

determined in our study. Therefore, these patients should merely be considered to represent cases of possible IPA only, and the 17% overall incidence thus is an upper bound estimate. Conversely, IPA incidence is known to be higher among immunocompromised patients, but our study population was too small to provide estimates stratified by prior immune status.

Our study demonstrates that plasma galactomannan measurement conveys insufficient evidence to confidently rule out influenza-associated IPA, as half of clinically recognized cases had undetectable plasma galactomannan levels in *post hoc* testing. This confirms previously reported sensitivities of galactomannan measurement in ICU patients of only 30–50% (6–8). True sensitivity may be even worse, as measurements in plasma are thought to result in higher optical indexes than in serum (9), and sensitivity to detect late-onset IPA was increased by measuring at two times. Sensitivity of galactomannan could probably be increased by a combination with β -glucan measurements, but unfortunately, our biorepository did not contain the required serum samples. It is also possible that patients with influenza predominantly suffer from airway-invasive *Aspergillus* infection rather than angio-invasive infection, which would explain negative plasma galactomannan results. Unfortunately, as computed tomography imaging of the lungs was performed in only 4 of 10 IPA cases, and findings were mostly nonspecific, it was not possible to confidently differentiate these pathologies. Furthermore, the daily processing and storage of leftover plasma did not guarantee sterile sample handling, which may have led to false-positive test results. This is supported by the absence of histopathological findings for invasive pulmonary mycosis in a patient with high levels of plasma galactomannan in *post hoc* determination.

In conclusion, our study confirms the higher risk of IPA in patients with sCAP with influenza, compared with other pathogens, and high mortality of influenza-associated IPA. Our findings also demonstrate the low diagnostic yield of routine plasma galactomannan measurement, which we, therefore, do not recommend as a screening tool for IPA in patients with sCAP caused by influenza. ■

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Maternal Asthma, Preeclampsia, and Risk for Childhood Asthma at Age Six

To the Editor:

Maternal asthma and preeclampsia have independently been reported to be associated with increased asthma incidence in children of affected mothers. Maternal asthma is also associated with increased

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risk for preeclampsia development. This evidence raised an unanswered question of whether maternal asthma alone might drive the association of preeclampsia with an increased risk for childhood asthma, or whether the two conditions could confer an independent and joint effect on child asthma risk (1, 2). Therefore, using the data from VDAART (Vitamin D Antenatal Asthma Reduction Trial), we prospectively investigated whether preeclampsia was associated with increased risk for early-life childhood asthma in children younger than 3 and 6 years, and whether the risk was even higher if the mother also had asthma. Eligible participants in the VDAART were nonsmoking adult women (18–39 yr old) with gestational ages of 10–18 weeks; with history of asthma or atopy, or whose partner (biologic father of the child) had a history of asthma or atopy; and with intent to participate for at least 4 years. After delivery, children were monitored by phone every 3 months and in person yearly for 6 years, where the children's health, respiratory symptoms, and medications were assessed. Asthma was defined as a maternal or caregiver report of physician-diagnosed asthma.

Maternal Asthma, Preeclampsia, and Asthma Diagnosis during the First 6 Years of Life

