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Impact of community respiratory viral infections in urban children with asthma

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Abstract

Background: Upper respiratory tract viral infections cause asthma exacerbations in children. However, the impact of natural colds on asthmatic children in the community, particularly in the high-risk urban environment, is less well-defined.

Objective: We hypothesized that children with high-symptom upper respiratory viral infections have reduced airway function and greater respiratory tract inflammation than children with virus-positive low-symptom illnesses or virus-negative upper respiratory tract symptoms.

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Conflicts of interest: none

Methods: We studied 53 asthmatic children from Detroit, Michigan during scheduled surveillance periods and self-reported respiratory illnesses for one year. Symptom score, spirometry, fraction of exhaled nitric oxide (FeNO) and nasal aspirate biomarkers, viral nucleic acid and rhinovirus (RV) copy number were assessed.

Results: Of 658 aspirates collected, 22.9% of surveillance samples and 33.7% of respiratory illnesses were virus-positive. Compared to the virus-negative asymptomatic condition, children with severe colds (symptom score ≥5) showed reduced forced expiratory flow at 25–75% of the pulmonary volume (FEF25–75), higher nasal mRNA expression of C-X-C motif chemokine ligand (CXCL)-10 and melanoma differentiation-associated protein 5, and higher protein abundance of CXCL8, CXCL10 and C-C motif chemokine ligands (CCL)-2, CCL4, CCL20 and CCL24. Children with mild (symptom score 1–4) and asymptomatic infections showed normal airway function and fewer biomarker elevations. Virus-negative cold-like illnesses demonstrated increased FeNO, minimal biomarker elevation and normal airflow. RV copy number was associated with nasal chemokine levels but not symptom score.

Conclusion: Urban asthmatic children with high-symptom respiratory viral infections have reduced FEF25–75 and more elevations of nasal biomarkers than children with mild or asymptomatic infections, or virus-negative illnesses.

Keywords

chemokine; cold; exhaled nitric oxide; MDA5; nasal; rhinovirus; spirometry; urban; viral load

Introduction

Viral infections are the most common cause of asthma exacerbation in children. Cross-sectional studies of outpatient children who are sick with asthma exacerbations have shown 61–81% positivity for viral infection compared to 21–42% of children who are well[1–4]. Rhinovirus (RV) makes up over 50% of viruses isolated. Viral detection is associated with asthma exacerbation treatment failure [5].

Nevertheless, apparently well children with asthma may also harbor respiratory viruses in their airways [1, 6–9]. Twenty-one percent of hospitalized children three years or older without wheezing tested positive for virus [3]. Similarly, 23% of children two years or older with well-controlled asthma tested positive for virus [4]. Virus detection rates in healthy children are higher in young children [10–15] and developing communities[16]. Given the high rate of RV transmission within families [17] and the one-to-three week duration of RV shedding after infection, most asymptomatic infections likely represent children convalescing from a symptomatic viral infection [18].

The impact of respiratory viral detection in children with asthma in a community environment is less well studied. In children from Madison, Wisconsin, virus-positive weeks were associated with greater asthma symptoms, as well as more frequent loss of asthma control [19, 20]. In a community cohort of asthmatic children from Randrick, Australia (a suburb of Sydney), Australia, RV was detected in 25.5% of nasal samples and associated with increased cough, phlegm, wheeze and chest tightness [21, 22]. There was no change in

peak expiratory flow (PEF) or forced expiratory volume in 1 second (FEV_1). However, effects of natural respiratory viral infections on lung function and symptoms in urban children with asthma remain largely undefined. Patterns of viral respiratory illnesses may differ between urban and suburban children. For example, sick inner-city infants have lower rates of viral detection than suburban infants. [23] In addition, asthma is undertreated in urban children [24], which may amplify the effects of viral infection. Finally, while we [25] and others [26–29] have examined nasal cytokine responses of children with asthma to natural colds, potential effects of asymptomatic or mild viral infections have not been studied.

We hypothesize that, children with asthma in an urban community environment who experience high-symptom upper respiratory viral infections have reduced pulmonary function as well as greater respiratory tract inflammation and viral copy number than children with virus-positive low-symptom illnesses or virus-negative upper respiratory tract symptoms. We therefore examined the influence of viral infection on respiratory symptoms, lung function and nasal cytokines in children with asthma from Detroit, Michigan.

Methods

Screening questionnaire.

This study was conducted by Community Action Against Asthma (CAAA), a community-based participatory research partnership, as part of an environmental epidemiology study evaluating the impact of roadway-associated air pollution on asthma health. Children with known or probable asthma living in Detroit, Michigan were recruited using a screening questionnaire [24] distributed at community venues and through door-to-door recruitment in neighborhoods near highways. The questionnaire asked about demographic information, symptoms, and if their child had ever been diagnosed by a medical care provider with any of the following conditions: asthma, bronchitis, bronchiolitis, reactive airways disease or pneumonia. Parents were also asked whether their child had taken prescription medication for these conditions. Classification of asthma severity was based on symptom frequency and reported inhaled steroid use (eTable 1). Children were classified as atopic if they reported having hay fever, nasal allergies or eczema. This study was approved by the University of Michigan IRB (ID# HUM00018442).

Data and sample collection.

Fifty-three children participated in a two-week surveillance assessment period of health status each season from fall 2010 to summer 2011. During each two-week surveillance period, staff obtained spirometry, symptom reports and nasal lavage samples during three home visits. Respiratory symptoms were assessed using a modified version of a previously published respiratory symptom score [30] assessing fever, cough, sore throat, nasal symptoms, wheezing, difficulty breathing and interference with activities (eTable 2). By definition, children with wheezing, difficulty breathing or breathing fast had symptom scores ≥5. Families were given a calendar and respiratory symptom scale to mark the level of their symptoms.

