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MAJOR ARTICLE

Early Amplified Respiratory Bioactive Lipid Response Is Associated With Worse Outcomes in Pediatric Influenza-Related Respiratory Failure

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Background. Biomarkers are needed for early identification of patients at risk of severe complications from influenza infection, including prolonged respiratory failure and death. Eicosanoids are bioactive lipid mediators with pro- and anti-inflammatory properties produced in response to infection. This study assessed the relationships between the host bioactive lipid response, influenza viral load, and clinical outcomes.

Methods. Influenza-positive, intubated children ≤18 years old were enrolled across 26 US pediatric intensive care units (PICUs). Mass spectrometry was used to measure >100 lipid metabolites in endotracheal and nasopharyngeal samples. Influenza viral load was measured by quantitative polymerase chain reaction.

Results. Age and bacterial co-infection were associated with multiple bioactive lipids ($P < .05$). Influenza viral load was lower in patients with bacterial co-infection compared with those without, and pro-inflammatory bioactive lipids positively correlated with viral load in bacterially co-infected children ($P < .05$). Lipids associated with disease resolution correlated with viral load in patients without bacterial co-infection ($P < .01$). After adjusting for age and bacterial co-infection status, elevated pro- and anti-inflammatory lipids measured early in the intensive care unit course were associated with higher mortality, whereas influenza viral load and endotracheal cytokine levels were not associated with clinical outcomes. Prostaglandin E_2, arachidonic acid, docosahexaenoic acid, and 12-hydroxyeicosatetraenoic acid measured within 72 hours of PICU admission predicted death or prolonged (≥28 days) mechanical ventilator support (area under the curve, 0.72–0.79; $P < .02$) not explained by admission illness severity.

Conclusions. Children with influenza-related complications have early bioactive lipid responses that may reflect lung disease severity. Respiratory bioactive lipids are candidate prognostic biomarkers to identify children with the most severe clinical outcomes.

Keywords. bacterial infection; influenza; lipids; mass spectrometry; pediatric care.

Influenza virus is a leading cause of hospitalization and acute respiratory failure in children. The majority of reported pediatric deaths in the United States occur in healthy children [1]. Despite annual vaccinations, influenza continues to be a public health threat, and seasons with poor vaccine–strain match lead to high hospitalization rates [1]. Aside from antivirals and supportive care, there are currently no approved therapies for severe influenza infection [2–5].

In hospitalized patients, lower respiratory tract infection (LRTI) can progress to acute respiratory distress syndrome (ARDS), which is associated with high morbidity and is often fatal [6]. Multiple influenza virus immune evasion mechanisms directly impede the host response [6, 7]. Secondary immune dysregulation can enable invasion by pathogenic bacteria that colonize the upper respiratory tract such as *Streptococcus pneumoniae* and *Staphylococcus aureus* [8–12]. Variability in host response to pathogen invasion is believed to strongly influence inflammation and lung disease severity [13, 14]. Understanding how bacterial co-infection differentially influences a dysregulated host immune response to influenza could facilitate development of targeted treatments.

Several studies have assessed the relationship between pro-inflammatory cytokines measured in blood and clinical outcomes in pediatric patients infected with either pandemic or severe strains of influenza [15–19]. In the multicenter Pediatric Intensive Care Influenza (PICFLU) cohort, a hyperinflammatory module of serum cytokines was associated with severe complications. However, >40 cytokines measured in the endotracheal (ET) aspirates of children with respiratory failure were surprisingly not associated with clinical outcomes [20]. Although it is

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appreciated that the lung is the primary organ affected in severe influenza infections, there is a disconnect between traditional respiratory cytokines as measures of inflammation and disease burden.

Eicosanoids are bioactive lipids comprised of arachidonic acid (AA) metabolites, such as prostaglandins and leukotrienes, that are known to have both pro-inflammatory and inflammation-resolving properties [21–26]. As depicted in Figure 1, AA and related polyunsaturated fatty acids (PUFAs) are released from phospholipid bilayers through the enzymatic activity of phosophlipase A2[26], and multiple pathways can further metabolize these precursor lipid molecules to produce hundreds of active lipid mediators capable of inducing a variety of cellular signals.

Several studies have suggested that bioactive lipids may complement existing influenza disease severity biomarkers and may improve our ability to identify patients at risk for more severe clinical outcomes [24, 27, 28]. In this study, the performance of bioactive lipids as disease-proximal biomarkers reflective of ongoing inflammation in the lung was examined. Bioactive lipids were assessed in matched ET aspirates and nasopharyngeal (NP) swabs collected from a cohort of children

