luminescence was measured with a FLUOstar OPTIMA microplate reader (BMG Labtech [Cary, NC]) and expressed as relative luminescence units.

Statistical Analysis

LDH concentrations (in NW and serum samples) and caspase activity were normalized by $\log_{10}$ transformation. The statistical evaluation of normally distributed continuous variables was performed with Student's *t* test or 1-way analysis of variance as appropriate. Differences

of proportions of non-parametric data were compared with the χ^2 test. Correlations were calculated by Pearson's or Spearman's coefficients as appropriate. Statistical significance was established with 2-tailed tests and an α level of $P < .05$.

The association between hospitalization (defined as hospital stay > 24 hours) and independent variables was investigated with multivariate logistic regression analyses. Factors identified by univariate analysis were included in the model if the association with the outcome had P value less than .25. The initial model contained the following categorical variables: age (≤ 3 or > 3 months); fever (yes or no), hypoxia (yes or no), administration of intravenous fluid (yes or no), and detection of a viral pathogen (yes or no). The NW LDH concentration and the caspase 3/7 activity were classified in quartiles for inclusion in the model ($\geq 75\%$ or $< 75\%$). We removed fever, detection of a virus, and caspase 3/7 activity from the final model because they did not contribute to the goodness-of-fit of the model.

All statistical analyses were performed using Stata 10.0 for Windows (Stata Corp, College Station, TX).

RESULTS

Etiologic Agents of Bronchiolitis in the Population

As described in detail in our previous study, 101 children with bronchiolitis were enrolled from the ED at Texas Children's Hospital. Two patients were excluded from the analyses (1 violated inclusion criteria, and another withdrew consent). One specimen was lost in transit to the laboratory although clinical information was retained. NW samples were available for viral diagnosis in 98 subjects. Clinical data was available in 97 patients with known caspase levels and 94 patients with known LDH concentration in NWs. The total numbers of children in the analyses reflect the missing data

Median age at presentation was 5.6 months (range: 0.46–23.9), and 64% were boys.¹⁵ Forty-six percent had a family history of asthma, and 22% had a history of eczema. Median duration of illness at the time of presentation to the ED was 4 days (mean $\pm$ SD: 5.2 ± 4.2 days).

A specific viral etiology was determined in 83.6% of the patients (Table 1). RSV infection was the leading cause of bronchiolitis, present in 66.3% of individuals. Rhinovirus was second in frequency, being detected in 19.4% cases. Of note, 16% of patients had dual infections.

Analysis of RSV subgroups revealed 52 cases of RSV-A and 13 cases of RSV-B. There were no differences in children with A or B strains with respect to hospitalization, need for mechanical ventilation, oxygen requirement > 24 hours, IVF requirement > 24 hours or mean concentrations of LDH in the NW samples (data not shown). The proportion of RSV infected subjects was comparable between those hospitalized versus those discharged home from the ED ($P = .3$).

Demographic and Clinical Factors

There were no differences in NW LDH concentrations according to age, gender, race, Hispanic or Latino ethnicity, family history of atopy, environmental exposure to tobacco smoke, day care attendance or exposure to young children (Table 2). The detection of a virus ($P = .01$ versus no virus) or the presence of dual viral infection ($P = .02$ versus single or no viral infection) were associated with higher concentration of NW LDH. Children with RSV infection had greater NW LDH concentration compared with children not infected with RSV ($P < .001$). This difference persisted after excluding the 15 patients with dual infection with

RSV and another viral pathogen (mean log₁₀ NW LDH: 2.15 [95% confidence interval (CI): 1.98–2.32] versus 1.58 [95% CI: 1.24–1.93] for patients with single RSV infection versus patients without RSV detection, respectively ($P = .001$).

NW LDH, Total Protein and RSV Concentration

NW LDH concentration was determined in the study population (Fig 1). Levels were not significantly different between children in the following age groups: <3, 3 to 5, 6 to 11, and 12 to 23 months (data not shown). No correlation was observed between concentration of LDH in serum and NW fluid (Spearman's $\rho = 0.06$; $P = .6$).

