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Nasopharyngeal CCL5 in infants with severe bronchiolitis and risk of recurrent wheezing: A multicenter prospective cohort study

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To the Editor

Bronchiolitis is the leading cause of hospitalizations in U.S. infants (1). Additionally, small cohort studies indicate that 30%–40% of infants hospitalized for bronchiolitis (severe bronchiolitis) will subsequently develop recurrent wheezing and childhood asthma (1). However, it remains unclear which infants with severe bronchiolitis will develop these chronic respiratory outcomes. Within the limited literature, a single-center analysis of 81 infants with respiratory syncytial virus (RSV) bronchiolitis demonstrated that upregulated expression of CCL5 (previously known as RANTES; a β -chemokine chemoattractant for inflammatory cells such as eosinophils) in nasal epithelium was associated with higher risk of developing childhood asthma (2). However, despite the heterogeneity of bronchiolitis and subsequent risks of adverse respiratory outcomes (1), we are not aware of another study that has investigated the role of airway CCL5 in other important populations (e.g., infants with rhinovirus bronchiolitis). In the current study, we examined the relationship between nasopharyngeal CCL5 levels and risks of developing recurrent wheezing, according to virus etiology, in a prospective cohort of infants with severe bronchiolitis.

Details of the study design, setting, participants, data collection, and analysis may be found in the Online Repository (Supplemental Methods). Briefly, this prospective cohort study, the 35th Multicenter Airway Research Collaboration (MARC-35)(3) enrolled infants (age <12 months) at an initial episode of severe bronchiolitis at 17 sites across 14 U.S. states (Table E1) during the 2011–2014 winter seasons. Bronchiolitis was defined according to the American Academy of Pediatrics guidelines – acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions (4). We excluded infants with previous enrollment during earlier bronchiolitis hospitalization, those who were transferred to a participating hospital >24 hours after the original hospitalization, those who were consented >24 hours after hospitalization, or those with known heart-lung disease, immunodeficiency, immunosuppression, or gestational age <32 weeks. All patients were treated at the discretion of the treating physician.

Using a standardized protocol, investigators collected nasopharyngeal samples within 24 hours of hospitalization. We tested the samples for 17 respiratory viruses (e.g., RSV, rhinovirus) using real-time PCR assays, and CCL5 levels using enzyme-linked immunosorbent assay. For the present analysis, to simplify data interpretation, we categorized CCL5 values into tertiles: low (<25.0 pg/ml), intermediate (25.0–74.0 pg/ml), and high (>74.0 pg/ml) CCL5. The primary outcome was recurrent wheezing by age 3 years. According to the 2007 NIH asthma guidelines (5), recurrent wheezing was defined as having at least two corticosteroid-requiring exacerbations in six months or having at least four wheezing episodes in one year that last at least one day and affect sleep. To examine heterogeneity of CCL5-outcome associations by type of viral infection, we constructed Cox proportional hazards model for each of four virus categories (solo RSV infection, solo rhinovirus infection, RSV-rhinovirus coinfection, and others)(3). In the models, we adjusted for 11 potential confounders (age, sex, race/ethnicity, parental history of asthma, gestational age, season of birth, history of breathing problems, daycare attendance, smoking exposure at home, use of positive pressure ventilation, and IgE sensitization at the enrollment) and

accounted for patient clustering at the hospital-level. To examine the robustness of the findings, we conducted a series of sensitivity analyses. First, to address potential dilutions of the specimens, we normalized the CCL5 level by using RNase P (host housekeeping gene) copy number, as we have previously described (6). Second, we performed a subgroup analysis excluding patients who had a breathing problem before the index hospitalization. Lastly, we modeled the outcome as a binary outcome by fitting a generalized linear mixed model for each of the four virology strata.