From winter 2010 to summer 2011, measurements were repeated during a one-week period whenever the child experienced a symptomatic respiratory illness as defined by a symptom score of two or higher (referred to as a “sick period”). We set a low symptom threshold in order to maximize sensitivity to detect viral illnesses. Families called when the child became ill. When symptoms reached the appropriate threshold, staff would begin a “sick period” assessment within 48 hours of the phone call (median time to first sample was 72 hours after symptom development). Staff also conducted weekly telephone calls to identify illnesses in progress that families may not have reported and initiated a “sick period” collection if the child had current symptoms.

Nasal lavage.

Nasal lavage samples were collected three times during a two-week surveillance period or a one-week sick period by field staff. Two squirts of isotonic 0.65% NaCl (B.F. Ascher, Lenexa, KS) were instilled into the child’s nostrils. Subjects then blew their nose into a zippered plastic bag, and three ml of M4RT viral transport medium (Remel, Lenexa, KS) were added. After collection, samples were placed in transport cooler at 0°C and transported to the Henry Ford Health System Epidemiology Lab for freezing to −70°C, and subsequently transported to Ann Arbor on dry ice.

Detection of respiratory viruses.

Nasal lavage samples were homogenized (Thermo Fisher Scientific, Waltham, MA) and nucleic acids extracted using TRIzol-LS (ThermoFisher), chloroform and an RNeasy Mini Kit (Qiagen, Valencia, CA). Samples were analyzed for viral nucleic acid by multiplex PCR (Seegene Seeplex RV-15 ACE detection kit, Concord, CA). This kit detects human adenovirus, bocavirus 1–4, coronaviruses 229E/NL63 and OC43, enterovirus, influenza A and B, metapneumovirus, parainfluenza viruses 1–4, respiratory syncytial virus (RSV) A and B and rhinovirus A, B and C. For surveillance samples, all specimens were analyzed for virus; for cold samples, specimens from the same sick week were pooled prior to viral detection analysis (samples from sick periods were not pooled for cytokine or viral copy number determination, see below).

Nasal lavage mRNA and protein expression.

All nasal samples were analyzed for mRNA and protein. cDNA was synthesized from total RNA by Taqman reverse transcriptase kit (Qiagen). DNA was digested with DNase I (Qiagen). C-X-C motif chemokine ligand (CXCL)-8, CXCL10, interferon regulatory factor 7 (IRF7), retinoic-acid-inducible protein 1 (RIG-I), melanoma differentiation-associated protein 5 (MDA5), Toll-like receptor 3 (TLR3) and interferon (IFN)-λ1 mRNA expression were measured by qPCR. Specific primers and probes spanning exon-exon junctions (intron splice-sites) were used to prevent amplification of genomic DNA. Expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the ΔΔCt method. Reactions with a GAPDH cycle number >35 were not analyzed. CXCL8, CXCL10, CCL2, CCL4, CCL5, CCL20, CCL24, interleukin (IL)-4, IL-13 and ICAM-1 protein levels were determined by multiplex immune assay (Affymetrix, Santa Clara, CA). Biomarkers were chosen based on previous studies showing elevations after RV infection, our interest in

examining biomarkers we had not previously studied, difficulty of detecting some biomarkers or cytokines in nasal aspirate fluid, cost and availability.

RV copy number and typing.

For samples testing positive for RV, copy number was determined by qPCR using previously published primers[31].

RV typing.

RV-positive surveillance samples and one sample each from RV-positive sick period were further analyzed to determine RV genotype. RV typing was performed by semi-nested PCR amplification of the P1-P2 region from gel purified PCR products [32]. The identity of each sequence was determined by comparison to known 5′ sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Spirometry.

Using protocols we developed for large-scale community-based asthma studies [33], staff conducted spirometry to assess lung function during home visits using the EasyOne spirometer (NDD, Andover, MA).

Fraction of exhaled nitric oxide in exhaled breath (FeNO).

FeNO was measured using the NIOX MINO (Aerocrine, New Providence, NJ).

Statistical analysis.

Our initial analysis identified that, in addition to anticipated viral-positive illnesses and virus-negative asymptomatic periods, there were surveillance samples in which viruses were detected and symptomatic illnesses during which no virus was detected. This prompted us to perform a post-hoc analysis looking for similarities and differences between six groups: virus-positive/severe symptoms (symptom score ≥5); virus-negative/mild symptoms (symptom score 1–4); virus-negative/asymptomatic (symptom score 0); virus-negative/severe symptoms (symptom score ≥5); virus-positive/mild symptoms (symptom score 1–4); and virus-positive/asymptomatic (symptom score 0). Mean and standard deviation were used to describe nasal biomarker protein levels, nasal mRNA levels and symptom score before and during viral illnesses. Distributions of continuous outcome variables were examined and appropriate transformations taken to achieve normality. TLR3 and IFN-λ1 mRNA levels were undetectable in a large number of samples (see eTable 3) and therefore these results were analyzed as a binary variable (detectable, undetectable).

Effects of viral/symptom state on individual symptoms, lung function, nasal aspirate mRNA and protein levels and RV copy number were determined using generalized estimating equations (GEE), with an exchangeable correlation structure using the identify link for continuous outcomes and the log link for binary ones. Analysis was performed using SAS software (Cary, NC). We evaluated and adjusted for age, gender, ethnicity/race, self-reported atopy, smoker in the home, caregiver educational attainment, season of sample collection and whether the sample was from a surveillance or sick collection period. Family income, proximity to high-traffic highways, baseline asthma severity and medication use were

evaluated but not included in final models as they were not significant predictors. Significance level was set at $P < 0.05$.

Results

Study participants.