In our initial report from the VDAART participants (806 children and their mothers [322/806, 40.0% with maternal asthma]), we demonstrated that the development of preeclampsia during pregnancy increased the risk for offspring recurrent wheeze (at least two occasions in 2 separate years, as previously defined [3]) accompanied by a physician diagnosis of asthma by age 3 years (risk ratio [RR] = 1.95; 95% confidence interval [CI], 1.13–3.38) (3). Recent data from the VDAART on the follow-up of 806 children by age 6 years confirms our prior observation at 3 years of follow-up. Overall, 119 (63.30%) of 188 children who had recurrent wheeze by age 3 years were diagnosed with asthma before age 6 years. These children demonstrated a higher risk for an asthma diagnosis if their mother had preeclampsia compared with those children born to mothers without preeclampsia (16/67 [23.88%] vs. 103/739 [13.94%], respectively; RR = 1.71; 95% CI, 1.10–2.70). This risk represents a higher probability of asthma diagnosis from birth to 6 years of age (cumulative incidence) among children born to mothers with preeclampsia compared with those born to mothers without preeclampsia. Similarly, the risk for recurrent wheeze by age 3 years, and an asthma diagnosis before age 6 years, was higher among children born to mothers with asthma (regardless of control status) compared with among those born to mothers without asthma (72/322 [22.36%] and 47/484 [9.71%], respectively; RR = 2.30; 95% CI, 1.64–3.23). Ninety-four (29.19%) of 322 children whose mothers had asthma were exposed to at least 1 month of uncontrolled asthma during prenatal period, as determined by Asthma Control Test (ACT) during pregnancy (ACT score $\leq$ 16) monthly, reported by their mothers (4). This exposure was associated with an increased risk for recurrent wheeze by age 3 years accompanied by an asthma diagnosis before age 6 years compared with those children whose mothers had controlled asthma throughout pregnancy (29/94 [30.85%] and 43/228 [18.86%], respectively; RR = 1.64; 95% CI, 1.10–2.45). However, having a mother with controlled asthma throughout pregnancy (ACT score $>$ 16) did not nullify an increased risk for child recurrent wheeze by age 3 years accompanied by an asthma diagnosis before age 6 years compared with children born to mothers without asthma (43/228 [18.86%] and 47/484 [9.71%], respectively; RR = 1.94; 95% CI, 1.33–2.85).

Joint Effect of Maternal Asthma and Preeclampsia on Active Asthma Status at Age 6 Years

Considering prior prospective investigations on early-life wheezing, asthma, and lung function at ages 3 and 6 years, and the fact that children with wheezing and an asthma diagnosis by age 3 years might outgrow their symptoms by age 6 years (5), we further investigated whether children born to mothers with preeclampsia were also at higher risk of remaining symptomatic and received treatment for asthma at age 6 years. Active asthma at age of 6 years, for children observed after their fifth birthday, was determined by report of physician-diagnosed asthma at any time in the first 6 years of life and child's use of any asthma medication after the fifth birthday with or without parental report of wheeze. Of 806 children with asthma diagnosis status before age 6 years, 707 (87.72%) had available data on active asthma status at age 6 years. Among these children, 79 (45.40%) of 174 children who had recurrent wheeze by age 3 years had active asthma at age 6 years. Six (8.96%) of 67 children whose mothers had preeclampsia during pregnancy were among those with unavailable data for active asthma at age 6 years. A total of 40.31% of children with available data (285/707) had mothers with a history of asthma.

In 806 pairs of children and mothers, we earlier showed there was a higher risk for child asthma diagnosis during first 6 years of life among pregnancies with preeclampsia (RR = 1.71). Therefore, we examined whether this risk remained high for active asthma status at 6 years of life among 707 children with available data for asthma treatment and respiratory symptoms at the age of 6 years (alternative hypothesis: RR $>$ 1). The incidence of active asthma at age 6 years in children born to mothers with preeclampsia during pregnancy whose parents also reported recurrent wheeze by age 3 years was 18.03% (11/61) versus 10.53% (68/646), with an RR of 1.71 compared with those born to mothers without preeclampsia (lower limit of one-sided 95% CI, 1.053; $P = 0.035$). This risk represents a trend toward a similar effect of preeclampsia on asthma diagnosis during the first 6 years of life and having an active asthma at the sixth year of life (RR = 1.71 for both child asthma diagnosis by age 6 yr and active asthma at age 6 yr). Of note, 81.82% of children (9/11) born to mothers with preeclampsia and active asthma at age 6 years had their asthma diagnosis by age 3 years.

To demonstrate the additive interaction between preeclampsia and maternal asthma, a new composite variable with four categories was constructed, indicating a category of joint exposure to both risk factors ($++$: maternal asthma with preeclampsia), a category of exposure to one of the risk factors only ($-+$ or $+-$: without maternal asthma with preeclampsia or with maternal asthma without preeclampsia), and the joint reference category of no exposure (background risk, $--$ or 1: without maternal asthma without preeclampsia). The logistic regression was used to calculate the odds ratios (ORs), using the new indicator variable. Test for trend was used to examine a dose-response association between the ordered variable and child active asthma at age 6 years. A test for trend allows a researcher to demonstrate a dose-response association between the risk factor and the outcome, even if the association is not statistically significant for any particular level of exposure (6). To evaluate interaction on an additive scale, three different measures were calculated: 1) relative excess risk resulting from interaction (RERI): $RERI = OR_{++} - OR_{+-} - OR_{-+} + 1$; 2) attributable proportion resulting from interaction (AP): $AP = RERI/OR_{++}$; and 3) the synergy index (S): $S = (OR_{++} - 1)/[(OR_{-+} - 1) + (OR_{+-} - 1)]$. In the absence of an interaction effect, RERI and AP equal 0, and S equals 1 (7).