Of 921 infants in the longitudinal cohort, the median age was 3.2 months (IQR, 1.6–5.9 months) and 60% were male. Additionally, 44% were non-Hispanic white, 23% non-Hispanic black, and 30% Hispanics. All had detectable CCL5 at the index hospitalization for bronchiolitis, with the median concentration of 18.3 pg/ml (IQR, 42.0–103.94 pg/ml). Overall, 32% developed recurrent wheezing by age 3 years. Between CCL5 strata, most patient characteristics did not differ while infants with low CCL5 levels were younger and more likely to have RSV infection (both $P < 0.05$; Table E2). There was a significant interaction between CCL5 levels and virus category with regard to risk of recurrent wheezing ($P_{\text{interaction}} = 0.01$), indicating that CCL5-outcome associations differ by virus. Indeed, among infants with solo RSV infection, compared to those with low CCL5 levels, the risk of recurrent wheezing was significantly higher in those with intermediate and high CCL5 levels (25%, 33%, and 33%, respectively) with corresponding adjusted HR of 1.57 (95% CI, 1.06–2.31; $P = 0.02$) and 1.56 (95% CI, 1.04–2.32; $P = 0.03$; Table 1 and Figure 1). In contrast, among the three other virus categories, CCL5 levels were not significantly associated with risks of recurrent wheezing (all $P \geq 0.15$; Table 1 and Figure E1). This heterogeneity persisted across the sensitivity analyses with the use of normalized CCL5 levels, exclusion of patients with prior breathing problems, and use of generalized linear mixed models (Table 1).

The observed association between CCL5 levels and risks of recurrent wheezing in RSV bronchiolitis is concordant with earlier reports. In a single-center study of 30 Korean infants with RSV infection, Chung *et al.* reported associations between higher nasal CCL5 levels and higher risks of recurrent wheezing during the 1-year follow-up period (7). In another single-center analysis of 81 infants with RSV bronchiolitis, Bacharier *et al.* reported associations between elevated CCL5 mRNA expression and higher risks of childhood asthma by age 6 years (2). Similarly, another single-center study of 320 Chinese infants with RSV bronchiolitis also demonstrated that a polymorphism in the *CCL5* gene is associated with higher serum CCL5 levels and subsequent risks of recurrent wheezing by age 3 years (8). Our current multicenter study of 921 infants with severe bronchiolitis corroborates these earlier reports on RSV bronchiolitis, and extends them by demonstrating the lack of association in infants with other virus infections (e.g., rhinovirus bronchiolitis).

Exploring the underlying mechanism of these findings is beyond the scope of the current analysis. However, the association in solo RSV infection may be causal – i.e., severe RSV infection promotes exuberant CCL5-induced airway inflammation thereby contributing to recurrent wheezing. Alternatively, the expression of CCL5 during RSV infection is genetically determined (8), and hence elevated levels may be simply a marker of infants who are predisposed to develop recurrent wheezing. Interestingly, the CCL5-recurrent wheezing

association was not observed in the other virus groups. The lack of significant association was also found in the RSV-rhinovirus coinfection group, while it is difficult to speculate whether rhinovirus infection modifies RSV-CCL5-recurrent wheezing associations given the limited sample size. Regardless, our findings lend additional support to the emerging concept that bronchiolitis is a heterogeneous condition with different pathobiological mechanisms and risks of adverse respiratory outcomes (1).

Our study has potential limitations, such as parental report of wheezing outcome and the absence of a “control” group. Additionally, our observations were based on nasopharyngeal CCL5 levels while bronchiolitis also involves lower airways. However, the literature has shown significant correlations between upper and lower airway inflammatory mediators, including CCL5 (9). The use of upper airway specimens is also unavoidable as lower airway sampling is overly invasive in young infants. Next, the current analysis investigated the relationship of airway CCL5 with risks of recurrent wheezing rather than incident asthma. However, the presence of recurrent wheezing carries a 5.5-fold increased risk of developing asthma by age 6 years.(10) Finally, while the study cohort comprised a racially/ethnically-diverse U.S. sample, we must cautiously generalize our inferences beyond infants with severe bronchiolitis. Still, our data remain directly relevant for 130,000 hospitalized U.S. infants each year, of which 30%–40% will develop asthma (1).

In sum, this pre-planned analysis in a multicenter cohort of 921 infants with severe bronchiolitis demonstrated that the relationship of nasopharyngeal CCL5 levels with risk of recurrent wheezing differs by infecting virus. Specifically, higher CCL5 levels were associated with 50% increased risk of recurrent wheezing only in infants with solo RSV bronchiolitis. Although causal inference remains premature, our observations – in conjunction with prior studies – advance research into the complex interplay between respiratory virus infection and host immunity in early infancy, and their integrated contribution to lung health in childhood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
HR	hazard ratio

IQR	interquartile range
MARC	Multicenter Airway Research Collaboration
PCR	polymerase chain assay
RSV	respiratory syncytial virus

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Figure 1. Proportion of patients with solo respiratory syncytial virus (RSV) infection who remained recurrent wheezing-free over a 36-month period

Among infants with solo RSV infection, the risks of developing recurrent wheezing were significantly higher in those with intermediate CCL5 (adjusted HR, 1.57; 95%CI, 1.06–2.31; $P=0.02$) and those with high CCL5 (adjusted HR, 1.56; 95%CI, 1.04–2.32; $P=0.03$), compared to infants with low CCL5 levels. The number at risk indicates the number of patients at risk for developing recurrent wheeze at each age. The numbers decrease with age as patients who develop the outcome are censored in the survival analysis. As requested, we have added this information to the Figure Legend section.