Fifty-three children with asthma were enrolled. Surveillance samples were collected from September 2010 to August 2011 and sick samples were collected from December 2010 to August 2011. Subjects were predominantly African-American (Table 1). Most subjects were atopic, exposed to tobacco smoke and had a household income less than $15,000. Based on symptom frequency and reported inhaled steroid use, most children had mild intermittent or mild persistent asthma. Approximately one-quarter had moderate-to-severe persistent disease and used inhaled corticosteroids within the last year. Of note, this community has a high rate of poorly-controlled asthma and undertreatment with inhaled corticosteroids 24, 34. Mean values of forced vital capacity (FVC), FEV1, and PEF measured at the time of the first surveillance visit were normal, but FEV_1/FVC ratio and forced expiratory flow at 25–75% of the pulmonary volume ($FEF_{25–75}$) were mildly reduced (Table 2). Average FeNO was elevated. Group mean surveillance nasal aspirate mRNA and protein values are shown in Table 3.

Participant respiratory illnesses.

From September 2010 to August 2011, 410 surveillance samples were collected, 94 (22.9%) of which were positive for one or more viruses. From December 2010 to August 2011, there were 83 self-reported respiratory illnesses, for which 248 samples were collected. Analysis of samples pooled within each individual sick period showed that 28 of 83 (33.7%) of these illnesses were positive for virus. Thus, subjects were only slightly more likely to have a virus during self-reported colds than during surveillance sample collection. RV was detected in 50 (53.2%) of virus-positive surveillance samples and 22 (78.6%) of virus-positive sick periods (Table 3). Due to the large number of virus-negative self-reported illnesses, we retested the 83 pooled sick period samples for RV using qPCR. Of the 20 Seegene samples with single RV infections, 17 (85%) were positive for RV by qPCR. Of the 61 Seegene RV-negative samples, 2 (3%) were positive for RV by qPCR. Seventy-four samples from single RV infections were examined for genotyping by qPCR. Sequences of 73 samples revealed a specific RV genotype. Median level of identity was 95%. Infections consisted of 60 species A infections, two species B infections and 11 species C infections.

Analysis of virus-positive high-symptom and low-symptom conditions.

During surveillance periods, virus-negative samples were associated with a symptom score of 2.6±4.5 (mean ± SD) and virus-positive samples were associated a symptom score of 3.1±4.2 (p=0.008, Wilcoxon rank sum test). During sick periods, virus-negative samples were associated with a symptom score of 6.4±5.7 and virus-positive samples were associated a symptom score of 5.7±5.1. Children with virus-positive, severe illnesses (symptom score ≥5) experienced reductions in $FEF_{25–75}$ (Figure 1A). Only the symptomatic virus-negative groups demonstrated a significant increase in FeNO (Figure 1B).

Next, we examined nasal aspirate mRNA and protein levels in the six conditions. A total of 607 samples were analyzed (Table 4). Of interest, 43 samples were virus-positive and had an associated symptom score of 0 (asymptomatic infection). Of these, 24 had at least one sample collected within 28 days prior to the asymptomatic infection. Eighteen of 24 (75%) were preceded by a cold within that time period, indicating that asymptomatic infections represented convalescence from an earlier symptomatic cold.

Compared to the virus-negative asymptomatic condition, samples from children with more severe colds (symptom score ≥5) showed higher nasal mRNA expression of CXCL10 and MDA5 (Figure 2A) and greater protein abundance of CXCL8, CXCL10, sICAM-1, CCL2, CCL4, CCL20 and CCL24 (Figure 2C). In addition, samples from children with more severe colds were more likely to have detectable levels of TLR3 mRNA (Figure 2B). Samples from children with mild and asymptomatic viral infections also showed significant increases in some nasal aspirate biomarkers, albeit fewer biomarkers than samples from more severe colds (Figure 2A, 2C). Samples from children with asymptomatic infections showed higher nasal CXCL10 mRNA and CCL20 protein expression, suggesting persistence of these cytokines after resolution of symptoms. Children with mild colds and asymptomatic infections, but not those with severe colds, also showed an increase in the number of aspirates positive for IFN-λ mRNA (Figure 2B). Finally, and unexpectedly, symptomatic virus-negative illnesses showed no significant increases in nasal biomarkers except for CXCL10 protein. When we re-examined data, restricting our analysis to children with persistent asthma (either mild or moderate-to-severe), nearly identical results were obtained, except that samples from all three virus-positive groups showed significant increases in IFN-λ compared to the virus-negative, asymptomatic condition (not shown).

The considerable number of virus-negative illnesses led us to examine the distribution of subjects with self-reported atopy and proximity to highways in the six viral detection/symptom score conditions. However, there was no difference in the percentage of children with atopy (chi-square, 0.24) or high-traffic exposure (0.46) in the virus-negative illness groups. Also, when we re-evaluated our data for effect modification using interaction modeling, interaction terms for atopy and high traffic were not statistically significant. Finally, when we re-ran our GEE models adjusting for the main effects of atopy or high traffic, there was no change in the associations of interest between virus/symptom group and cytokine level.

Relationships of RV copy number to nasal aspirate biomarkers and respiratory symptoms.

For RV infections, we examined the association between viral copy number with nasal aspirate mRNA, protein and overall symptom scores. Nasal aspirate RV copy number was positively associated with mRNA expression of CXCL10 and MDA5 (Figure 3A). Viral load also was associated with protein abundance of CXCL8, CXCL10, ICAM-1, CCL2, CCL4 and CCL20 (Figures 3B, 3C). which together attract neutrophils and monocytes to the airways. However, levels of IL-4, IL-13, CCL5 and CCL24, which promote eosinophil chemotaxis and allergic airways disease, were not associated with viral load. There was no significant difference in viral copy number between symptomatic and asymptomatic infections (symptomatic, $1.22 \times 10^5 \pm 125$ copies/ml; asymptomatic, $0.54 \times 10^5 \pm 54$

copies/ml; geometric mean ± geometric SD, p=0.62, Wilcoxon rank sum test). Nor was there an association between viral copy number and overall symptom score (p=0.42). The study did not have statistical power to correlate copy number with lung function.

