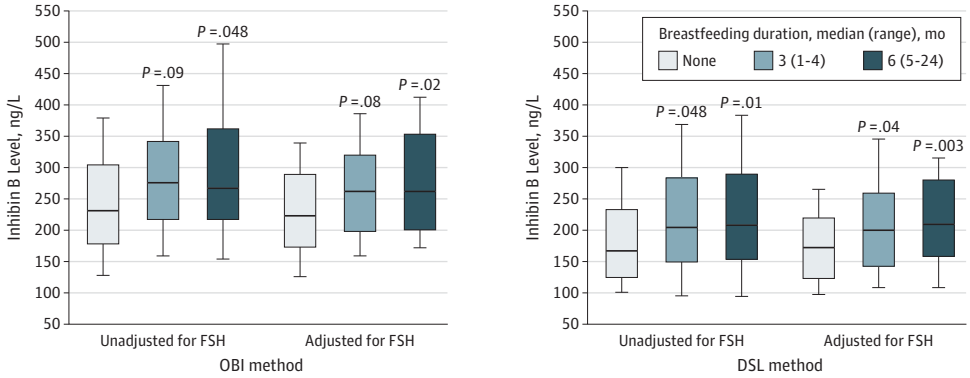
Figure. Serum Inhibin B Levels in School-Aged Adolescents According to the Total Duration of Breastfeeding



The middle horizontal bars represent the median values; the upper and lower limits of the boxes, the interquartile range; and the whiskers, the 10th and 90th percentiles. Inhibin B level was adjusted for age, time of blood sampling, and the cumulative attendance at indoor chlorinated pools before the age of 10 years. The group of breastfed adolescents was dichotomized at the median split of breastfeeding duration. *P* values represent the Bonferroni-adjusted *P* values for the comparison of breastfed groups (n = 61 in each group) with the nonbreastfed group (n = 76). DSL indicates Diagnostic System Laboratories; FSH, follicle-stimulating hormone; and OBI, Oxford Bio-Innovation.

Discussion | Serum inhibin B is a marker of the Sertoli cell number that determines testis size and sperm production in adults. Sertoli cells develop during fetal and neonatal life and also at puberty when their number is definitively set. Our study suggests that breastfeeding is important for neonatal testes development because the inhibin B increase associated with breastfeeding was of the same magnitude as the proportion of Sertoli cells formed during the first year of life.[5]

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Study concept and design: Bernard.
Acquisition of data: All authors.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: Bernard.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: All authors.
Obtained funding: All authors.
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Study supervision: Bernard.

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Effect of Routine Vaccination on Aluminum and Essential Element Levels in Preterm Infants

Parenteral feedings containing more than 4 to 5 µg/kg/d of aluminum have been shown to result in neurodevelopmental delay in preterm infants.[1] However, an infant at the 2-month checkup receives multiple aluminum-containing vaccines that in combination may have as high as 1225 µg of intramuscular aluminum; this is a much higher intramuscular aluminum dose than the safely recommended intravenous aluminum dose.[2] Our first objective was to measure prevaccine and postvaccine levels of aluminum in preterm infants, a population at higher risk of aluminum neurotoxic effects. Our second objective was to measure prevaccine and postvaccine levels of essential elements (EE). Inflammation from trauma can cause declines in serum levels of specific EE such as zinc and selenium[3-5]; there may be similar EE perturbations secondary to vaccination-induced inflammation.

Methods | After institutional review board approval and parental consent, 15 preterm infants scheduled for routine 2-month vaccinations while still hospitalized were recruited in the Sparrow Hospital neonatal intensive care unit in East Lansing, Michigan. One day prior to scheduled vaccination, 0.25-mL blood and 12-hour urine collections were obtained. Prevnar 13, PedvaxHIB, and Pediarix vaccines were administered, in total containing 1200 µg of aluminum, as determined by company literature and confirmed by testing a set of these vaccines in our laboratory. One day postvaccination, 0.25-mL blood and 12-hour urine collections were obtained. Aluminum and EE concentrations were quantified by inductively coupled plasma

