HHS Public Access

Author manuscript

J Pediatr. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

J Pediatr. 2017 August ; 187: 323–327. doi:10.1016/j.jpeds.2017.04.061.

Low Retinol Binding Protein and Vitamin D Levels are Associated with Severe Outcomes in Children Hospitalized with Lower Respiratory Tract Infection and RSV or hMPV detection

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Abstract

Retinol binding protein and vitamin D were measured in children <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 children and are associated with significantly elevated risk of intensive care unit admission and invasive mechanical ventilation.

Keywords

respiratory infection; respiratory tract; vitamin levels; paramyxovirus; mechanical ventilation; intensive care

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, 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.

The authors declare no conflicts of interest.

Respiratory syncytial virus (RSV) and the related human metapneumovirus (hMPV) are responsible for considerable morbidity and mortality due to lower respiratory tract infections (LRTI) in young children in the United States (9–12). Contradictory findings exist in the literature concerning the correlations of serum vitamin levels, evaluated independently, with RSV disease outcome (13–20). Here we sought to determine if vitamin A and/or D deficiencies, examined in parallel, were present among U.S. children <5 years of age who were hospitalized with LRTI in Memphis, TN and with RSV or hMPV detections, and if one or a combination of both vitamin levels correlated with disease outcome. 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 (St. Jude) to develop vaccines for these two pathogens (9). The Centers for Disease Control (CDC) Etiology of Pneumonia in the Community (EPIC) study provided a unique opportunity to pose questions about serum vitamin levels and respiratory disease (21).

Methods

The EPIC study was a population-based study of the incidence and etiology of community-acquired pneumonia (CAP) among hospitalized patients. Detailed methods of the EPIC study have been described elsewhere (21). This analysis included a subset of pediatric patients with RSV or hMPV detection for whom residual samples were available, who were enrolled in Memphis, TN, 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, and CDC. 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 for bacterial culture and whole blood polymerase chain reaction (PCR), and nasopharyngeal and oropharyngeal (NP/OP) swabs for RT-PCR or PCR for respiratory viruses and atypical pathogens (*Mycoplasma* or *Chlamydophila*) respectively were collected; blood for acute serology was collected at enrollment and convalescent serology three to seven weeks later for 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 radiograph; however, a dedicated study radiologist made the final determination of pneumonia based on a blinded review and some children were excluded from the main EPIC study analysis (21). For the analysis described here, we included all enrolled children regardless of study radiologist final determination (patients hereafter 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 <5 years old with LRTI and detection of RSV, hMPV, or both by NP/OP RT-PCR or a ≥ fourfold 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 co-detection if there was detection of one or

more viruses in addition to RSV or hMPV by RT-PCR or serology, and viral-bacterial co-detection 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 ETA culture within 48 hours of intubation [10]. Retinol binding protein (RBP) was measured from acute sera as a surrogate for vitamin A (due to the typical 1:1 molar ratio in serum between retinol and RBP, and the relative instability of retinol) by enzyme-linked immunosorbent assay (ELISA, R&D Systems human RBP4 Quantikine ELISA Kit); VAD was defined as RBP <15,000 ng/mL or <0.7 μmol/L per World Health Organization recommendations (22–24). Serum vitamin D was measured from acute sera by a Clinical Laboratory Improvement Amendments (CLIA)-approved Roche Elecsys Vitamin D assay that measured 25-hydroxylated metabolites of vitamin D; VDD was defined as 25-hydroxy-vitamin D <20 ng/mL, although the precise cut-offs for vitamin insufficiencies and deficiencies remain a topic of continued debate (25, 26).

The Fisher exact test was first performed to compare the proportions of VAD, VDD, or combined deficiencies (VAD+VDD) with outcomes including intensive care unit (ICU) admission, invasive mechanical ventilation (MV), and length of hospital stay (LOS) (dichotomized based on median). Odds ratios and 95% CI 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 may affect the association in an observational study (27). A primary advantage of IPWE is that this approach could potentially make deficient and sufficient subsamples more comparable, with important factors balanced. For IPWE, a propensity score (PS) model was first built to balance the vitamin deficient and non-deficient 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 may influence disease severity including age groups (0–2 and 2–5 years), presence of radiographically-confirmed pneumonia, viral-viral co-infection, viral-bacterial co-infection and co-morbid 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 <0.05 was considered statistically significant. All statistical analyses were performed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC).