Time course of viral-induced cytokine expression.

Although we did not design the study to examine the time course of cytokine expression, our collections included three samples from each of 28 sick period single viral infections, as well as baseline surveillance samples for these individuals. mRNA expression of CXCL10, IRF7 and MDA5 and protein abundance of CXCL10 peaked 1–3 days after the onset of infection (Figure 4A). Protein abundance of CXCL8, CXCL10, CCL4, CCL20 and ICAM1 peaked at 4–6 days (Figure 4B). IL-4, IL-13, CCL5, CCL24, each of which promote type 2 inflammation, peaked 7–14 days after infection.

Discussion

This study was undertaken to examine the influence of natural upper respiratory tract viral infection on respiratory symptoms, airway function and inflammation in children with asthma from the urban community, and determine possible mechanisms by correlating these outcomes with nasal aspirate cytokines and other biomarkers. We hypothesized that children with high-symptom severe colds have greater airflow obstruction, respiratory tract inflammation and viral copy number than those with milder colds and asymptomatic infections, as well as virus-negative conditions. In contrast to children from a suburban setting [22], high-symptom viral infections were associated with reduced small airway function, as evidenced by changes in $FEF_{25–75}$. Compared to the virus-negative asymptomatic condition, children with severe colds showed elevations of nasal mRNA and protein biomarkers. Children with mild and asymptomatic infections showed fewer elevations. We found associations between viral load and nasal aspirate levels of chemokines which together attract neutrophils and monocytes to the airways, but not those which promote eosinophil chemotaxis and allergic airways disease. This is the first study to correlate viral load and respiratory tract cytokine levels during natural colds. Finally, urban children with asthma experienced many virus-negative symptomatic illnesses which were associated with increased exhaled nitric oxide but not reduced airway function or elevated nasal biomarkers.

We found that subjects were only slightly more likely to have a virus during self-reported colds (33.7%) than during surveillance sample collection (22.9%). The high rate of viral detection during surveillance periods is consistent with previous studies in well children with asthma [1–4] and unlikely to be due to false positives, as RV detection was confirmed by amplification and sequencing of gel purified PCR products in 92% of cases. Conversely, we believe the low viral detection rate during symptomatic episodes can be explained by the fact that samples were not collected in the fall and instead were only collected from January to August, when rhinovirus infections are less prevalent. The low symptom threshold for “sick” sample collection and financial reimbursement for each sick period assessment, which offset time and effort needed to participate, could also have contributed to a low viral detection rate. While our rate of viral detection is lower than reported previously [1, 2], it is consistent

with the lower frequency of viral detection in urban children with respiratory illnesses compared to suburban children [23]. Finally, our data indicate that urban children with asthma experience frequent virus-negative upper respiratory tract illnesses. Cold-like illnesses were unlikely to represent false-negative viral infections, as they were unaccompanied by reduced pulmonary function or increases in nasal aspirate MDA5, a double-stranded RNA pattern recognition receptor which was increased in virus-positive samples and has been shown to be induced following RV infection [35]. Finally, when we retested a subset of our initial multiplex PCR viral detection results using standard RV qPCR, we found a false negative rate of only 3%. Like previous studies [23, 36], RV was the most common virus detected and that, overall, inner-city infants had low rates of viral detection. However, we did not appreciate higher adenovirus rates as observed previously [23]. While RV-C is associated with severe asthma exacerbations [32, 37–43], we did not recruit enough patients to discern a difference in symptom severity between species.

Next, we evaluated the significance of viral infection on respiratory symptoms, lung function and respiratory tract inflammation. One-third of viral infections were associated with severe colds (symptom score ≥5), which by definition included children with wheezing, difficulty breathing or breathing fast. In contrast to children with asthma from a suburb of Sydney [22], children from Detroit with severe colds demonstrated significant reductions in $FEF_{25–75}$, consistent with small airways involvement [44]. This discrepancy could relate to the frequent undertreatment of urban children with asthma [24]. Since inhaled corticosteroid use reduces the rate of exacerbations [45], urban children may be more susceptible to viral-induced reductions in airway function than suburban children. This hypothesis is consistent with previous studies in which the impact of pollution on asthma was seen predominantly in children not using steroids [46, 47].

In addition, we found that high-symptom severe viral infections, but not virus-negative illnesses, were associated with significant elevations in nasal aspirate mRNA expression of CXCL10, MDA5 and TLR3 and protein abundance of CXCL8, CXCL10, sICAM-1, CCL2, CCL4, CCL20 and CCL24. Previous studies of children with natural colds have shown increases in nasal chemokines [25–29]. Increases in MDA5, TLR3 and ICAM-1 are noteworthy, as each functions as a receptor for RV. MDA5 and TLR3 are cytoplasmic and endosomal receptors for viral double-stranded RNA, respectively (reviewed in [48]), whereas ICAM-1 is a receptor for major group RV [49]. Samples from children with mild and asymptomatic viral infections also showed significant increases in nasal aspirate biomarkers, albeit fewer than samples from more severe colds. One-quarter of virus-positive samples were not associated with symptoms. Collection of these samples often followed symptomatic viral infections, indicating that asymptomatic infections represented convalescence from more severe colds. Samples from children with asymptomatic infections showed higher nasal CXCL10 mRNA and CCL20 protein expression, suggesting persistence of these cytokines after resolution of symptoms.