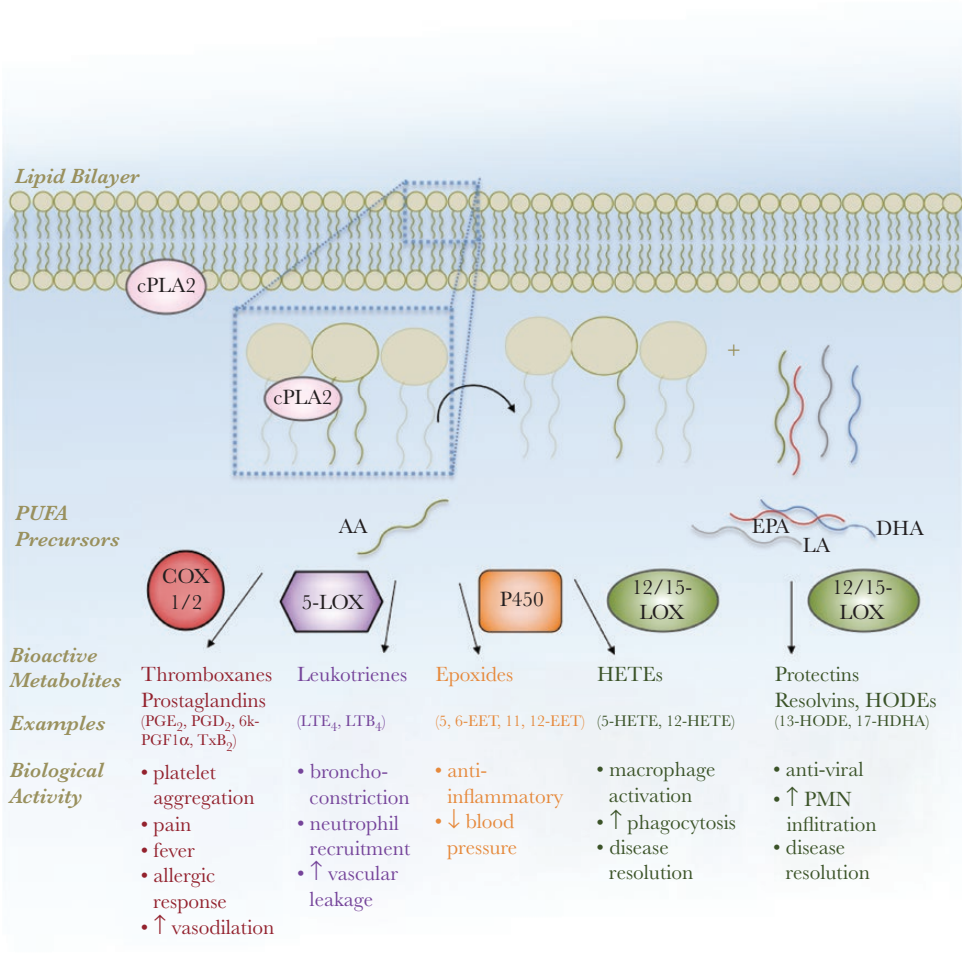

Figure 1. Metabolic processing of bioactive lipids and their role in host immune response to infection. Bioactive lipids are local signaling molecules produced from the metabolism of PUFA precursors (eg, AA, DHA, EPA, and LA) released from the phospholipid bilayer through the lipase activity of $cPLA_2$. These biologically inert precursors can be rapidly further metabolized through several enzymatic pathways to produce hundreds of bioactive mediators with distinct biological activity depending on cell types and receptor expression patterns in the local environment. For example, AA can be metabolized by COX-1/2 and 5-LOX to produce inflammatory mediators including thomboxanes, prostaglandins, and leukotrienes, or it can be metabolized by cytochrome P450 and 12/15-LOX enzymes to produce mediators with anti-inflammatory properties. Similar to AA, other PUFA precursors including EPA, DHA, and LA can also be metabolized via different enzymatic pathways including the 12/15-LOX pathway, which can lead to production of resolvins and protectins, a new class of bioactive lipids associated with disease resolution. Abbreviations: 11,12-EET, 11,12-epoxyeicosatrienoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 12/15-LOX, 12/15-lipoxygenase; 13-HODE, 13-hydroxyoctadecadienoic acid; 17-HDHA, 17-hydroxydocosahexaenoic acid; 5-LOX, 5-lipoxygenase; 5-HETE, 5-hydroxyeicosatetraenoic acid; 5,6-EET, 5,6-epoxyeicosatrienoic acid; 6k-PGF_1alpha, 6-keto-prostaglandin F_1alpha; AA, arachidonic acid; COX-1/2, cyclooxygenase-1/2; $cPLA_2$, cytoplasmic phopholipase A_2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HETEs, hydroxyeicosatetraenoic acids; HODEs, hydroxyoctadecadienoic acids; LA, linoleic acid; LTB_4, leukotriene B_4; LTE_4, leukotriene E_4; P450, cytochrome P450; PGD_2, prostaglandin D_2; PMN, polymorphonuclear neutrophils; PGE_2, prostaglandin E_2; PUFA, polyunsaturated fatty acid; TxB_2, thromboxane B_2.

with acute respiratory failure from influenza virus infection (PICFLU), and associations with clinical complications such as ARDS and septic shock were examined.