Results

Among the 90 tested children <5 years old hospitalized with LRTI and RSV and/or hMPV detections, median age was 19 months (interquartile range (IQR) 10–30 months), and 61 (68%) were black (Table I). There were 68 (76%) patients with RSV alone, 19 (21%) with hMPV alone, and 3 (3%) with RSV-hMPV co-detections; co-detections with other viruses (39%) or bacteria (9%) were also observed. There were 41 (46%) children with VAD and 11 (12%) with VDD; most (64%) children with VDD were also VAD.

There were 15 patients (17%) admitted to ICU and 6 (7%) requiring mechanical ventilation. On bivariate analysis, a greater proportion of patients with VAD had ICU admission (27% vs

8%, p=0.02) but this was not seen for mechanical ventilation (Fisher exact test, Table I). There was no significant difference in the proportion of patients with VDD admitted to ICU (55% vs. 36%, p=0.08), but once admitted to the ICU, a greater proportion of VDD patients received mechanical ventilation (27% vs 4%, p=0.02). LOS >3 days was not associated with VAD or VDD. The number of patients with combined VAD and VDD was small (n=7) and no significant differences were observed when these patients were compared with patients with single deficiencies. However, the proportion of patients with combined VAD and VDD (n=7) was greater among those with ICU admission (43% vs 7%, p=0.03) and mechanical ventilation (29% vs 2%, p=0.04) compared with patients without either deficiency (Table II).

In the IPWE model, VAD was not significantly associated with ICU admission (OR 2.54 (95% CI 0.59–10.88)), mechanical ventilation (OR 2.36 (95% CI 0.26–21.74)), or LOS >3 days (OR 0.74 (95% CI 0.32–1.69)) (Table II). VDD was significantly associated with ICU admission (OR 3.29 (95% CI 1.20–9.02)) and mechanical ventilation (OR 11.20 (95% CI 2.27–55.25)), but not LOS >3 days (OR (OR 1.09 (85% CI 0.48–2.52)) (Table II).

Discussion

The study described in this report is unique in its examination of both VAD and VDD in children hospitalized with RSV and/or hMPV LRTI. In our population of young, predominantly black, hospitalized children, VAD and VDD were common. This association of black race and low vitamin D levels has been described previously (28). Although in bivariate analysis both deficiencies were associated with severe illness, IPWE model results demonstrated that only VDD was significantly associated with ICU admission and mechanical ventilation, although numbers were small and CIs were wide. VDD was independently associated with severe illness in an IPWE model that adjusted for age, viral-viral co-detections, viral-bacterial co-detections, radiographically-confirmed pneumonia, and co-morbidities; in the same model, VAD did not have a significant independent association with severe illness. The dependence of VAD on these confounders was not surprising, given the complex relationships that exist among vitamin A, age, the immune system, and the lung (6, 8, 26, 29–34).

Small animal studies previously showed that VAD is associated with poor IgA responses to respiratory viruses and that vitamin supplementation restores IgA responses (31, 35, 36). Apart from immunological deficiencies, VAD animals are characterized by a partial loss of cilia as well as squamous metaplasia within the epithelial lining of the respiratory tract (6–8). In preliminary studies (unpublished results), we found that VAD animals experienced more weight loss and death than vitamin-sufficient animals upon infection with two respiratory pathogens (in this case influenza virus and pneumococcus (37)).

Previous literature has described VDD associations with pneumonia severity. For example, among children in low-income countries, VDD (25-hydroxy-vitamin D<20 ng/mL) and vitamin D insufficiency (25 hydroxy vitamin D=20–29 ng/mL), or the presence of rickets, were associated with severe pneumonia as measured by the need for hospital admission, prolonged LOS, or hypoxemia (38). In Japan, children with VDD and LRTI often required supplemental oxygen or mechanical ventilation(2).

Although the numbers were small in the current study, we found that children with both VAD and VDD experienced the highest frequency of ICU admissions (43%) compared with individuals with a single deficiency or no deficiency. Consistent with this finding, we have observed that laboratory animals with both VAD and VDD had weaker immune responses toward respiratory virus antigens than animals with either VAD or VDD alone; supplementation with vitamins A and D corrected these responses (39).