We hypothesized that viral copy number determines asthma symptoms and respiratory tract inflammation after RV infection. We found that viral load was associated with protein abundance of CXCL8, CXCL10, CCL2, CCL4 and CCL20, chemokines which together attract neutrophils and monocytes to the airways, but not with IL-4, IL-13, CCL5 or CCL24,

cytokines which promote eosinophil chemotaxis and allergic airways disease. In contrast, there was no association between viral copy number and symptoms. Our data are consistent with a previous study in adults with asthma showing no correlation between severity of lower respiratory tract symptoms and viral load [50]. These data suggest that respiratory symptoms in asthmatic children with natural colds may not depend on viral load alone. Other factors may determine asthma control after viral infection, including environmental and genetic factors [51].

The exact nature of the observed virus-negative respiratory tract illnesses is unclear. Unlike virus-positive illnesses, virus-negative symptomatic sicknesses were accompanied by increased eNO. Previous studies in asthmatic subjects have shown that experimental exposure to allergen and particulate matter increases eNO in contrast to experimental RV infection which does not [52, 53], suggesting that virus-negative illnesses could have been precipitated by environmental exposures. We did not find an association between viral-negative illnesses and self-reported atopy or proximity to high-traffic roadways. However, since the six groups we studied do not represent different subjects but different disease states according to viral detection and symptoms, it is still possible that these illnesses represent acute exposures to allergen, traffic or other pollutants.

African-American children living in low-socioeconomic-status urban environments continue to experience higher asthma morbidity than white children [54]. Racial disparities are observed in asthma prevalence, emergency department visits, hospital readmissions and death rates [55, 56]. Therefore, while data from Detroit may not be generalizable to other settings, they provide new insight into the effect of viral infections on an important pediatric population.

There are a number of limitations to our study. First, we used nasal aspirates to sample respiratory tract inflammation, allowing repeated collection of samples from children in a non-invasive manner. We did not validate our method by comparing our results with lower respiratory tract specimens. However, gene expression among asthmatic children is altered similarly in nasal and bronchial airways [57]. Second, there was a lag period between onset of respiratory symptoms and nasal aspirate collection. Third, symptoms may have been caused or prevented by any number of unmeasured covariates including body mass index, mucus production or anti-inflammatory cytokines. Fourth, our assessments of symptom score, asthma severity and atopy were based on self-report and were not independently validated, allowing the possibility of measurement error.

We conclude that, in urban children with chronic asthma, high-symptom respiratory viral infections reduce airway function. Children with more severe colds demonstrate more elevations of nasal biomarkers than children with mild colds, asymptomatic infections or virus-negative illnesses. However, many children experienced virus-negative cold-like illnesses associated with increased eNO but not nasal aspirate biomarkers or lung function change. Further studies are needed to understand the precise factors which determine respiratory tract symptoms in children with asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations/Acronyms:

CAAA	Community Action Against Asthma
CCL	C-C motif chemokine ligand
CXCL	C-X-C motif chemokine ligand
$FEF_{25–75}$	forced expiratory flow at 25–75% of the pulmonary volume
FeNO	fraction of exhaled nitric oxide
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GEE	generalized estimating equations
IFN	interferon
IL	interleukin
IRF7	interferon regulatory factor-7
MDA5	melanoma differentiation-associated protein 5
PCA	principal component analysis
PEF	peak expiratory flow
qPCR	quantitative polymerase chain reaction
RIG-I	retinoic-acid-inducible protein 1

RSV	respiratory syncytial virus
RV	rhinovirus
sICAM	soluble intercellular adhesion molecule
TLR3	Toll-like receptor 3.

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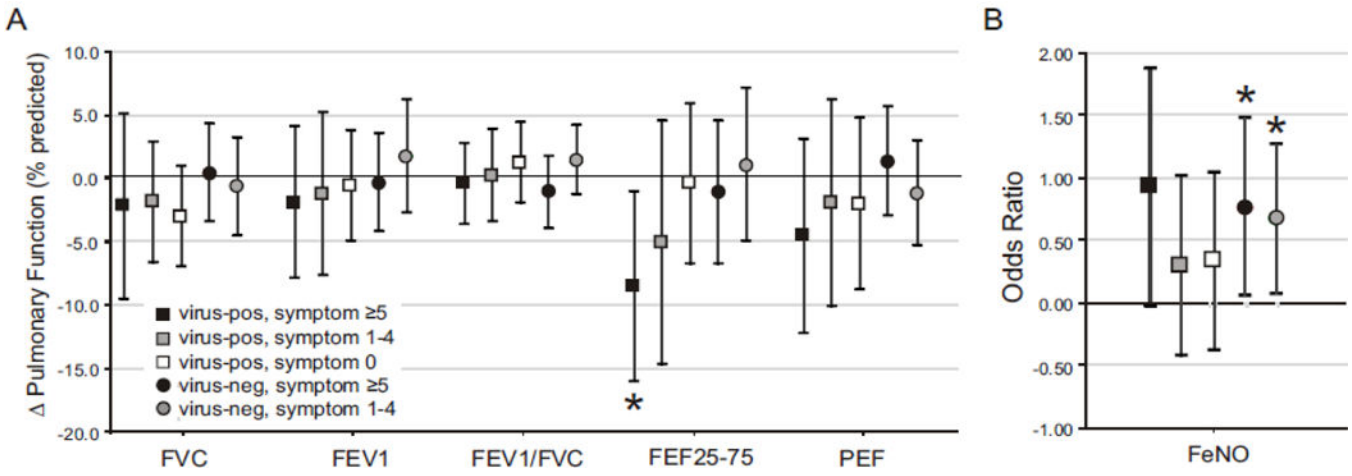

Figure 1. Comparisons of lung function and exhaled NO (eNO) between the six groups of conditions.