Table 1. Trend in Risk for Child Active Asthma at Age 6 Years Preceded by Recurrent Wheeze by Age 3 Years across Pregnant Women Groups according to Their History of Physician-diagnosed Asthma and Preeclampsia Status

Maternal (Co)morbidity Status	Offspring Active Asthma at Age of 6 yr			Trend in Risk Estimates	
	Yes	No	Total	Odds Ratio	P Value for Trend Test*
No asthma					
Without preeclampsia	26	361	387	1 (Reference)	
With preeclampsia	5	30	35	2.30	
Asthma					
Without preeclampsia	42	217	259	2.70	
With preeclampsia	6	20	26	4.17	
Total	79	628	707	—	<0.0001

*Extended Mantel-Haenszel chi square for linear trend = 27.47; *P* value for trend (1 degree of freedom) with continuity correction < 0.0001.

The incidence of active asthma at age 6 years preceded by recurrent wheeze by age 3 years was 6.72% (26/387), 14.29% (5/35), 16.22% (42/259), and 23.08% (6/26) among children born to mothers without asthma without preeclampsia, mothers without asthma with preeclampsia, mothers with asthma without preeclampsia, and mothers with asthma with preeclampsia, respectively. The trend test indicated that an increase in maternal morbidity across ordered levels (without asthma without preeclampsia, without asthma with preeclampsia, with asthma without preeclampsia, and with asthma

with preeclampsia) increased the risk for child active asthma at age 6 years. The highest risk was observed among mothers with asthma who developed preeclampsia during their pregnancy relative to mothers without asthma without preeclampsia (OR, 4.17; *P* for trend < 0.0001; Table 1). This risk was relatively lower if the mother had asthma and did not have preeclampsia, or did not have asthma but developed preeclampsia (OR, 2.70 and 2.30, respectively; Table 1). The additive interaction measures were RERI = 1.17, AP = 0.28, and S = 1.06. The trend in association of the composite ordered variable of maternal

Table 2. Estimated Risk for Active Asthma at 6-Year Follow-up in the Offspring by Maternal Asthma and Preeclampsia Status

Variable	Univariable Analysis			Stepwise Multivariable Analysis		
	OR	95% CI	P Value	Adjusted OR	95% CI	P Value
Maternal asthma and morbidity status						
Mothers without asthma						
Without preeclampsia	1 (Ref)*	—	—	1 (Ref)*	—	—
With preeclampsia	2.30	0.83–6.46	0.10	1.40	0.42–3.87	0.56
Mothers with asthma						
Without preeclampsia	2.70	1.60–4.50	<0.001	2.60	1.53–4.40	<0.001
With preeclampsia	4.17	1.54–11.27	0.002	3.70	1.20–10.07	0.014
Maternal age	0.95	0.91–0.99	0.015	—	—	—
Gestational age at delivery, ≥34 vs. <34 wk	0.24	0.09–0.70	0.008	0.27	0.1–0.85	0.017
Trial group, vitamin D intervention vs. placebo	0.96	0.60–1.54	0.87	—	—	—
Maternal baseline vitamin D, ≥30 vs. <30 ng/ml	0.55	0.27–1.03	0.043	—	—	—
Clinical center						
Boston, Massachusetts	1 (Ref)	—	—	—	—	—
San Diego, California	0.69	0.29–1.25	0.18	0.86	0.38–1.91	0.71
St. Louis, Missouri	1.86	1.07–3.35	0.03	1.84	1.03–3.39	0.044
Race						
White	1 (Ref)	—	—	—	—	—
African American	2.24	1.33–3.87	0.003	—	—	—
Other	0.73	0.28–1.67	0.48	—	—	—
Maternal marital status, married vs. unmarried or divorced	0.43	0.26–0.71	0.001	0.61	0.33–1.08	0.098
Maternal education, college and above vs. less than college	0.53	0.30–0.90	0.02	—	—	—
Body mass index at first appointment, <25 vs. ≥25 kg/m ²	0.54	0.31–0.92	0.014	—	—	—
Gestational diabetes, yes vs. no	0.18	0.01–0.86	0.10	0.27	0.02–1.34	0.21
Mode of delivery, vaginal vs. cesarean delivery	1.13	0.68–1.94	0.64	—	—	—
Child's sex, M vs. F	1.73	1.07–2.85	0.027	1.67	1.01–2.78	0.048
Parity, 1 vs. >1	0.73	0.42–1.42	0.23	—	—	—
Child's weight at birth, ≥2.5 vs. <2.5 kg	0.9994	0.9990–0.9998	0.003	—	—	—
Paternal asthma, yes vs. no	1.22	0.70–2.04	0.47	—	—	—