Table. Characteristics of 15 Study Participants

	Mean (SD)
Nutrition: breast milk (alone or supplemented), No./total No. (%)	11/15 (73.3)
White, No./total No. (%)	13/15 (86.7)
Male, No./total No. (%)	8/15 (53.3)
Gestational age, wk	27.1 (1.7)
Age at vaccination, d	71.6 (31.9)
Postconceptional age, wk	37.4 (4.3)
Birth weight, g	1021 (334)
Weight at vaccination, g	2254 (962)
Blood creatinine level, mg/dL	0.4 (0.2)
Hemoglobin, g/dL	10.7 (1.5)
Hematocrit, %	31.9 (4.4)
Prevaccination serum level of aluminum, ng/mL	11.1 (10.3)
Prevaccination serum levels of essential elements associated with inflammation-induced perturbations	
Iron, ng/mL	4.3 (6.5)
Manganese, ng/mL	2.7 (1.8)
Copper, µg/mL	0.5 (0.2)
Selenium, ng/mL	47.4 (13.4)
Zinc, µg/mL	1.1 (0.6)

SI conversion factors: To convert copper to micromoles per liter, multiply by 0.157; creatinine to micromoles per liter, multiply by 88.4; hematocrit to proportion of 1.0, multiply by 0.01; hemoglobin to grams per liter, multiply by 10; iron to micromoles per liter, multiply by 0.179; manganese to nanomoles per liter, multiply by 18.202; selenium to micromoles per liter, multiply by 0.0127; and zinc to micromoles per liter, multiply by 0.153.

mass spectrometry in serum and urine. Urine data were normalized using creatinine concentration. Two-tailed *P* values <.05 on paired *t* tests (SAS software; SAS Institute Inc) were considered significant.

Results | No significant change in levels of urinary or serum aluminum were seen after vaccination (**Table**). Significant declines were noted postvaccination in serum iron (58.1%), manganese (25.9%), selenium (9.5%), and zinc (36.4%) levels, as was a significant increase in serum copper level (8.0%). A rise in selenium level was the only significant urine change. No significant postvaccine urinary or serum level changes were noted for phosphorus, sulfur, potassium, cobalt, nickel, molybdenum, nickel, or sodium. All participants had normal serum creatinine levels.

Discussion | We were reassured to find no significant postvaccine rise in serum aluminum level after vaccination of preterm infants with vaccines containing a total of 1200 µg of aluminum. The average study infant weighed 2200 g at vaccination and thus received about 545 µg/kg of intramuscular aluminum. Thus far, infant aluminum-adjuvant dosage safety has relied on animal-to-human extrapolations[6] and modeling of infant pharmacokinetics based on extrapolation from adult pharmacokinetic data to infant glomerular filtration rates.[7] We know of no study prior assessing actual aluminum blood level responses to vaccination in human infants.

Our study is small (N = 15), but one of the key studies for examining the postvaccine rise in aluminum blood levels is a study of only 6 rabbits.[6] That study showed that postvaccination serum aluminum levels rose 1%, peaking within 24 hours of vaccination. We thus chose 24 hours as our postvaccine measurement point.[6]

We observed a significant decline of serum levels of iron, manganese, zinc, and selenium and a significant increase in copper level (a marker of inflammation) on the day after vaccination. These same EE have been described as declining after inflammation from trauma or burns.[3-5] Of the EE that are not known to be associated with inflammation-induced perturbations (ie, sodium and potassium), we found stability of these levels after vaccination.

None of our participants had changes in nutrition type, medications, and/or blood transfusions during the course of the study. Therefore, it is reasonable to assume that the EE changes were a result of vaccination. Because of the aforementioned stability of aluminum-influencing factors (ie, nutrition and medications), postvaccine aluminum changes, if they were to have occurred, could have been attributed to vaccination dosage.