We have observed in vitro that increased RSV replication in respiratory epithelial cells results in greater concentrations of LDH in the supernatant, and that this effect is potentiated by the presence of proinflammatory cytokines.²¹ The correlation between NW LDH and infectious RSV measured by plaque assay failed to reach statistical significance ($P = .05$, Fig 2A), however, a significant correlation was noticed when the RSV load was quantified by subgroup-specific real-time PCR (RSV-A, $r = -0.48$, $P < .001$; RSV-B: $r = -0.62$, $P = .015$, Fig 2 B and C).

Total protein content in the NW fluid may be a combination of proteins released during virus-induced epithelial cell injury and the host immune response. As expected, a significant correlation was observed between NW total protein and NW LDH concentration ($r = 0.48$; $P < .001$; Fig 2D).

Cytokines, Chemokines and Apoptosis

T helper (Th) 1 cytokines (Interleukin-2 [IL-2], interferon γ , tumor necrosis factor α , and IL-1 β), Th2 cytokines (IL-4, IL-6, and IL-10), and other proinflammatory cytokines (macrophage inflammatory protein 1 β , monocytes chemoattractant protein 1, IL-8, granulocyte-macrophage colony-stimulating factor [GM-CSF], and granulocyte colony-stimulating factor) detected in NW fluid samples were all strongly correlated with LDH in NW samples (Table 3). A positive linear correlation was observed between NW LDH and NW caspase 3/7, a measure of apoptosis activity ($r = 0.75$; $P < .001$) (Fig 3).

Analysis of Indicators of Severe Bronchiolitis

Classic indicators of bronchiolitis severity are shown in Table 4 with their respective levels of NW LDH. Children with bronchiolitis who were discharged home from the hospital from the ED had significantly higher NW LDH concentrations than those who were admitted for supportive care (2.36 vs. 1.85 U/mL; $P = .002$) (Fig 4). Hospitalization occurred in 82%, 71%, 50%, and 58% of children aged 0 to 2, 3 to 5, 6 to 11 and 12 to 23 months, respectively.

Approximately half of the hospitalized patients required supplemental oxygen. Patients receiving oxygen >24 hours had lower NW LDH levels compared to children who received $\leq$ 24 hours of oxygen, although this difference disappeared when considering only those children who were hospitalized (data not shown). Patients with high levels of NW LDH received shorter duration of oxygen supplementation or none (Wilcoxon rank-sum test $z = 2.76$; $P = .006$). Intubation was not associated with differences in NW LDH, but only 6 patients required mechanical ventilation. Collectively, these data indicate that a more severe clinical presentation leading to hospitalization was associated with lower levels of NW LDH concentration.

Odd Ratios for Bronchiolitis Severity Leading to Hospitalization According to Multivariate Analysis

Quartile distribution of NW LDH was as follows: low-quartile group, <31.2 U/mL; mid-quartile, 31.2 to 364.8 U/mL; high-quartile, ≥ 364.8 U/mL. The univariate analysis identified the following variables for inclusion in the multivariate logistic regression analysis for hospitalization: presence of fever (odds ratio [OR]: 0.48; $P = .12$), need for intravenous fluids (OR: 13.2; $P < .001$), detection of a viral pathogen (OR: 0.41; $P = .19$), age ≤ 3 months (OR: 3.19; $P = .02$), NW LDH concentration above 75% percentile (OR: 0.14; $P < .001$), NW-caspase activity above 75% percentile (OR: 0.47; $P = .13$), and presence of hypoxia (OR: 9.80; $P = .03$). The final multivariate prediction model for hospitalization among children presenting to the ED with bronchiolitis is presented in Table 5. In this model, age ≤ 3 months and the presence of hypoxia were associated with ~ 5- and 12-fold increase in hospitalization, respectively. High concentrations of NW LDH (≥ 365 U/mL) were associated with an 81% reduction in the need for admission ($P = .011$).

The final model gave an area under the receiver operating characteristic (ROC) curve of 0.87, with a Pearson goodness-of-fit test statistic of 12.85 ($P = .12$) (Fig 5). Assuming a predicted probability of hospitalization cutoff value of 0.5, the model correctly classified 79.6% of patients when all observations were available for inclusion in the logistic regression. Twelve patients would have been incorrectly sent home (false negative rate, 19%). The sensitivity, specificity, positive predictive value and negative predictive value were 81%, 77%, 88% and 66%, respectively.