METHODS

Study Design

This study cohort included prospectively enrolled children (≤18 years of age) with severe influenza infection who were intubated and received invasive mechanical ventilator support at 26 pediatric intensive care units (PICUs) in the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network participating in the PICFLU study. Children with underlying heart, lung, immune, or other medical conditions predisposing to severe influenza infection were excluded. Patients were enrolled from February 2009 through May 2016, and all had at least 1 positive influenza test by polymerase chain reaction (PCR). Details of the PICFLU study have been published [29]. All PICFLU patients who were intubated at an enrolling center where endotracheal samples could be rapidly processed to obtain supernatant for analysis and whose samples were collected within 72 hours of PICU admission were included in this study. The institutional review boards at each site approved the study, and written informed consent was obtained from at least 1 parent or guardian.

The Pediatric Risk of Mortality (PRISM) III score was used to assess illness severity upon admission to the PICU [30]. ARDS was defined as acute onset of hypoxia (P/F ratio of ≤300 = mild, ≤200 = moderate, and ≤100 = severe, respectively) with bilateral infiltrates on chest radiograph and no evidence of left heart failure [31]. Vasopressor-dependent septic shock was defined as receiving dopamine >5 μg/kg/min and/or any epinephrine or norepinephrine [32]. Ventilator-free days was defined as days alive and free of mechanical ventilation up to 28 days [33]. Bacterial co-infections were defined as the growth of respiratory pathogen from the ET aspirate, blood, and/or pleural fluid within 72 hours of PICU admission (some grew from multiple sites) that were diagnosed as a co-infection at the clinical site and treated with a course of antibiotics, as previously described [34].

Sample Collection

NP flocked swabs, ET aspirates, and blood were collected using standard universal precautions and processed using BSL2 conditions. NP flocked swabs were inserted into the nares and back to the posterior nasopharynx with a standardized method and then placed in universal transport media. ET aspirate samples were obtained using standardized methods with 1.5 mL of sterile normal saline down the endotracheal tube, then 3–4 breaths before obtaining the sample with 2 suctioning attempts, aiming to collect at least 2 mL of aspirate. A variable amount of ET aspirate was retrieved from patients after what was needed for clinical purposes was removed. The sample was put on ice and transferred immediately to the laboratory for processing (median volume processed, ~2 mL per sample). One aliquot was immediately frozen (–80°C), and the rest were centrifuged; the supernatant was aliquoted and frozen at –80°C. Samples were obtained at enrollment and ~72 hours later, if still intubated. The paired NP swab and ET aspirate samples were collected in as close of a time frame as possible. See the Supplementary Data for information on sample processing.

Influenza Load Quantification

RNA was isolated from NP and ET samples with the MagNA Pure Compact Nucleic Acid Isolation kit (Roche Diagnostics, Penzberg, Germany). Viral RNA was transcribed to cDNA, and real-time quantitative PCR was performed using universal primers for influenza A and B (Supplementary Methods) in the same well using TaqMan FastVirus 1-Step Master Mix (Life Technologies, Carlsbad, CA, USA).

Bioactive Lipid Quantification

Quantification of bioactive lipid mediators was evaluated from human ET samples and NP swabs by liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS). Detailed information regarding sample preparation and the LC-MS/MS methods is reported in the Supplementary Data.

Statistical Analyses

Lipid concentrations (ng/mL) were log-transformed for all analyses. Spearman correlations were used to assess correlations between lipid mediators and continuous clinical outcomes. Differences in lipid concentrations among different demographic or clinical subsets were assessed using analysis of variance. Logistic regression was used to evaluate the association between lipids or cytokines (measured by enzyme-linked immunosorbent assay [20]) and mortality, ARDS, and shock. This was performed with or without adjustment for age, co-infection status, or both. Odds ratios (ORs) were reported, with significance set at $P < .05$, after adjustment for false discovery rate (FDR). Graphpad, version 7.0 (La Jolla, CA, USA), was used to generate receiver operating characteristic curves.

RESULTS

Characteristics of the 105 intubated pediatric patients included are shown in Table 1. There were 103 patients with ET samples, and 67 patients had matching NP swab samples (2 patients were included with NP samples only). The majority of children were male, 76.2% were white, and 21.9% were of Hispanic ethnicity. The majority were previously healthy (61.0%) with no prior medications. Influenza A virus predominated (79.0% influenza A, 21.9% influenza B), with H1N1pdm09 identified in 44.8% and H3N2 in 30.5%. Slightly over half of the influenza-positive children (n = 56, 53.3%) were diagnosed with bacterial co-infection, most commonly with *Staphylococcus aureus* (n = 37, 66.1%; n = 16 methicillin-resistant, n = 21

Table 1. Characteristics, Clinical Course, and Complications of Critically Ill Children Infected With Influenza Virus With or Without a Bacterial Coinfection (n = 105)

