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Serum cathelicidin level is associated with viral etiology and severity of bronchiolitis

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To the Editor:

Bronchiolitis is a common childhood acute respiratory infection (ARI) and the leading cause of hospitalization for US infants.¹ The two most common viral etiologies of severe bronchiolitis (i.e. bronchiolitis requiring hospitalization) are respiratory syncytial virus (RSV) and human rhinovirus (HRV).²

Although low serum 25-hydroxyvitamin D (25[OH]D) levels have been associated with increased incidence and severity of ARI, randomized controlled trials (RCTs) of vitamin D supplementation have demonstrated mixed results for the prevention of ARIs.³ Nonetheless, vitamin D status continues to garner attention as a modifiable risk factor for infectious diseases since it plays a central role in the innate immune system. Hydroxylation of 25(OH)D creates 1,25-dihydroxyvitamin D (1,25[OH]₂D) which *in vitro* increases the transcription of the innate immune protein, human cathelicidin antimicrobial peptide (hCAP-18).⁴ Bronchial epithelial cells and circulating white blood cells produce hCAP-18,⁵ which is capable of killing a wide variety of viral and bacterial pathogens.³ Although 25(OH)D levels are the best measure of overall vitamin D status, it is 1,25-(OH)₂D that drives the production of hCAP-18. Therefore, when examining the association between vitamin D and infectious disease outcomes, hCAP-18, may provide additional data.

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As part of a prospective, multicenter study enrolling children age <2 years hospitalized with bronchiolitis November through March,² study investigators at one Boston site also enrolled children with bronchiolitis discharged home from the emergency department (ED). For all participants at this site, investigators collected clinical data, blood samples and nasopharyngeal aspirates (NPA). Serum 25(OH)D concentration was measured by liquid chromatography-tandem mass spectrometry (LC- MS/MS). ELISA was used to measure serum levels of hCAP-18 (Online Repository). The NPAs were tested for 15 viruses using real time PCR.² We hypothesized that hCAP-18 levels would not be associated with the viral etiology of bronchiolitis, but that children with low hCAP-18 levels would more likely be hospitalized ≥ 24 hours. For the latter hypothesis we divided the children into two groups: 1) discharged home from the ED or hospitalized <24 hours and 2) hospitalized ≥ 24 hours. Analysis used descriptive statistics and multivariable logistic regression. This study was approved by the Boston Children's Hospital institutional review board.

Of 82 enrolled children, 33 (40%) were discharged home from the ED or hospitalized <24 hours, and 49 (60%) were hospitalized ≥ 24 hours. Overall, the median age was 5 months (interquartile range [IQR] 2–10); 46 (56%) were male; 39 (47%) were white, 21 (26%) black, and 22 (27%) were other or unknown; 35 (43%) were Hispanic ethnicity. Furthermore, median (IQR) levels of key serum tests were: 25(OH)D 34 ng/mL (28–40) and hCAP-18 218 ng/mL (130–382). The two most commonly identified viruses were RSV (76%) and HRV (18%). Both RSV and HRV were identified in 6% of children and no virus was identified in 5% of children. There was neither a significant correlation between 25(OH)D and hCAP-18 levels ($\rho=-0.12$; $P=0.26$), nor a significant association between 25(OH)D and hospitalization ≥ 24 hours ($P=0.50$).

Median hCAP-18 levels were lower among children with RSV only or RSV in combination with non-HRV viruses than among children with HRV only or HRV in combination with non-RSV viruses (median [IQR]: 159 ng/mL [118–243] vs 497 ng/mL [401–577], $P<0.001$). Interestingly, the median hCAP-18 level for the 5 children in whom both RSV and HRV were identified was between these two values (382 ng/mL [296–406]). There was no association between 25(OH)D levels and viral etiology.

As shown in Table 1, children with hCAP-18 levels less than the median (<218 ng/mL) were significantly more likely to be hospitalized ≥ 24 hours than children with hCAP-18 levels above the median (78% vs 41%; $P=0.001$). In multivariable analysis (Table 2) adjusting for age, gender, and race, children with serum hCAP-18 levels <218 ng/mL were more likely to be hospitalized ≥ 24 hours (adjusted odds ratio 5.7; 95% confidence interval 2.0–16.2, $P=0.001$). Premature birth was excluded from the final multivariable model since it was not significantly associated with hCAP-18 values (Table 1) and did not materially change the results of the other model factors. We did not collect information about prior ARIs.