Our results supplement previously described clinical findings in which VAD and VDD were evaluated independently for associations with disease caused by RSV. Associations between low vitamin levels and disease have been reported, but results have been contradictory (13–20). It is likely that discrepancies reflect differences among patient populations. The characteristics of a child's environment, background, and diet may modify the influence of vitamin levels on disease and/or the effects of disease on vitamin levels. Because we found that a greater proportion of patients with combined deficiency (VAD+VDD) were admitted to ICU and required mechanical ventilation, we encourage continued study of both VAD and VDD in future analyses.

What are vitamin levels among non-hospitalized children and adults? Again, the answer varies, because diets, breast-feeding practices and sun exposures differ across time periods, populations, and world regions (40). Studies often report the percentages of individuals who score as vitamin deficient or insufficient based on cut-off values (described above) rather than a normal range. For example, in a study of 4-month-old infants in New England conducted from 2005–2007, 37.5% of all newborns who were sampled within 72 hours of birth exhibited VDD, and 11.9% of 4-month-old infants exhibited VDD (41). In an earlier study, when vitamin A was measured among children with measles, and within a reference group in New York City, low vitamin levels were only recognized among the infected children and not within the reference group (42). The same was true in an independent study in California (43). In our more recent study in Memphis of non-hospitalized (and in some cases influenza virus-infected or virus-exposed) children and adults, deficiencies and insufficiencies for vitamin A (measured using the RBP surrogate) and vitamin D were frequently observed (34). The World Health Organization has estimated that moderate to severe VAD affects approximately 250 million preschool-age children worldwide (44). Overall, results suggest that vitamin insufficiencies and deficiencies frequently characterize both hospitalized and non-hospitalized individuals, in both developed and developing countries.

The current study has limitations, in that cause-effect relationships could not be discerned. Possibly, changes in RBP and vitamin D levels were a consequence of, rather than a cause of disease. RBP and vitamins A and D levels are negative acute phase reactants and can decline in response to inflammation (45, 46). The study was also limited in that it involved only one hospital with a small sample size. A larger study could help to further evaluate different infections, age groups, and geographical locations, and to dissect the influences of our selected covariates on outcome. The relatively high median age for RSV and hMPV infections makes these data less generalizable to the underlying population of infants with these infections. This anomaly likely occurred for two reasons: we did not actively enroll patients with bronchiolitis but instead enrolled patients with pneumonia or suspected

pneumonia, and we used residual blood from the EPIC study, which often was unavailable from the youngest children. Lastly, we were not able to include a control group without RSV or hMPV detections for comparison due to resource limitations.

In conclusion, we demonstrate that VAD and VDD were commonly detected among children <5 years old in Memphis, TN hospitalized with LRTI and RSV and/or hMPV detection. Low vitamin levels correlated with severe disease in this Memphis study, encouraging the continued evaluation of both vitamin A and D levels in U.S. children.

Acknowledgments

We thank Dr. Jay for overseeing vitamin D measurements in St. Jude's Department of Pathology. We acknowledge and fondly remember Dr. Russell W. Chesney for discussions supportive of this work.

Funded by NCI P30 CA21765 and ALSAC (to J.H.). The EPIC study was supported by the Influenza Division in the National Center for Immunizations and Respiratory Diseases at the Centers for Disease Control and Prevention through cooperative agreements with each study site and was based on a competitive research funding opportunity. The Memphis site for patient sample collection was supported by 1U18 IP-000489 to J.M.

Abbreviations

RSV	Respiratory syncytial virus
hMPV	human metapneumovirus
VAD	vitamin A deficiency
VDD	vitamin D deficiency
VAD+VDD	vitamin A deficiency and vitamin D deficiency
RBP	retinol binding protein
PCR	polymerase chain reaction
RT-PCR	reverse transcriptase-PCR
ETA	endotracheal aspirates
LOS	length of hospital stay
ICU	intensive care unit
IPWE	inverse probability-weighted estimation
PS	propensity score
OR	odds ratio
IQR	interquartile range

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Table 1

Characteristics of children hospitalized with lower respiratory tract infection and respiratory syncytial virus or human metapneumovirus detections (n=90)