	All Patients (n = 105)	Influenza—No Bacterial Coinfection(n = 49)	Influenza—Bacterial Coinfection[a] (n = 56)	*P* Value
Male	69 (65.7)	33 (67.4)	36 (64.3)	.74
Hispanic ethnicity	23 (21.9)	11 (22.5)	12 (21.4)	.9
Race				.84
White	80 (76.2)	36 (73.5)	44 (78.6)	
Black	17 (16.2)	9 (18.4)	8 (14.3)	
Mixed/other	8 (7.6)	4 (8.2)	4 (7.1)	
Age, y	6.9 (2.5–11.4)	5.7 (2.1–9.9)	8.0 (3.1–12.6)	.13
Baseline health status				
Previously healthy	64 (61.0)	24 (49.0)	40 (71.4)	.02
Asthma/asthma medications	21 (20.0)	13 (26.5)	8 (14.3)	
Other underlying respiratory disorder	6 (5.7)	4 (8.2)	2 (3.6)	
Other underlying condition	22 (21.0)	14 (28.6)	8 (14.3)	
Influenza type				
Influenza A	83 (79.0)	41 (83.7)	42 (75.0)	.28
Influenza A(H1N1)pdm09	47 (44.8)	23 (46.9)	24 (42.9)	
Influenza A H3N2	32 (30.5)	15 (30.6)	17 (30.4)	
Influenza A seasonal H1	2 (1.9)	1 (2.0)	1 (1.8)	
Influenza A other	2 (1.9)	2 (4.1)	0 (0)	
Influenza B	23 (21.9)	9 (18.4)	14 (25.0)	.41
Time to presentation, d	3 (2–5)	2 (1–4)	3 (2–5)	.21
Secondary complications				
Extracorporeal life support	19 (18.1)	2 (4.1)	17 (30.4)	.0005
Acute respiratory distress syndrome	51 (48.6)	19 (38.8)	32 (57.1)	.06
Shock requiring vasopressors	59 (56.2)	23 (46.9)	36 (64.3)	.07
Illness severity & outcomes				
PRISM III score	8 (3–16)	5 (3–10)	9 (5–21)	.004
Duration of mechanical ventilation, h	138.0 (68.0–271.0)	129.5 (76.5–212.0)	156.5 (65.3–362.9)	.30
Duration of PICU stay, h	192 (113–340)	174 (101–265)	240.5 (118.0–431.0)	.12
Hospital mortality	8 (7.6)	1 (2.0)	7 (12.5)[b]	.06
Ventilator-free days	22.2 (15.2–25.2)	22.4 (19.1–24.8)	21.5 (11.6–25.3)	.30

Data are presented as No. (%) or median (interquartile range).

Abbreviations: PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality.

[a]Bacterial infections: methicillin-resistant *Staphylococcus aureus* (n = 16), methicillin-susceptible *Staphylococcus aureus* (n = 21), group A strep (n = 3), *Pneumococcus* (n = 7), *Haemophilus influenzae* type b (n = 4), *Haemophilus influenzae* nontypeable (n = 3), gram-negative rods–unspecified (n = 3), gram-positive cocci–unspecified (n = 2), *Moraxella catarrhalis* (n = 1), *Mycoplasma pneumoniae* (n = 1).

[b]One death in the influenza-bacterial co-infection category only had a nasopharyngeal sample.

methicillin-sensitive). Other bacterial subtypes identified are listed at the bottom of Table 1. Information on development of symptoms before presentation was available for 93 children (88.6%), with a median time from presentation to PICU admission (interquartile range) of 3 (2–4.5) days. Children with influenza and bacterial co-infection were more frequently previously healthy (71.4%) and more likely to receive extracorporeal membrane oxygenation (ECMO) support (30.4%); bacterial co-infection was diagnosed in 7 of 8 deaths. Other characteristics and outcomes were not significantly different between influenza-infected children with and those without bacterial co-infection.

Viral Load and Bioactive Lipid Profiles in Respiratory Samples

Bioactive lipid concentrations in ET aspirates and NP swabs from the PICFLU cohort were assessed using mass spectrometry (Supplementary Figure 1). Of 144 lipids tested, 15 lipids were quantifiable in ET aspirate specimens: prostaglandin E_2 (PGE_2), 11-beta-prostaglandin E_2 (11b-PGE_2), prostaglandin D_2 (PGD_2), 6-keto-prostaglandin F_1alpha (6k-PGF_1alpha), 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), 12-hydroxyeicosatetraenoic acid (12-HETE), 17-hydroxydocosahexaenoic acid (17-HDHA), leukotriene B_4 (LTB_4), leukotriene E_4 (LTE_4), thromboxane B_2 (TxB_2), 5-hydroxyeicosatetraenoic acid (5-HETE), arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). In NP swab eluates, 9 lipids were quantifiable: PGE_2, 11b-PGE_2, LTB_4, 9-HODE, 5-HETE, 13-HODE, 12-HETE, 17-HDHA, AA, DHA, and EPA. Quantifiable was defined as any value above the lowest point included in the calibration curve and each calibration curve was required to have > 7 points with CV <20%. Only lipids that met this criterion in >50% of samples for each matrix were used for subsequent analyses.

