Low Retinol-Binding Protein and Vitamin D Levels Are Associated with Severe Outcomes in Children Hospitalized with Lower Respiratory Tract Infection and Respiratory Syncytial Virus or Human Metapneumovirus Detection

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Retinol binding protein and vitamin D were measured in children aged <5 years hospitalized with lower respiratory tract infection and respiratory syncytial virus and/or human metapneumovirus detections. Low vitamin levels were observed in 50% of the children and were associated with significantly elevated risk of the need for intensive care unit admission and invasive mechanical ventilation. *(J Pediatr 2017;187:323-7).*

Vitamins A and D have immunomodulatory properties, and deficiencies of each have been associated with increased morbidity and mortality in children and adults with respiratory infections.[1-3] Vitamin A deficiency (VAD) has been associated specifically with secondary bacterial infections and mortality in measles-associated pneumonia, as well as with other complications of measles virus infection.[4] Vitamin D deficiency (VDD) has been associated with prolonged and complicated illness as well as death in children and adults with all-cause pneumonia.[2,3] Vitamins A and D are highly interactive and cross-regulated and influence multiple organ systems, including the lung and its epithelial cell lining.[1,5-8] Despite these important relationships, vitamins A and D are not usually measured simultaneously.

Respiratory syncytial virus (RSV) and the related human metapneumovirus (hMPV) are responsible for considerable morbidity and mortality owing to lower respiratory tract infections (LRTIs) in young children in the US.[9-12] Contradictory findings have been published concerning the correlations of serum vitamin levels, evaluated independently, with RSV disease outcome.[13-20] Here we sought to determine whether VAD and/or VDD, examined in parallel, were present in children aged <5 years who were hospitalized with LRTI in Memphis, Tennessee with RSV or hMPV, and whether VAD and/or VDD correlated with disease outcomes. RSV and hMPV were of particular interest to us because of their frequent associations with hospitalizations, and because of current efforts at St Jude Children's Research Hospital to develop vaccines for these 2 pathogens.[9] The Centers for Disease Control and Prevention's Etiology of Pneumonia in the Community (EPIC) Study provided a unique opportunity to pose questions about serum vitamin levels and respiratory disease.[21]

EPIC	Etiology of Pneumonia in the Community
hMPV	Human metapneumovirus
ICU	Intensive care unit
IPWE	Inverse probability-weighted estimation
LOS	Length of stay
NP/OP	Nasopharyngeal and oropharyngeal
PCR	Polymerase chain reaction
RBP	Retinol-binding protein
RSV	Respiratory syncytial virus
RT-PCR	Reverse-transcriptase polymerase chain reaction
VAD	Vitamin A deficiency
VDD	Vitamin D deficiency

Methods

The EPIC study was a population-based study of the incidence and etiology of community-acquired pneumonia among hospitalized patients. The methodology of the EPIC study has been described in detail elsewhere.[21] The analysis described here included a subset of pediatric patients with RSV or hMPV for whom residual samples were available. These patients were enrolled between January 2010 and June 2012. Informed consent was obtained before enrollment, and the study protocol was approved by the Institutional Review Boards at the University of Tennessee, St Jude Research Hospital, and the Centers for Disease Control and Prevention. Patients were enrolled if they had clinical and radiographic evidence of LRTI. Children with recent hospitalization, cystic fibrosis, severe immunosuppression, or tracheostomy were excluded.

Demographic data were collected by caregiver interview. Clinical and laboratory data were collected by abstraction from the medical record. Blood was collected for bacterial culture and whole blood polymerase chain reaction (PCR), and

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nasopharyngeal and oropharyngeal (NP/OP) swabs were collected for reverse-transcriptase (RT)-PCR or direct PCR for respiratory viruses and atypical pathogens (*Mycoplasma* or *Chlamydophila*). Blood for acute serology was collected at enrollment and blood for convalescent serology was collected 3-7 weeks later to test for antibodies to select viral pathogens (adenoviruses, influenza A and B viruses, hMPV, parainfluenza viruses 1-3, and RSV). If required for clinical care, pleural fluid and endotracheal aspirates were tested by bacterial culture and/or PCR.