DISCUSSION

To the best of our knowledge, this is the first description of LDH in NW specimens of young children with bronchiolitis. NW LDH was found to be a significant independent biochemical marker in reducing the risk for hospitalization. We observed that NW LDH levels were significantly higher in children sent home from the ED compared to those admitted for bronchiolitis ($P = .002$), and strongly correlated with the apoptosis marker caspase 3/7 and with cytokines and chemokines involved in the innate and adaptive immune response. The detection of viruses was associated with higher NW LDH values. It is important to note that NW LDH did not correlate with serum LDH, implying that NW LDH originated from cellular events that occurred in the respiratory airways.

We did not observe a correlation between NW and serum LDH. This indicates that LDH in nasal secretions is unlikely to be derived from capillary extravasation of protein into the airways. Supporting this notion, studies evaluating LDH concentration in bronchoalveolar fluid concluded that its source is different from serum.^{22, 23} When considering the relation of NW-to-serum LDH as a ratio, a factor ≥ 0.6 was more likely to be present in children being sent home from the ED compared to those admitted for supportive care ($P = .003$, data not shown). Thus, it is reasonable to hypothesize that LDH originates from widespread airway epithelial cell injury and apoptosis^{21, 22, 24} or from leukocytes (largely polymorphonuclear cells)²⁵ present in the NW fluid. The strong correlation between NW LDH, IL-8 and GM-CSF supports polymorphonuclear cells and other leukocytes as a source for LDH, while the near linear correlation with caspase 3/7 suggest apoptosis is contributing to NW LDH. A better understanding of the source of the NW LDH may provide additional information on the early innate mechanisms involved in virus-induced bronchiolitis.

RSV is the major etiologic agent of bronchiolitis and our data reflect this fact with 66% of cases attributed to RSV. Intuitively, elevated concentrations of RSV should be closely linked to more severe bronchiolitis disease. Previous studies support this theory.^{26–28} DeVincenzo et al analyzed 141 infants infected with RSV who were hospitalized during 4

winter seasons. Markers of disease severity included requirement for intensive care, respiratory failure, and duration of hospitalization. An increased NW RSV load was a significant independent predictor of all 3 markers of disease severity.²⁷ Buckingham et al²⁶ studied with RSV infection who were hospitalized. Mean NW concentrations were significantly higher in patients who required mechanical ventilation (5.06 ± 0.34) compared to those who did not (3.91 ± 0.35). In contrast, Wright et al²⁹ examined RSV titers in NW samples from 77 infants who were hospitalized with bronchiolitis, and did not find demonstrable correlation between viral titer and severity of illness. Martin et al³⁰ described patients with respiratory infection attributed to RSV where, after adjusting for age and the presence of comorbidities, the frequency of hospitalization decreased with each log increase in viral load (OR: 0.8 [95% CI: 0.7–0.997]; $P = .047$). The strong correlation we observed between NW LDH and RSV viral load (as quantified by real-time PCR) was not present when only infectious virus was assessed ($P = .05$) with the plaque assay (Fig 1A–C). We attribute this discrepancy to the possible presence of RSV neutralizing antibodies in nasal secretions [disproved by Wright et al²⁹] or an increased precision of PCR over traditional methods [disproved by Bae et al³¹].

Significant correlations were observed between NW LDH and various cytokines and chemokines, which may reflect the activation of parallel biological responses related to viral infection and innate and adaptive immune system activation. Aberrant Th2 immune responses³² and mutations in genes involved in the innate immunity have been linked to RSV bronchiolitis susceptibility and severity.^{33, 34} However, recent data suggest that cytokine levels do not necessarily correlate with disease presentation.³⁵ Welliver et al²⁴ showed that infants with fatal RSV lower respiratory tract disease succumbed to overwhelming viral infection and epithelial cell apoptosis and not to a cytokine storm. Lung sections were characterized by a paucity of lymphocytes and natural killer cells, unchecked viral replication, and apoptotic crisis. These recent studies support the concept of a protective effect derived from a robust innate immune response during an episode of RSV bronchiolitis, where inflammatory markers inversely correlate with disease severity.^{15, 24, 35}