For each airway sample type, lipid profiles were compared between the following categorical variables: gender, ethnicity, patient age group, and influenza subtype. Only patient age and bacterial co-infection status had a significant impact on bioactive lipid levels from ET aspirates. On average, older children had elevated levels of polyunsaturated fatty acid (PUFA) lipid precursors as well as cyclooxygenase metabolites PGE_2 and 6k-PGF_1alpha, the 12-lipoxygenase metabolite 12-HETE, and 15-lipoxygenase metabolites 13-HODE and 17-HDHA in ET aspirate supernatants (Supplementary Figure 2).

Similarly, children with bacterial co-infection had elevated ET aspirate lipid levels compared with children with no bacterial co-infection (Supplementary Figure 2). Specifically, patients with bacterial co-infection had elevated levels of the PGE_2 metabolite 11b-PGE_2, and similar trends were seen with PGE_2 wheras the prostaglandin PUFA precursor AA was unchanged. Patients with no detectable bacterial co-infection had elevated levels of 17-HDHA and lower levels of its PUFA precursor DHA. Bacterially co-infected children also had lower levels of 12-HETE, a 12/15-lipoxygenase metabolite thought to play a role in dampening inflammation [27, 28, 35]. Influenza subtype, gender, and ethnicity did not significantly contribute to differences in the described lipid profiles (Supplementary Figure 2).

NP swab samples were also compared between the same categorical variables: gender, ethnicity, patient age group, and influenza subtype. No statistically significant differences in lipid levels based on age and co-infection status were observed in NP swabs, although trends similar to the ET aspirates were seen (Supplementary Figure 3). Because of reduced sensitivity in NP swab eluates compared with ET aspirates, only ET aspirate lipid concentrations were used for the remaining analyses.

Influenza-infected patients with no evidence of bacterial co-infection had on average a >10-fold higher influenza viral load in their ET aspirates compared with those with bacterial co-infection (Figure 2A). Parent-reported time from symptom onset to presentation was used to approximate illness duration, and there was no significant difference between patients infected with influenza alone and patients with bacterial co-infection (Supplementary Figure 4). Viral load was not associated with reported clinical complications of septic shock, moderate–severe ARDS, or mortality (Supplementary Figure 4).

Associations between influenza viral load in the lung and levels of bioactive lipids were assessed. Levels of PGE_2, PGD_2, LTB_4, and 12-HETE correlated with influenza viral load in influenza-infected patients co-infected with bacteria (Figure 2B, D; Supplementary Figure 5). Levels of 17-HDHA, 13-HODE, and DHA correlated with viral load in patients without bacterial co-infection (Figures 2C, D; Supplementary Figure 5). Spearman correlations between viral load and individual lipids differed by bacterial co-infection status, and these correlations are summarized in a heatmap (Figure 2D).

Association of Bioactive Lipids With Clinical Outcomes

Specific bioactive lipids were modestly associated with the number of days alive and free of mechanical ventilator support (VFDs) differentially in patients with and without bacterial co-infection (Figure 3A). In children with bacterial co-infection, levels of DHA, EPA, and 9-HODE were inversely associated with fewer VFDs (r = –.36, –.36, and –.35, respectively; all P < .01), indicating that higher levels of bioactive lipids were associated with worse clinical outcomes. Higher levels of LTB_4 were observed to be associated with more VFDs and improved clinical outcome in bacterially co-infected patients. In influenza-infected children without bacterial co-infection, only TxB_2 was associated with fewer VFDs (r = .39; P < .01). Scatterplots and correlation results for all lipids are shown in the Supplementary Data (Supplementary Figure 6).

None of the lipids tested were associated with shock or ARDS; however, many of the individual bioactive lipids were prognostic for mortality in the 7 patients who died. Importantly, death was associated with lipids reported to have pro-inflammatory and pro-disease resolution immunomodulatory properties including PGE_2, 12-HETE, 13-HODE, 9-HODE, AA, EPA, DHA, and LTB_4. Patients with elevated levels of these bioactive lipids in ET samples detected within 72 hours of PICU admission had a significantly increased risk for mortality after controlling for patient age and bacterial co-infection (Figure 3B). In contrast, a post hoc analysis of the PICFLU cohort ET aspirate cytokine data, which included 41 cytokines and total protein, showed no associations with shock, ARDS, or death after adjusting for age and bacterial co-infection (Supplementary Figure 7).

Changes in bioactive lipid levels in ET aspirates over time were evaluated in 38 of the 103 patients (37%) who had a second sample taken ~72 hours after the initial sample (initial sample was taken within 72 hours of PICU admission). Levels of bioactive lipids collected at later time points were mostly lower or equal to levels observed in the initial sample taken closer to PICU admission (Supplementary Figure 8). There was no clear correlation between lipid level change from baseline and duration of ventilation or length of stay in the PICU. This is a limited sample set, and additional longitudinal samples from patients who died are needed to determine whether a decrease in lipid levels is indicative of clinical improvement.