The virus-negative/high symptom group (symptom score ≥5, black squares), virus-positive/mild symptom group (symptom score 1–4, grey squares), virus-positive/asymptomatic (symptom score 0, white squares), virus-negative/high symptom group (symptom score ≥5, black circles) and virus-negative/mild symptom group (symptom score 1–4, grey circles) are each compared to the virus-negative/asymptomatic group. A. Changes in lung function (percent predicted) compared to the virus-negative/low symptom group. Adjusted mean estimates and 95% confidence intervals are shown. B. Changes in eNO (ppb) compared to the virus-negative/no symptom group. Adjusted odds ratios and 95% confidence intervals are shown.

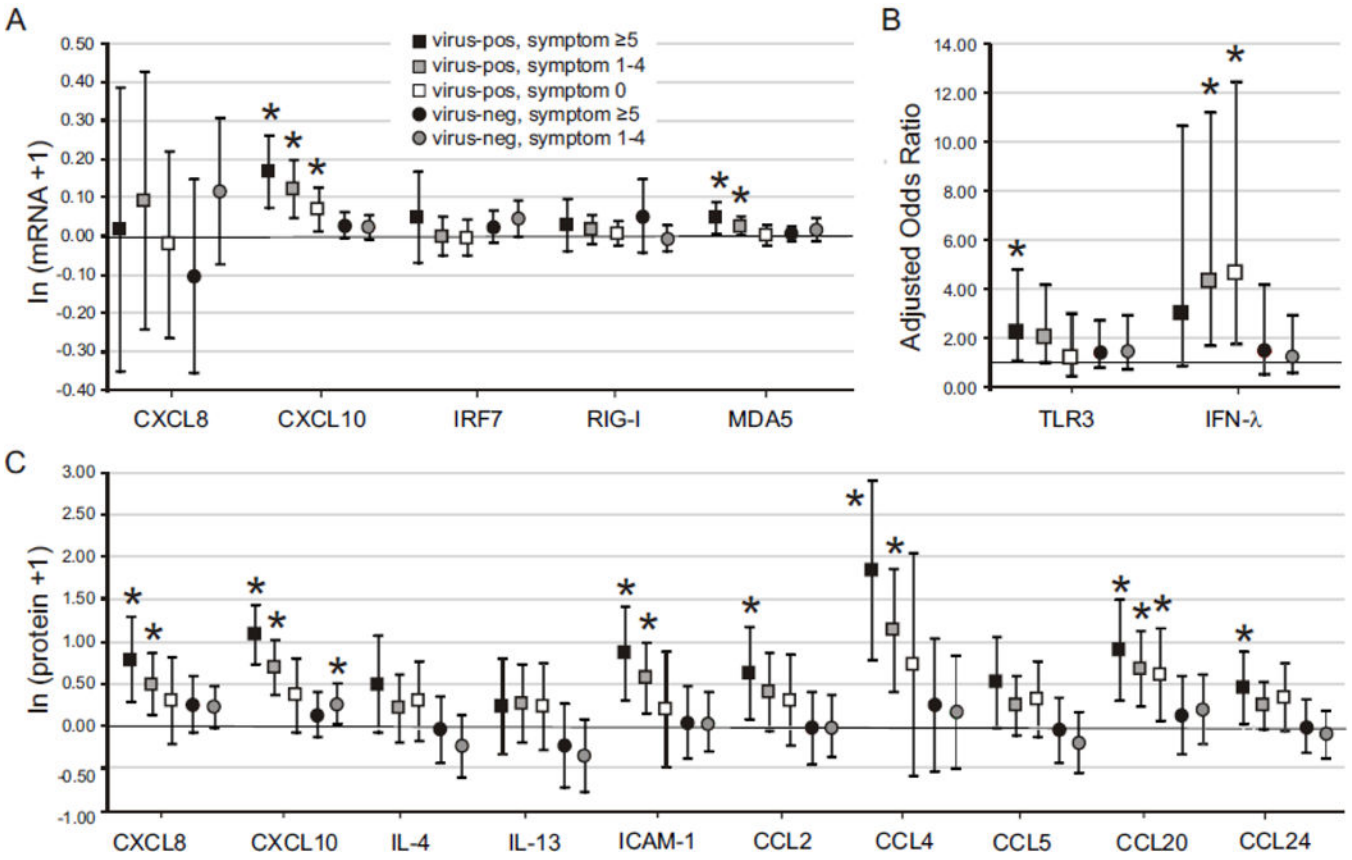

Figure 2. Comparison of nasal aspirate mRNAs and proteins between the six groups of conditions.

The virus-negative/high symptom group (symptom score ≥5, black squares), virus-positive/mild symptom group (symptom score 1–4, grey squares), virus-positive/asymptomatic (symptom score 0, white squares), virus-negative/high symptom group (symptom score ≥5, black circles) and virus-negative/mild symptom group (symptom score 1–4, grey circles) are each compared to the virus-negative/asymptomatic group. A. Differences in log transformed mean mRNA values for CXCL8, CXCL10, IRF7, RIG-I and MDA5 compared to the virus-negative/low symptom group. 95% confidence intervals are also shown (**P*<0.05). B. TLR3 and IFN-λ1 mRNAs were analyzed as a binary variable (detectable, undetectable). Odds ratios and 95% confidence intervals compared to the virus-negative/low symptom group are shown (**P*<0.05). C. Differences in log transformed mean mRNA values for CXCL8, CXCL10, IL-4, IL-13, sICAM-1, CCL2, CCL4, CCL5, CCL20 and CCL24 compared to the virus-negative/low symptom group. 95% confidence intervals are also shown (**P*<0.05).