To date there is no biochemical marker predictive of disease severity in children with bronchiolitis. In our study, the sensitivity and specificity of NW LDH for predicting hospitalization was 81% and 77%, respectively. These values are comparable to many of the point-of-care tests used in diagnosing a viral infection. The LDH assay is easy to perform, inexpensive, and available in most clinical laboratories. At our institution, the expected turnaround time for serum LDH is 1 hour, and NW samples should not be treated differently. Currently the presence of hypoxemia, significant respiratory distress and clinical judgment are the main consideration for determining the need to hospitalize a child with bronchiolitis. Having a validated biochemical marker predictive for hospitalization can provide another objective parameter to the physician, and would be valuable in difficult-to-assess cases.

A limitation of our study was that a single NW sample was obtained from each patient, and severity of disease was determined by the final disposition of the patient and the need for therapeutic interventions. Therefore, disease progression may have occurred in patients who were discharged from the hospital. The data were collected during a single season when RSV was most prevalent (and, therefore, having more weighted impact on the results), and originated from a single center. We recognize that repeat sampling and/or longer follow-up of these patients would strengthen the conclusions. Despite these limitations, our results were consistent for multiple clinical outcomes and support the validity of our conclusions.

This study is strengthened by our comprehensive effort to characterize viral agents and the assessment of the complex interplay between RSV concentration, the inflammatory

response, patient age, and severity of bronchiolitis. In addition, we included children with mild to severe bronchiolitis in the ED setting which was critical for a more comprehensive analysis. Additional evaluation of our hypothesis is warranted and will add to a better understanding of the immunopathogenesis of bronchiolitis.

CONCLUSIONS

The determination of LDH in NW has the potential of being a useful clinical biochemical marker of disease severity among children with bronchiolitis. Studies validating our observations will be needed before NW LDH can be incorporated into the clinical decision of whether to safely discharge from the hospital a young child presenting to the ED with mild to moderately severe bronchiolitis.

Acknowledgments

This study was supported in part by the National Institutes of Health (NIH) Baylor Research Training Program for Pediatricians grant T32 HD42977 (Dr. Laham; Morey W. Haymond, principal investigator) and Viral Respiratory Pathogen Research Unit contract N01-AI-30039, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH (Drs. Piedra and Garofalo), NIH N01 HV28184 (Dr. Garofalo), and P01 AI062885 (Dr. Garofalo).

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Figure 1.
NW LDH concentrations of children with bronchiolitis

Figure 2.

(A) Correlations between NW LDH and RSV titer, as determined by plaque assay, or by RSV-A (B) and RSV-B (C) subgroup-specific real-time PCR. (D) Correlation between NW LDH and NW total protein. Ct indicates crossing threshold; PFU, plaque-forming units.

Figure 3.
Correlation between LDH and caspase 3/7 activity in NW samples. RLU indicates relative luminescence units.

Figure 4.
NW LDH concentrations according to hospitalization status.

Figure 5.
ROC curve for the multivariate logistic analysis presented in Table 5.

Table 1

Viral Etiology of Bronchiolitis in Children <2 Years Old

Virus	No. of Patients
RSV	50
Rhinovirus	8
RSV + rhinovirus	11
Adenovirus	2
Picornavirus	2
Cytomegalovirus	1
Other coinfections:	5
RSV + HMPV	1
RSV + Adenovirus	2
HMPV + Adenovirus	1
RSV + Parainfluenza type 2	1
No virus	16
Total	96

A viral pathogen could not be further identified in 2 patients for whom tissue culture cells exhibited cytopathic effect.