There were 11 patients who died and/or remained on mechanical ventilator support in the PICU on day 28 (0 VFD). Nine of these patients had bacterial co-infection. Receiver operating characteristics were assessed for PGE_2, 12-HETE, 17-HDHA, AA, and DHA to determine their ability to predict 0 VFD. DHA, PGE_2, AA, and 12-HETE were strongly predictive of patients with 0 VFD, with an area under the curve (AUC) of 72–79 (Figure 4); however, the PRISM III admission score had higher predictive power (AUC, 0.84). In bivariate analyses controlling for PRISM (Supplementary Table 2), DHA, AA, and

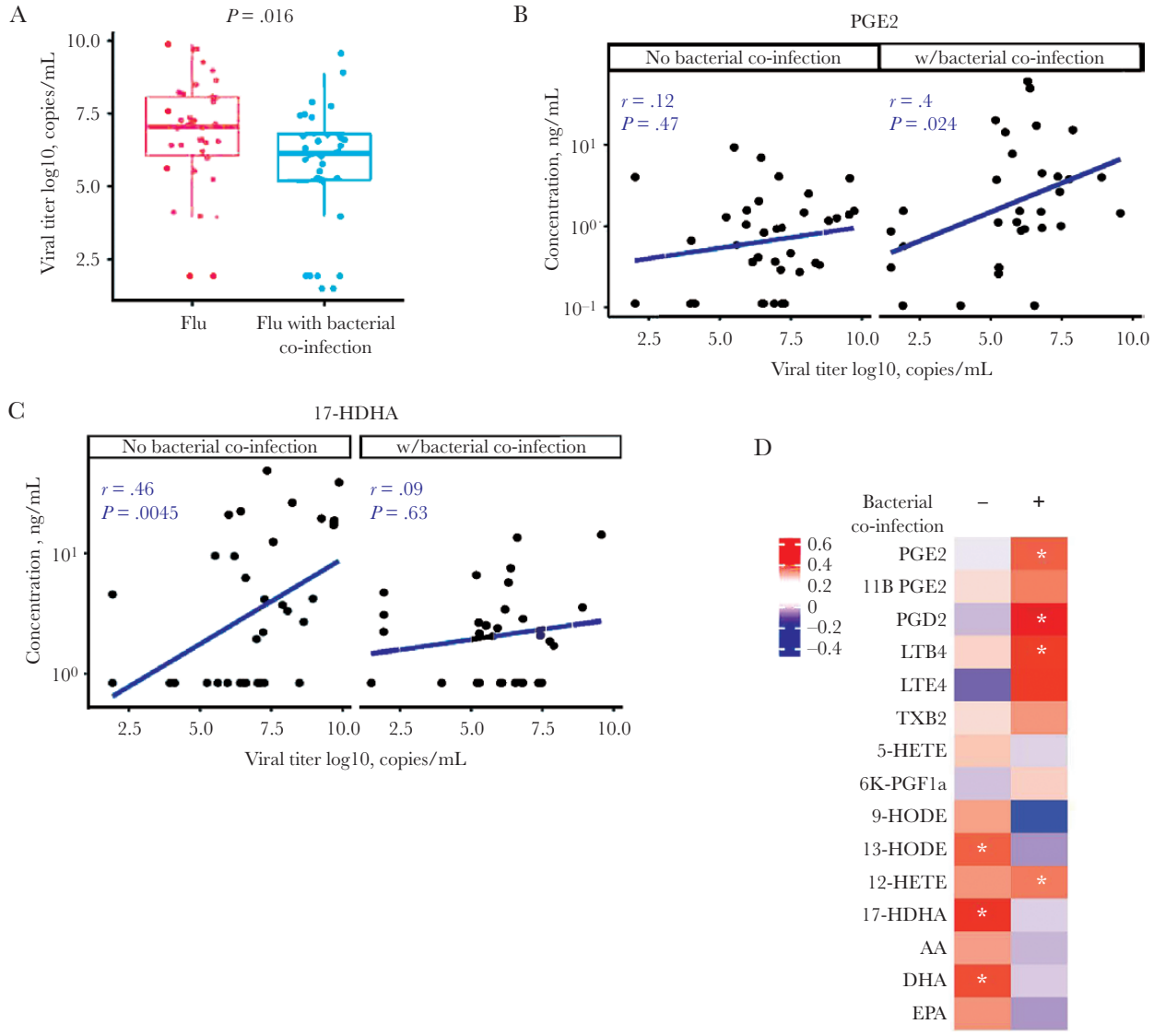

Figure 2. Correlations between viral titer and bioactive lipids are impacted by bacterial co-infection status. A, Viral titers in ET samples are grouped by bacterial co-infection status. Patients with no bacterial co-infection are indicated in red, and patients with bacterial co-infection are indicated in blue. Spearman correlations between concentrations of (B) PGE_2 and (C) 17-HDHA and viral titer in ET samples grouped by co-infection status are plotted. Lipid concentrations and viral titer are plotted on a log scale with correlation coefficients and *P* values labeled on each plot. D, Heatmap summarizing correlation values between viral titer and lipid levels in ET samples. Red indicates higher correlation and purple indicates lower correlation. Asterisks indicate a *P* value <.05. Abbreviations: 12-HETE, 12-hydroxyeicosatetraenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; 17-HDHA, 17-hydroxydocosahexaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; 6k-PGF_1alpha, 6-keto-prostaglandin F_1alpha; 9-HODE, 9-hydroxyoctadecadienoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ET, endotracheal; LTB_4, leukotriene B_4; LTE_4, leukotriene E_4; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; TxB_2, thromboxane B_2.

PGE2 remained significant (*P* = .008, .01, and .02, respectively), whereas 12-HETE was above the margin (*P* = .06).

DISCUSSION