Variable	No. (%)
Sex (female),	43 (48)
Race/ethnicity,	
White, non-hispanic	16 (18)
Black, non-hispanic	61 (68)
Hispanic	9 (10)
Any high school education (parent or guardian)	53 (58.8)
Age groups, n (%)	
<2 years	59 (66)
≥2 to <5 years	31 (34)
Age in months, median (IQR)[a]	19 (10 to 30)
Weight percentile < 5th	8 (9)
Any comorbid condition	57 (63)
Asthma	42 (47)
Preterm birth[b]	18 (40)
Congenital heart disease	5 (6)
Neurological disorder	2 (2)
Radiographic pneumonia[c]	71 (79)
Viral-viral co-detection[d]	35 (39)
Viral-bacterial co-detection[d]	8 (9)
Vitamin D deficiency	11 (12)
Vitamin D level (ng/mL), median (IQR)	32 (24 to 38)
Vitamin A deficiency[e]	41 (46)
RBP level (ng/mL), median (IQR)	15,522 (9749 to 21,630)
Both vitamin A and vitamin D deficiency[e]	7 (8)
Length of stay (LOS) more than 3 days	40 (44)
Length of stay in days, median (IQR)	3 (2 to 6)
Admission to intensive care unit (ICU), n (%)	15 (17)

Variable	No. (%)
Invasive mechanical ventilation	6 (7)

[a] IQR, interquartile range.

[b] Preterm birth (<37 weeks gestation) was only determined for children under 2 years of age at the time of enrollment (n=45).

[c] Radiographic pneumonia based on final criteria by study radiologist.

[d] Detection of one or more viruses or bacteria (both typical and atypical pathogens) in addition to RSV or hMPV. See text for details.

[e] RBP level less than 15,000 ng/ml indicates vitamin A deficiency. Vitamin D level less than 20 ng/ml indicates vitamin D deficiency. When a value for vitamin D was <5 ng/ml, it was assigned a numerical value of 1.

Table 2

Bivariate comparison of clinical outcomes between vitamin deficiency groups*

	LOS>3 days, n (%)	ICU admission, n (%)	Mechanical ventilation, n (%)
Vitamin A			
VAD (n=41)	18 (44%)	11 (27%)	4 (10%)
Non-VAD (n=49)	22 (45%)	4 (8%)	2 (4%)
P-value (VAD vs. Non-VAD)	1.00	0.02	0.41
OR (95% CI)	0.96 (0.42, 2.21)	4.13 (1.20, 14.17)	2.54 (0.44, 14.64)
Vitamin D			
VDD (n=11)	6 (55%)	4 (36%)	3 (27%)
Non-VDD (n=79)	34 (43%)	11 (14%)	3 (4%)
P-value (VDD vs. Non-VDD)	0.53	0.08	0.02
OR (95% CI)	1.59 (0.45, 5.64)	3.53 (0.89, 14.1)	9.5 (1.64, 55.13)
Vitamin A and Vitamin D			
VAD+VDD (n=7)	4 (57%)	3 (43%)	2 (29%)
Neither deficiency (n=45)	20 (44%)	3 (7%)	1 (2%)
P-value (VAD+VDD vs. Neither deficiency)	0.69	0.03	0.04
OR (95% CI)	1.67 (0.33, 8.32)	10.5 (1.57, 70.25)	17.6 (1.34, 230.5)

*LOS, length of hospital stay; ICU, intensive care unit; MV, invasive mechanical ventilation; VAD, vitamin A deficiency; VDD, vitamin D deficiency; OR, odds ratio; CI, confidence interval. Fisher's exact test was applied to compare proportions.

Association between vitamin deficiency and clinical outcomes with inverse probability-weighted estimation IPWE*

	LOS>3 days		ICU admission		MV	
	OR (95 CI)	**P-value**	**OR (95 CI)**	**P-value**	**OR (95 CI)**	**P-value**
VAD	0.74 (0.32–1.69)	0.469	2.54 (0.59–10.88)	0.198	2.36 (0.26–21.74)	0.437
VDD	1.09 (0.48–2.51)	0.834	3.29 (1.20–9.02)	0.018	11.20 (2.27–55.25)	<.001

* LOS, length of hospital stay; ICU, intensive care unit; MV, invasive mechanical ventilation; VAD, vitamin A deficiency; VDD, vitamin D deficiency; OR, odds ratio; CI, confidence interval.