Table 1

Associations of nasopharyngeal CCL5 levels with development of recurrent wheezing by age 3 years, according to virus groups

Virus groups and CCL5 levels	Model 1		Model 2		Model 3		Model 4	
	Cox proportional hazards model*	HR (95% CI)	P-value	Sensitivity analysis using normalized CCL5 levels [†]	HR (95% CI)	P-value	Sensitivity analysis excluding patients who had a breathing problem episode before index hospitalization (n=734) [*]	OR (95% CI)
Sole RSV infection								
Low CCL5 (n=197)	Reference	Reference	-	Reference	Reference	-	Reference	-
Intermediate CCL5 (n=180)	1.57 (1.06–2.31)	0.02	0.04	1.52 (1.03–2.23)	1.83 (1.16–2.89)	0.01	1.82 (1.12–2.94)	0.02
High CCL5 (n=164)	1.56 (1.04–2.32)	0.03	0.045	1.50 (1.01–2.24)	1.66 (1.03–2.66)	0.04	1.70 (1.04–2.79)	0.03
Sole rhinovirus infection								
Low CCL5 (n=20)	Reference	Reference	-	Reference	Reference	-	Reference	-
Intermediate CCL5 (n=15)	0.77 (0.21–2.84)	0.69	0.44	0.62 (0.18–2.10)	0.44 (0.13–1.52) [§]	0.19	0.57 (0.09–3.80)	0.56
High CCL5 (n=17)	1.45 (0.45–4.61)	0.53	0.16	2.50 (0.69–9.09)	0.80 (0.27–2.37) [§]	0.68	1.27 (0.21–7.52)	0.79
RSV + rhinovirus coinfection								
Low CCL5 (n=23)	Reference	Reference	-	Reference	Reference	-	Reference	-
Intermediate CCL5 (n=39)	1.23 (0.39–3.84)	0.72	0.44	1.56 (0.50–4.84)	0.70 (0.18–2.72)	0.61	1.35 (0.31–5.85)	0.69
High CCL5 (n=29)	0.55 (0.14–2.20)	0.40	0.45	0.60 (0.16–2.25)	0.23 (0.03–1.55)	0.13	0.47 (0.08–2.80)	0.41
Others								
Low CCL5 (n=67)	Reference	Reference	-	Reference	Reference	-	Reference	-
Intermediate CCL5 (n=72)	0.90 (0.50–1.61)	0.72	0.68	1.13 (0.63–2.03)	0.91 (0.43–1.90)	0.80	0.95 (0.43–2.08)	0.89
High CCL5 (n=98)	0.66 (0.38–1.16)	0.15	0.30	0.73 (0.41–1.32)	0.61 (0.28–1.34)	0.22	0.67 (0.32–1.41)	0.29

Bold results are statistically significant

Abbreviations: CI, confidence interval; HR, hazard ratio; OR, odds ratio; RSV, respiratory syncytial virus

* Cox proportional hazards model adjusting for patient-level confounders (age, sex, race/ethnicity, parental history of asthma, gestational age, season of birth, history of breathing problems, daycare attendance, smoking exposure at home, use of mechanical ventilation during the index hospitalization, and IgE sensitization [aeroallergen and/or food] at the enrollment) and accounting for potential patient clustering within the sites.

[†] CCL5 levels normalized by RNase P copy number.

[§] Generalized linear mixed model adjusting for patient-level confounders (age, sex, race/ethnicity, parental history of asthma, gestational age, season of birth, history of breathing problems, daycare attendance, smoking exposure at home, use of mechanical ventilation during the index hospitalization, and IgE sensitization [aeroallergen and/or food]) and accounting for potential patient clustering within the sites.

Unadjusted Cox proportional hazards model because of the relatively small sample size.

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