Table 2

Demographic and Clinical Features of Children <2 Years Old with Bronchiolitis and LDH Concentration in NWs

Condition	n	Mean NW-LDH Concentration Log 10 (95% CI), U/mL	P
Host			
Age, mo			
0–3	31	1.99 (1.73–2.24)	.36
3–6	21	2.23 (1.91–2.54)	
6–12	31	2.00 (1.73–2.29)	
12–24	12	1.74 (1.12–2.36)	
Gender			
Boy	61	2.00 (1.80–2.20)	.86
Girl	33	2.03 (1.76–2.16)	
Race			
Black	14	1.93 (1.47–2.39)	.59
Asian	1	1.08 (NA)	
White	73	2.02 (1.84–2.20)	
Other	4	2.23 (0.85–3.60)	
Ethnic category			
Hispanic or Latino	45	2.03 (1.81–2.26)	.71
Not Hispanic or Latino	47	1.98 (1.75–2.21)	
Atopy			
Family history of asthma	42	2.03 (1.82–2.25)	.63
No family history of asthma	49	1.96 (1.72–2.19)	
Patient history of wheezing	26	1.95 (1.62–2.29)	.66
No patient history of wheezing	67	2.03 (1.85–2.21)	
Environment			
Exposure to cigarette smoke	25	1.95 (1.60–2.30)	.64
No exposure to cigarette smoke	68	2.03 (1.86–2.21)	
Daycare	16	1.99 (1.61–2.37)	.85
No daycare	75	2.03 (1.85–2.20)	
Exposure to children <5 y old	58	2.05 (1.86–2.24)	.62
No exposure to children <5 y old	33	1.97 (1.67–2.26)	
Clinical presentation			
Duration of illness, d			
1–2	21	2.07 (1.70–2.44)	.29
3–4	31	2.00 (1.72–2.27)	
5–6	19	1.97 (1.58–2.35)	
7–10	16	2.24 (1.95–2.52)	
>10	7	1.48 (0.66–2.31)	
Virus detection			
No virus identified	14	1.42 (0.89–1.95)	

Condition	n	Mean NW-LDH Concentration Log 10 (95% CI), U/mL	P
Single infection	65	2.05 (1.87–2.22)	.001
Dual infection	16	2.40 (2.15–2.67)	
RSV status			
RSV	64	2.21 (2.07–2.36)	<.001
Non-RSV	32	1.63 (1.30–1.96)	

NA indicates not applicable.

Table 3

Correlations Between Cytokine/Chemokines and NW LDH Concentrations

Cytokine/Chemokine	Correlation Coefficient	P
Th 1 cytokines		
IL-2	0.46	<0.001
Tumor necrosis factor α	0.79	<0.001
Interferon γ	0.80	<0.001
IL-1 β	0.84	<0.001
Th 2 cytokines		
IL-10	0.61	<0.001
IL-4	0.67	<0.001
IL-6	0.75	<0.001
Chemokines and other proinflammatory cytokines		
GM-CSF	0.63	<0.001
Monocyte chemoattractant protein 1 (CCL4)	0.66	<0.001
Granulocyte colony-stimulating factor	0.78	<0.001
Macrophage inflammatory protein 1 β (CCL2)	0.80	<0.001
IL-8 (CXCL8)	0.86	<0.001

Table 4

Univariate Analysis for Indicators of Severe Bronchiolitis

Severity Parameter	n	Mean NW LDH Concentration (95% CI)	P
Hospitalization			
Yes	64	1.85 (1.65–2.04)	.002
No	30	2.36 (2.14–2.58)	
Oxygen supplementation			
≤24 h	64	2.13 (1.94–2.31)	.03
>24 h	30	1.76 (1.49–2.04)	
Intubation			
Yes	6	1.76 (0.87–2.64)	.40
No	88	2.03 (1.87–2.19)	
Intravenous fluids			
Yes	39	1.87 (1.61–2.12)	.12
No	55	2.11 (1.91–2.31)	
Intravenous fluids			
≤24 h ^a	71	2.09 (1.91–2.27)	.06
>24 h	23	1.75 (1.45–2.05)	

^a indicates patients who did not receive intravenous fluids.

Table 5

Multivariate Logistic Model to Ascertain Factors Related to Hospitalization Among Children Treated in the ED for Bronchiolitis

Characteristic	OR	(95% CI)	P
Age $\leq$ 3 mo	5.45	1.42–20.91	0.013
Need for intravenous fluids	10.70	2.55–44.87	0.001
Hypoxia	12.16	1.09–135.62	0.042
Log ₁₀ NW LDH $\geq$ 2.56 U/mL ^a	0.19	0.05–0.68	0.011

^aUpper quartile, corresponding to values $\geq$ 365 U/mL; $P < 0.001$; pseudo- $R^2 = 0.35$.