Definition of abbreviations: CI = confidence interval; OR = odds ratio; Ref = reference.

**P* for trend across levels < 0.0001.

asthma and preeclampsia status with active asthma status in the 6-year-old children remained significant after adjustment for potential confounders (the same variables as in the earlier analysis [3]) used in multivariable logistic regression models (P for trend < 0.0001; Table 2). Higher risk for active child asthma was observed among mothers without asthma with preeclampsia and with asthma without preeclampsia compared with mothers without asthma without preeclampsia (adjusted OR, 1.40 [95% CI, 0.42–3.87] and 2.60 [95% CI, 1.53–4.40], respectively). However, the estimated risk for asthma was relatively greater among mothers with asthma and preeclampsia (adjusted OR, 3.70; 95% CI, 1.20–10.07; Table 2). In the multivariable adjusted model (Table 2), the additive interaction measures were $RERI = 1.70$, $AP = 0.46$, and $S = 1.63$, demonstrating a positive additive interaction between maternal asthma and preeclampsia in relationship with active asthma at age 6 years.

Implications

These results further confirm our prior observation in 3-year-old children that preeclampsia is a risk factor for early childhood wheeze and asthma and has an additive effect to maternal asthma as a strong prenatal risk factor among pregnant women. Furthermore, the observation implicates a higher risk for asthma persistence in late childhood of those born to mothers with high-risk pregnancies (i.e., preeclampsia with or without asthma). In the VDAART cohort, children born to asthmatic mothers with controlled asthma throughout pregnancy were at higher risk for recurrent wheeze before age 3 years and asthma diagnosis during the first 6 years of life compared with those born to mothers without asthma. However, these children had a lower risk for recurrent wheeze before age 3 years and asthma diagnosis during the first 6 years of life compared with those born to mothers with at least 1 month of uncontrolled asthma during their pregnancy. This observation highlights the role of genetics in asthma and places further emphasis on the improvement of asthma control during pregnancy to reduce the risk for adverse pregnancy and offspring outcomes, including preeclampsia and childhood asthma (2, 8). It is noted that the VDAART cohort included nonsmokers pregnant with a 40% prevalence of maternal asthma compared with an 8% prevalence of asthma in pregnancy generally. This feature might have affected the estimated risks, as opposed to the general population.

Conclusions and Future Direction

Despite lack of full agreement with results from prior observational studies investigating the association of preeclampsia and increased risk for wheezing and asthma in offspring, together, the weight of evidence from these investigations favors preeclampsia as a risk factor for offspring wheeze and asthma (9). Our study adds further to the literature on this relationship and highlights the role of *in utero* programming in the pathobiology of asthma. There is scant evidence on the potential mechanisms involved. One area of research to be explored in the interaction of preeclampsia and maternal asthma in child asthma development is the role of impaired vascular endothelial growth factor in pregnancies with preeclampsia, and how fetal exposure to increased sFlt-1 (soluble fms-like tyrosine kinase 1) could affect fetal respiratory and immune cell maturation during affected pregnancies (10–12) and how nutritional deficiencies in pregnancy and childhood such as vitamin D deficiency might interact (13). Patterns of preschool lung growth and the characterization of immune system responses in children born to mothers with preeclampsia should also be further explored (14, 15).