All enrolled children had evidence of LRTI on admission chest radiography; however, a dedicated study radiologist made the final determination of pneumonia based on a blinded review, and some children were then excluded from the main EPIC study analysis.[21] For the analysis described here, we included all enrolled children regardless of the study radiologist's final determination (patients hereinafter are referred to as having LRTI), and we refer to patients meeting the final radiographic definitions as having radiographically confirmed pneumonia. We only included children aged <5 years with LRTI and detection of RSV, hMPV, or both by NP/OP RT-PCR or a ≥4-fold rise in specific IgG for RSV or hMPV between acute and convalescent samples[21] and for whom residual acute serum was available. Children were considered to have viral-viral codetection if there was detection of 1 or more viruses in addition to RSV or hMPV by RT-PCR or serology, and viral-bacterial codetection if there was detection of a typical or atypical bacterial pathogen in addition to RSV or hMPV (and possibly other viruses) by blood or pleural fluid culture or PCR, NP/OP RT-PCR, or endotracheal aspirate culture within 48 hours of intubation. Retinol-binding protein (RBP) was measured in acute serum samples as a surrogate for vitamin A (owing to the typical 1:1 molar ratio in serum between retinol and RBP, as well as the relative instability of retinol) by enzyme-linked immunosorbent assay (Human RBP4 Quantikine ELISA Kit; R&D Systems, Minneapolis, Minnesota). VAD was defined as an RBP <15 000 ng/mL (equivalent to <0.7 μmol/L) in accordance with World Health Organization recommendations.[22-24] Serum vitamin D was measured in acute serum samples with a Clinical Laboratory Improvement Amendments–approved Vitamin D assay (Elecsys; Roche Diagnostics, Indianapolis, Indiana) that measured 25-hydroxylated metabolites of vitamin D; VDD was defined as a 25-hydroxy-vitamin D level <20 ng/mL, although the precise cutoffs for vitamin insufficiencies and deficiencies remain a topic of continued debate.[25,26]

We first applied the Fisher exact test to compare the proportions of VAD, VDD, or combined deficiencies (VAD+VDD) with outcomes, including intensive care unit (ICU) admission, invasive mechanical ventilation, and hospital length of stay (LOS) (dichotomized based on the median value). ORs and 95% CIs were also estimated for these comparisons. To further examine the impact of VAD and VDD, we applied inverse probability-weighted estimation (IPWE) to adjust for factors that could affect the association in an observational study.[27] A primary advantage of IPWE is that this approach can potentially make deficient and sufficient subsamples more comparable, with important factors balanced. For IPWE, a propensity score model was first built to balance the vitamin-deficient and -nondeficient groups with respect to baseline confounders or covariates that could affect the association of VAD or VDD with outcomes. More specifically, a logistic model with VAD/VDD as a response variable and a pool of covariates that could influence disease severity, including age groups (0-2 and 2-5 years), presence of radiographically confirmed pneumonia, viral-viral coinfection, viral-bacterial coinfection, and comorbid conditions (**Table I**), was fitted to estimate the predicted probability of VAD or VDD for each individual. In the second step, each individual was weighted by the inverse predicted probability of observed deficiency status. In the last step, the weights were used in a logistic regression model to characterize the effect of VAD or VDD on disease severity outcomes. A P value <.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.3 for Windows (SAS Institute, Cary, North Carolina).

Results

Among the 90 tested children aged <5 years hospitalized with LRTI and RSV and/or hMPV, the median age was 19 months

Table I. Characteristics of children hospitalized with lower respiratory tract infection and RSV or hMPV detections (n = 90)

Variables	Value
Female sex, n (%)	43 (48)
Race/ethnicity, n (%)	
White, non-Hispanic	16 (18)
Black, non-Hispanic	61 (68)
Hispanic	9 (10)
Any high school education (parent or guardian), n (%)	53 (58.8)
Age group, n (%)	
<2 y	59 (66)
≥2 to <5 y	31 (34)
Age, mo, median (IQR)	19 (10-30)
Weight <5th percentile, n (%)	8 (9)
Any comorbid condition, n (%)	57 (63)
Asthma	42 (47)
Preterm birth*	18 (40)
Congenital heart disease	5 (6)
Neurologic disorder	2 (2)
Radiographic pneumonia, n (%)†	71 (79)
Viral-viral codetection, n (%)‡	35 (39)
Viral-bacterial codetection, n (%)‡	8 (9)
Vitamin D deficiency, n (%)	11 (12)
Vitamin D level, ng/mL, median (IQR)	32 (24-38)
Vitamin A deficiency, n (%)§	41 (46)
RBP level, ng/mL, median (IQR)	15 522 (9749-21 630)
Both vitamin A and vitamin D deficiency§	7 (8)
Length of stay >3 d, n (%)	40 (44)
Length of stay, d, median (IQR)	3 (2 to 6)
Admission to intensive care unit, n (%)	15 (17)
Invasive mechanical ventilation, n (%)	6 (7)

*Preterm birth (<37 weeks gestation) was determined only for children aged <2 years at the time of enrollment ($n = 45$).
†Radiographic pneumonia based on final criteria by study radiologist.
‡Detection of 1 or more viruses or bacteria (both typical and atypical pathogens) in addition to RSV or hMPV; see text for details.
§RBP level <15 000 ng/mL indicates vitamin A deficiency. Vitamin D level <20 ng/mL indicates vitamin D deficiency. A value for vitamin D <5 ng/mL was assigned a numerical value of 1.