Bioactive lipids from ET samples collected early in the PICU stay of children with influenza-related respiratory failure were predictive of death or very prolonged (≥28 days) mechanical ventilator support. DHA, AA, and PGE_2 remained predictive after controlling for the patient's admission illness severity. Specifically, the host bioactive lipid response in influenza-infected children with acute respiratory failure showed an elevated bioactive lipid profile, especially in the presence of bacterial co-infection. The bioactive lipid profile associated with influenza viral load differed markedly in patients with bacterial co-infection versus those without. Specifically, the bioactive lipid response to influenza was more inflammatory in the presence of bacterial co-infection. However, viral load and cytokines from the respiratory compartment were not predictive of disease complications, whereas elevated levels of bioactive lipids with opposing

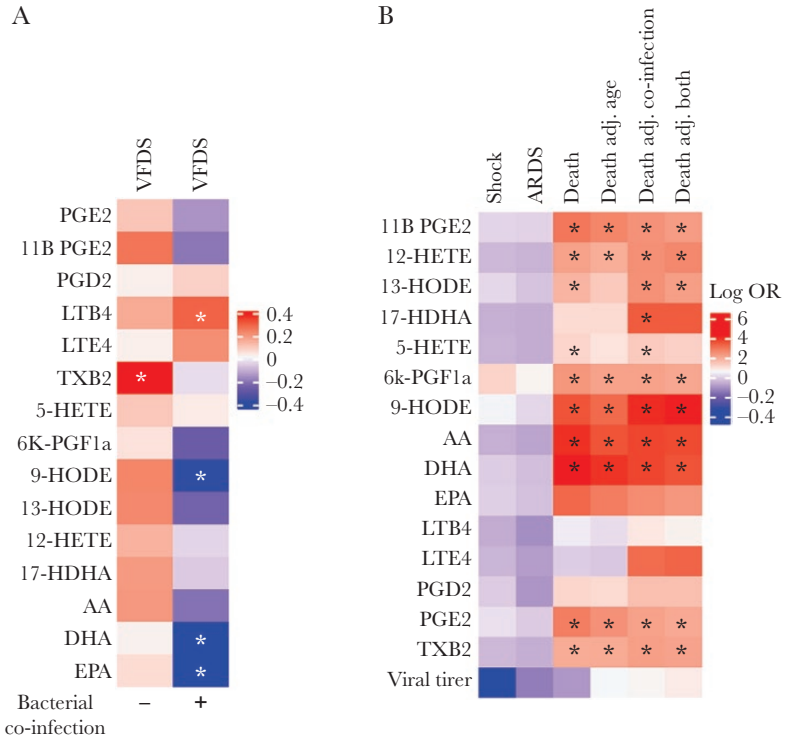

Figure 3. Bioactive lipids in ET aspirates are prognostic for clinical outcomes in influenza-infected children. A, Heatmap of Spearman correlations between number of days alive and free of mechanical ventilation and lipid concentrations subgrouped by bacterial co-infection status. Red indicates higher correlation and purple indicates lower correlation with days free of mechanical ventilation. Asterisks indicate a *P* value <.05. B, Heatmap of odds ratios for clinical outcomes shock, ARDS, death, death adjusted for age, death adjusted for bacterial co-infection, or death adjusted for both patient age and bacterial co-infection status. Red indicates a higher odds ratio of progression to shock, ARDS, death, death adjusted for age, death adjusted for bacterial co-infection, or death adjusted for both age and bacterial co-infection. Asterisks indicate a *P* value <.05. Abbreviations: 12-HETE, 12-hydroxyeicosatetraenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; 17-HDHA, 17-hydroxydocosahexaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; 6k-PGF_1alpha, 6-keto-prostaglandin F_1alpha; 9-HODE, 9-hydroxyoctadecadienoic acid; AA, arachidonic acid; ARDS, acute respiratory distress syndrome; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ET, endotracheal; LTB_4, leukotriene B_4; LTE_4, leukotriene E_4; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; TxB_2, thromboxane B_2; VFDS, ventilator-free days.

immunomodulatory properties were predictive of poor clinical outcome.