In conclusion, preeclampsia is associated with increased risk for early- and late-life wheeze and asthma in children younger than 6

years, this risk could be higher if the child's mother has asthma, and asthma control during pregnancy could be beneficial in reducing risk for preeclampsia and childhood asthma. The results further implicate the interplay between maternal factors as strong predictors of offspring asthma and *in utero* maternal–fetal immune perturbations and developmental dysregulations associated with preeclampsia. New approaches such as application of systems-level omics tools to unravel the underlying mechanisms during pregnancy and offspring's early life are warranted (16). ■

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6 The Effect of Acclidinium on Symptoms Including Cough in Chronic Obstructive Pulmonary Disease: A Phase 4, Double-Blind, Placebo-controlled, Parallel-Group Study

To the Editor:

Cough and sputum production are very common and troublesome symptoms for patients with chronic obstructive pulmonary disease (COPD) (1), and those symptoms are

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associated with lung function decline, increased exacerbation risk, and poor prognosis (2–4). To date, few clinical studies have investigated the efficacy of a long-acting muscarinic antagonist (LAMA) on cough (5, 6). Acclidinium is a LAMA approved as a twice-daily maintenance bronchodilator treatment for patients with COPD (7). This study assessed the efficacy of acclidinium on symptoms, including cough, in patients with moderate COPD. This was a phase 4, double-blind, placebo-controlled, parallel-group study (clinical trial registered with www.clinicaltrials.gov: NCT02375724) in 30 centers across five European countries between March 23, 2015, and November 17, 2015. Patients were randomly assigned 1:1 to receive twice-daily acclidinium 400 µg or placebo, administered via a multidose, dry powder inhaler (Genuair/Pressair, a registered trademark of the AstraZeneca group of companies, for use within the United States as Pressair and as Genuair within all other licensed territories). The study comprised a 1- to 2-week run-in period followed by an 8-week treatment period. Efficacy endpoints were measured at Week 4 and Week 8; the data over the course of 8 weeks are shown as an average of the scores measured at Week 4 and Week 8. Patients were aged at least 40 years with moderate COPD (post-bronchodilator FEV₁ ≥50% and <80% predicted; FEV₁/FVC <70%). The primary endpoint was change from baseline in Evaluating Respiratory Symptoms in COPD (E-RS: COPD, formerly the Exacerbations of Chronic Pulmonary Disease Tool [EXACT]-Respiratory Symptoms Scale [E-RS; <http://www.exactproinitiative.com/instrument-descriptions/>], is owned by Evidera; permission to use this instrument may be obtained from Evidera [<http://www.exactproinitiative.com/instrument-descriptions/exactpro@evidera.com>]) total score over the course of 8 weeks (minimal clinically important difference [MCID], 2.0) (8). Secondary efficacy endpoints were change from baseline in E-RS cough and sputum domain score over the course of 8 weeks (MCID, 0.7) (8) and change from baseline in Leicester Cough Questionnaire (LCQ; MCID, 1.3) (9) at Week 8. Exploratory endpoints included change from baseline in COPD Assessment Test (CAT) score (MCID, 2.0) (10), cough severity visual analog scale (VAS) score, E-RS total score, and E-RS cough and sputum domain score at Week 4 and Week 8, and E-RS breathlessness (MCID, 0.1) and chest domain scores (MCID, 0.7) (8) at Week 4 and Week 8 and over the course of 8 weeks. A *post hoc* analysis stratified patients by baseline cough severity (VAS; >30 mm, more severe; ≤30 mm, less severe) to assess the effect of acclidinium on cough-related endpoints. All patients provided written informed consent; study protocols and amendments were approved by local ethics committees. The primary endpoint was analyzed using a mixed model for repeated measures.

Overall, 269 patients were randomized; all had chronic bronchitis, 135 received acclidinium, and 134 received placebo. Sixty percent of patients were male, 64% were current smokers, and mean age was 62 years, with mean post-bronchodilator FEV₁ 64.2% predicted. In addition, patients had previously received short-acting β₂-agonist (74%) and LAMA (26%). Mean baseline E-RS breathlessness, cough and sputum, and chest domain scores were 6.0, 3.7, and 2.9, respectively, and total E-RS was 12.5. Baseline CAT and LCQ scores were 19.4 and 14.5, respectively.

Significant improvements in E-RS total score were observed with acclidinium versus placebo (Figure 1). Acclidinium