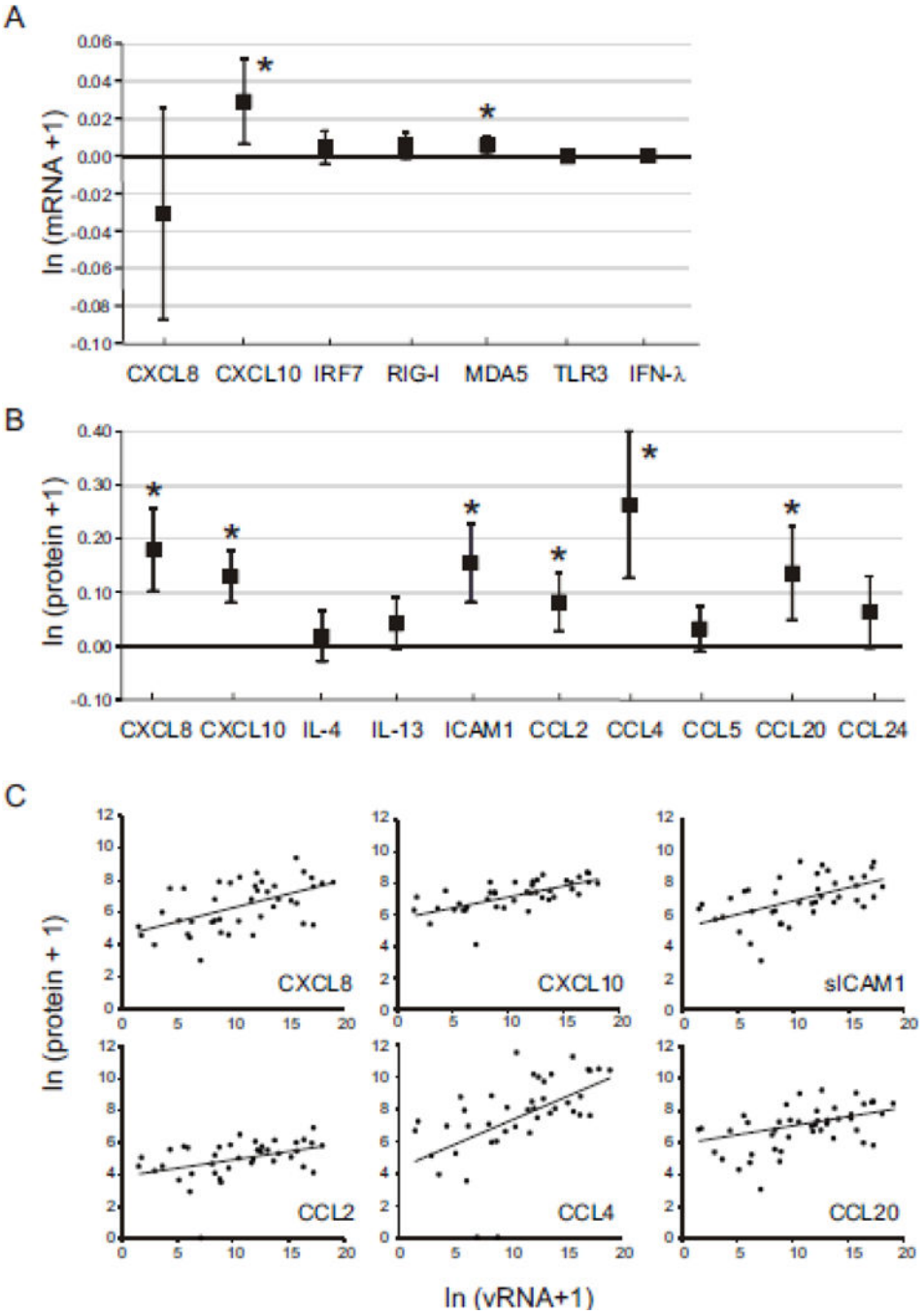

Figure 3. Associations between nasal aspirate biomarkers and rhinoviral RNA.

A. Nasal aspirate mRNAs are represented as ln (mRNA +1). Adjusted means and 95% confidence intervals are shown. B. Nasal aspirate proteins represented as ln (protein level +1). Adjusted means and 95% confidence intervals are shown. The association of viral copy number and nasal biomarker was determined using the GEE method (*P<0.05). C-G. Individual adjusted correlations of viral copy number and selected nasal aspirate cytokines.