Although our cohort is more severe, these findings support those of prior studies in influenza-infected patients, including Tam et al. and Morita et al., showing that specific AA and DHA bioactive lipid metabolites are detectable and elevated in patients with greater symptom burden [27, 28]. Our results support those of Dalli et al., who found significantly higher inflammation-initiating mediators including AA metabolites and pro-resolving DHA metabolites (eg, 17-HDHA) in nonsurviving sepsis patients [24]. It may be that patients at risk of mortality due to severe viral or bacterial infection have amplified levels of both inflammatory and pro-resolution bioactive lipids, indicating dysregulation of the host response, which may serve as an important biomarker of patient immune phenotype (Supplementary Figure 9).

This study differed from prior studies in that previous influenza studies used human nasal washes [27] or human cells lines [28], and this study focused on ET aspirates from children with respiratory failure. NP swab eluates were also examined; however, lower levels of bioactive lipids were detected in samples collected from the nose compared with those collected from the trachea. This observation could be attributed to differences in sample processing such as dilution volume or could reflect that ET samples are more proximal to the site of disease activity and therefore more appropriate for comparisons. This study is the first to evaluate the impact of bacterial co-infection on the bioactive lipid profile. Bacterial co-infection is a common comorbidity with influenza-infected patients with acute respiratory failure and it is associated with worse clinical outcomes [36, 37].

The strengths of this study include that the cohort of influenza-infected children came from multiple centers representing all US regions, that influenza subtyping was performed, and that detailed patient phenotyping was provided. The methods used for the lipid analyses were also rigorous and replicable. Although some lipids were detectable, many were either absent or present at low concentrations, thus precluding their analyses. One limitation of this study is that the cohort is

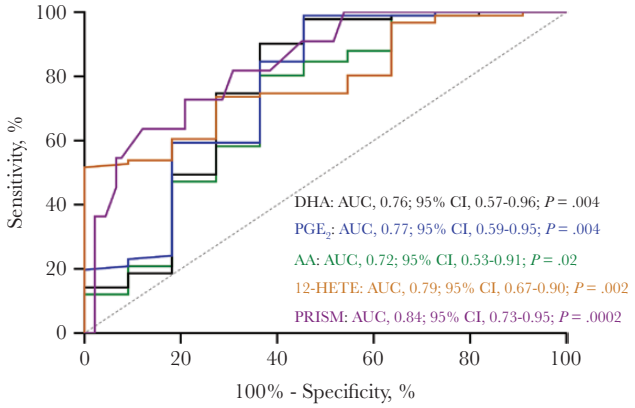

Figure 4. Evaluation of bioactive lipids as predictors of clinical outcome. Performance of PGE_2, 12-HETE, DHA, AA, and PRISM III admission illness severity score for prediction of 0 days free of mechanical ventilation (includes patients that died or were still on mechanical ventilation at day 28). The area under the receiver operating characteristic curve and classification accuracy are provided in each panel. Abbreviations: 12-HETE, 12-hydroxyeicosatetraenoic acid; AA, arachidonic acid; AUC, area under the curve; DHA, docosahexaenoic acid; PGE_2, prostaglandin E_2; PRISM, Pediatric Risk of Mortality.

limited in size, with ~10% of the children having VFD of 0, restricting the confidence in the resulting associations. Although there was no correlation with ARDS, all patients were intubated and hypoxic, and there may not have been sufficient variability in their lung disease severity to detect an association. Other indicators, such as oxygenation index, may have been more accurate. Additionally, there is no standardized approach for reporting analyte concentrations from ET supernatant samples, and multiple methods have been utilized in the literature, making comparability across studies a challenge. Lack of serial samples in all patients impeded accurate evaluation of the patients' lipid profiles over time. Unfortunately, we did not have a validation cohort of critically ill children with influenza virus–related acute respiratory failure to validate our findings.

CONCLUSIONS

In this study, a targeted bioactive lipidomics approach was applied to understand associations between bioactive lipid mediators and influenza-related host inflammation, viral replication, and clinical outcomes. The bioactive lipid profiles of children with influenza LRTI are strongly influenced by the presence of a bacterial co-infection, which may have important implications for future immune-modulatory therapeutic strategies. Early production of pro-inflammatory and pro-resolving bioactive lipid mediators in the lower respiratory compartment in the PICU course was associated with increased risk of the worst clinical outcomes. In summary, bioactive lipids found in ET aspirates may serve as important biomarkers of lung-specific disease activity, and the possibility exists that modulation of these bioactive lipid mediators in influenza-infected patients could be explored to understand the underlying influence on disease outcome.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. V.A., A.R., and J.M. conceived the study; V.A., X.Y., A.N., and M.N. analyzed the data; A.R. and M.N. oversaw patient recruitment and data management and performed the clinical assessments; all authors were involved in interpretation of experimental results, wrote parts of the manuscript, edited and reviewed the manuscript, and approved the final version.

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