One possible reason that we found no relationship between the 25(OH)D and hCAP-18 levels is that the majority of children had 25(OH)D levels >30 ng/mL.^{6, 7} In a study of 60 healthy adults, Bhan and colleagues found no significant correlation between 25(OH)D and hCAP-18 among adults with 25(OH)D levels >32 ng/mL ($r=0.12$, $P=0.58$), but among adults with 25(OH)D ≤ 32 ng/mL there was a significant correlation ($r=0.45$, $P=0.005$).

Associations between 25(OH)D levels and specific viral etiology have been recognized,⁸ but the association between hCAP-18 levels and viral etiology is novel. In our data children with lower hCAP-18 levels were significantly more likely to have RSV bronchiolitis than HRV bronchiolitis. Although the pathogenesis of this relationship is beyond the scope of our data, children with low hCAP-18 levels may be more susceptible to RSV or alternatively, the hCAP-18 levels may be lowered in the presence of RSV.

Importantly, hCAP-18 levels have been associated previously with clinical outcomes. For example, lower serum hCAP-18 levels (mean $619 \pm \text{SD } 329 \text{ ng/mL}$) at the initiation of chronic hemodialysis were independently associated with increased 1-year mortality (odds ratio, 2.6; 95% confidence interval, 1.4–5.0) due to infection.⁹ Furthermore, in adults hospitalized with community-acquired pneumonia, lower values of serum cathelicidin (median 69 ng/mL, [range 13–263]) were marginally associated with a higher 30-day mortality in unadjusted analyses ($P=0.053$).⁷ In our data, lower hCAP-18 levels were independently associated with bronchiolitis hospitalization ≥ 24 hours, but the reasons for the variability in serum hCAP-18 values between the two cited studies and our data is unclear.

In this prospective study of children presenting to the ED with bronchiolitis, most children had normal 25(OH)D levels. However, their hCAP-18 levels varied widely and, for the first time, we identified that low levels of hCAP-18, rather than 25(OH)D levels, were associated with RSV only infections and an increased severity of bronchiolitis. When examining the association between vitamin D status and infectious disease outcomes, hCAP-18 levels may provide an additional, clinically relevant biomarker, especially in populations with normal 25(OH)D levels.

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Abbreviations

ARI	Acute respiratory infections
RSV	respiratory syncytial virus
HRV	human rhinovirus
25(OH)D	25-hydroxyvitamin D
RCTs	randomized controlled trials
1,25(OH)₂D	1,25-dihydroxyvitamin D
hCAP-18	human cathelicidin antimicrobial peptide
ED	emergency department
NPA	nasopharyngeal aspirates
LC-MS/MS	liquid chromatography-tandem mass spectrometry

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Characteristics of children presenting to the emergency department with bronchiolitis by serum human cathelicidin antimicrobial peptide levels

Table 1

	hCAP-18 <218 ng/ml (n=41)		hCAP-18 ≥218 ng/ml (n=41)		
	n	%	n	%	P
Age in months					0.39
0-19	9	22%	6	15%	
2-59	17	41%	14	34%	
6-24	15	37%	21	51%	
Gender					1.00
Male	23	56%	23	56%	
Female	18	44%	18	44%	
Race					0.04
White	15	37%	24	59%	
Black	10	24%	11	27%	
Other or missing	16	39%	6	15%	
Hispanic	18	44%	17	41%	0.82
Insurance					0.10
Private	22	54%	20	50%	
Public	17	41%	12	30%	
None or unknown	2	5%	8	20%	
Premature Birth					0.12
37 weeks	13	32%	7	17%	
>37 weeks	28	68%	34	83%	
Admission status					
<24 hours or ED-only	9	22%	24	59%	0.001
≥24 hours	32	78%	17	41%	

Abbreviations: hCAP-18 indicates human cathelicidin antimicrobial peptide

Table 2

Multivariable predictors of hospitalization 24 hours among children presenting to the emergency department with bronchiolitis

Characteristics	Odds Ratio	95% CI	P
Age in months			
0–1.9	1.8	0.4–7.6	0.43
2–5.9	1.0	0.4–3.1	0.93
6–24	1.0	(reference)	
Gender			
Male	1.0	(reference)	
Female	1.0	0.4–2.5	0.92
Race			
White	1.0	(reference)	
Non-White	0.6	0.2–1.7	0.32
hCAP-18 (serum), ng/			
<218	5.7	2.0–16.2	0.001
218	1.0	(reference)	

Abbreviations: hCAP-18 indicates human cathelicidin antimicrobial peptide.