Sequestration of certain EE into tissues with a subsequent reduction of serum levels of corresponding EE is an important component of the innate immune system.[8] This immune response has likely evolved as a host defense mechanism to deprive microbial organisms of their nutrients.[8] While this vaccine-induced homeostatic shift in EE levels has not previously been described in humans, it has been documented in horses. After vaccination of horses, iron and other EE become temporarily sequestered within hepatocytes and other cell types.[9,10] As has also been found in other studies of EE after inflammation,[3-5] we found a significant rise in postvaccine urinary selenium levels, suggesting that selenium is at least partially excreted, rather than completely sequestered like other micronutrients for which there was no postvaccination urinary rise.

Limitations of our study include its small sample size and single postvaccine measurement, as well as the absence of markers of inflammation to help quantify the inflammatory response. However, because trace elements play important roles in neurodevelopment and the immune system, the effect of vaccination on EE should be investigated in more detail.

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Drafting of the manuscript: Movsas, Paneth, Zyskowski.
Critical revision of the manuscript for important intellectual content: Movsas, Paneth, Rumbeiha, Gewolb.
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COMMENT & RESPONSE

Questions Concerning Nasal Intermittent Positive-Pressure Ventilation vs Nasal Continuous Positive Airway Pressure

To the Editor It was with great interest that we read the article by Meneses et al.[1] After reading the article carefully, we have 2 questions. In the review and meta-analysis, Meneses et al concluded that it is plausible that nasal intermittent positive-pressure ventilation (NIPPV) may reduce the incidence of bronchopulmonary dysplasia (BPD) as compared with nasal continuous positive airway pressure (NCPAP). The data were obtained from 3 trials. We noticed that 1 of the trials by Meneses et al[2] reported that the total number of infants was 200, 100 in each arm who were randomly assigned. However, Figure 4 (incidence of BPD) of their meta-analysis[1] showed that the total number in the NIPPV group was 83, and the total number in the NCPAP group was 80. Why are the total numbers in the 2 articles not consistent? Also, in the meta-analysis,[1] Meneses et al chose the number of infants who had moderate or severe BPD but excluded mild BPD. The results of the incidence of BPD showed no significant difference between the groups (risk ratio, 0.56; 95% CI, 0.09-3.49), but significant heterogeneity was found among the trials (P = .04; I^2 = 69%). We suggest that all the infants who had BPD (mild, moderate, and severe) should be included for meta-analysis. The results from this meta-analysis (**Figure**) showed that there was still no difference between the groups (risk ratio, 0.50; 95% CI, 0.13-2.00), and no significant heterogeneity was found among the trials (P = .09; I^2 = 58%) in the random-effect model. There was a trend of a relatively low rate of BPD in infants randomized to NIPPV as compared with NCPAP in our meta-analysis. Why did Meneses et al exclude infants who had mild BPD?

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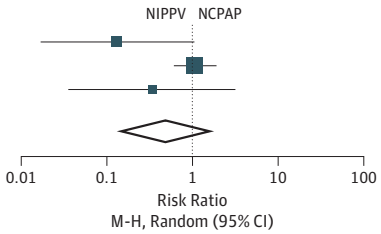
Figure. Incidences of Bronchopulmonary Dysplasia

Source, Year	NIPPV Events	NIPPV Total	NCPAP Events	NCPAP Total	Weight, %	Risk Ratio M-H, Random (95% CI)
Kugelman et al,[3] 2007	1	43	7	41	25.2	0.14 (0.02-1.06)
Meneses et al,[2] 2011	22	100	20	100	51.8	1.10 (0.64-1.88)
Sai Sunil Kishore et al,[4] 2009	1	37	3	39	23.0	0.35 (0.04-3.23)
Total (95% CI)		**180**		**180**	**100.0**	**0.50 (0.13-2.00)**
Total events	24		30			

Heterogeneity: $\tau^2 = 0.89$; $\chi^2_3 = 4.75$ (P = .09); I^2 = 58%
Test for overall effect: $z = 0.98$ (P = .33)



Defined as the need for supplemental oxygen, assessed at 36 weeks' postmenstrual age. M-H indicates Mantel-Haenszel test; NCPAP, nasal continuous positive airway pressure; and NIPPV, nasal intermittent positive-pressure ventilation.