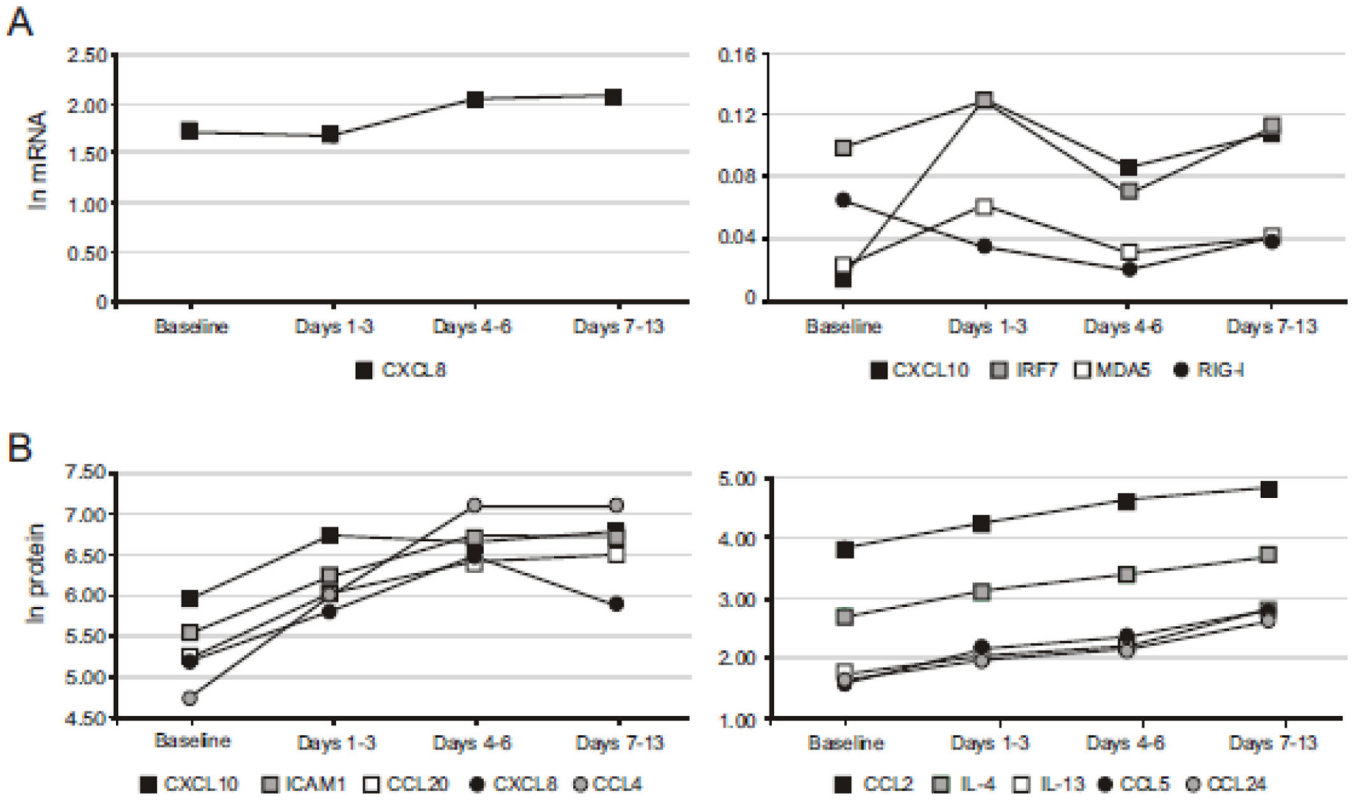

Figure 4. The time course of nasal biomarker changes from 28 virus-positive sick period weeks.

A. Nasal aspirate mRNAs are represented as ln mRNA. B. Nasal aspirate proteins are represented as ln protein. For easier readability, SD are not shown.

Table 1.

Participant baseline demographic characteristics (n=53).

Age, mean (SD)	9.7 (2.1)
Gender, % female	43.4
Race, % Non-Hispanic African-American	86.8
Household income, % ≤ $15,000	56.3
Caregiver years of education, % ≤ 12	56.9
Caregiver depression CESD score, mean (SD)	8.8 (5.1)
Smoker in household, % yes	67.9

Table 2.

Initial surveillance period health measures.*

Asthma severity, N (%)	
Moderate or severe persistent	14 (26.9)
Mild persistent	27 (51.9)
Mild intermittent	11 (21.2)
Atopy (self-reported), % yes	38 (73.1)
Asthma medication use in last 12 months (N, %)	
Inhaled corticosteroids	12 (23.1)
Short acting bronchodilator only	21 (40.4)
No asthma medication	19 (36.5)
Asthma control test (ACT) score, mean ± SD	20.0 ± 4.2
Symptom score, median (range)	2.3 (0, 27)
Lung function (% of predicted), mean ± SD (range)	
FVC (N=43)	93.0 ± 16.2 (56.7, 136.7)
FEV_1 (N=43)	80.3 ± 17.9 (30.2, 125.4)
FEV_1/FVC ratio (N=43)	75.5 ± 11.9 (38.9, 93.0)
$FEF_{25–75}$ (N=42)	55.8 ± 20.3 (17.6, 109.3)
PEF (N=42)	83.7 ± 20.9 (31.3, 122.7)
FeNO (ppb), mean ± SD (N=50)	26.8 ± 25.7 (6.0, 147.7)

*N=53 except when noted

Abbreviations: FEF25–75, forced expiratory flow at 25–75% of the pulmonary volume; FeNO, fraction of exhaled nitric oxide; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow.

Table 3.

Participant viral infections.

Surveillance collection (N=410)	**N**	**%**
No virus	288	70.2 (of total samples)
Virus	94	22.9
Single infections	85	20.7
RV	46	48.9 (of viral infections)
Coronavirus 229E/NL63	9	9.6
RSV A	8	8.5
Coronavirus OC43	5	5.3
RSV B	4	4.3
Influenza A	4	4.3
Influenza B	3	3.2
Adenovirus	2	2.1
Metapneumovirus	2	2.1
PIV 2	2	2.1
Multiple infections	9	9.6
Without RV	5	5.3
With RV	4	4.3
Sick collection (N=248 samples, number of sick periods = 83)		
No. of sick periods	%	
No virus	55	66.3 (of total sick periods)
Virus	28	33.7
Single infections	26	26.1
RV	20	71.4 (of viral infections)
Influenza A	2	7.1
Influenza B	1	3.6
Coronavirus 229E/NL63	1	3.6
PIV 2	1	3.6
RSV B	1	3.6
Multiple infections	2	7.1
Without RV	0	0.0

Surveillance collection (N=410)	**N**	**%**
With RV	2	7.1

Abbreviations: RSV, respiratory syncytial virus; RV, rhinovirus.

Table 4.

Distribution of samples based on viral detection and symptom score.

Virus-positive conditions	N	Percent
1=symptom score ≥ 5	55	32.4
2=symptom score 1–4	72	42.3
3=symptom score 0	43	25.3
Virus-negative conditions		
4= symptom score ≥ 5	130	21.42
5= symptom score 1–4	136	22.41
6= symptom score 0	171	28.17