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ORIGINAL REPORT

Number of antigens in early childhood vaccines and neuropsychological outcomes at age 7–10 years

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ABSTRACT

Purpose Concerns have been raised that children may be receiving too many immunizations under the recommended schedule in the USA. We used a publicly available dataset to evaluate the association between antibody-stimulating proteins and polysaccharides from early childhood vaccines and neuropsychological outcomes at age 7–10 years.

Methods Children aged 7–10 years from four managed care organizations underwent standardized tests for domain-specific neuropsychological outcomes: general intellectual function, speech and language, verbal memory, attention and executive function, tics, achievement, visual spatial ability, and behavior regulation. Vaccination histories up to 24 months of age were obtained from medical charts, electronic records, and parents' records. Logistic regressions and structural equation modeling (SEM) were used to determine associations between total antigens up to 7, 12, and 24 months and domain-specific outcomes.

Results On average, children ($N = 1047$) received 7266, 8127, and 10 341 antigens by ages 7, 12, and 24 months, respectively. For adjusted analyses, increase (per 1000) in the number of antigens was not associated with any neuropsychological outcomes. Antigen counts above the 10th percentile, compared with lower counts, were also not associated with any adverse outcomes. However, children with higher antigen counts up to 24 months performed better on attention and executive function tests (odds ratio for lower scores = 0.51, 95% confidence interval = 0.26, 0.99). Similar results were found with SEM analysis ($b = 0.08$, $p = 0.02$).

Conclusions We did not find any adverse associations between antigens received through vaccines in the first two years of life and neuropsychological outcomes in later childhood. Published 2013. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS—antigens; pediatrics; neuropsychology; epidemiology; child health; pharmacoepidemiology

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INTRODUCTION

Over the last hundred years, the number of recommended pediatric vaccines has increased from 1 to 11 with more than 20 doses of vaccines by 2 years of age. Although scientific evidence to date indicates that clinically significant adverse health outcomes following childhood immunizations are rare, concerns regarding vaccine safety persist.[1] Although a large majority of parents believe that vaccines protect their children from disease, many have concerns about serious adverse effects of vaccines,[2] and concerns about vaccine safety have led many parents to refuse certain vaccines for their children or delay immunization beyond the Centers for Disease Control and Prevention (CDC) recommended schedule.[3] In a survey among parents of young children, 13% reported following a vaccine schedule other than that recommended by CDC; of these, 82% reported delaying one or more vaccine doses, believing this to be a safer practice with fewer side effects.[4] In another nationally representative telephone survey conducted in 1999, 25% of parents believed that receipt of too many immunizations weakened children's immune system, and 23% thought that children were getting too many immunizations.[5]

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Vaccine-related immune response is primarily induced by antigens (i.e., antigenic or antibody-stimulating proteins and polysaccharides in vaccines). The number of antigens present in vaccines varies widely ranging from as low as one antigen (hepatitis B vaccine) to more than 3000 antigens (whole cell pertussis-containing vaccines) (Appendix 1). Considering this variation, Offit et al. suggested that taking into account the number of antibody-stimulating proteins and polysaccharides in vaccines might provide a better assessment of the level of immunologic stimulation rather than simply counting the number of vaccines received.[1] We assessed the association between the number of antigens received from vaccines in the first 2 years of life and development of neuropsychological outcomes later in childhood.

METHODS

We used a public use dataset (http://www.cdc.gov/vaccinesafety/Concerns/Thimerosal/neuropsychological_Outcomes.html) from a 2003–2004 CDC study led by Thompson et al. (2007) that evaluated the association between thimerosal exposure from vaccines in the first 7 months of life and neuropsychological outcomes at age 7 to 10 years.[6] A follow up study was conducted using a structural equation modeling (SEM) technique to verify and reassess some of the findings from the original study.[7] By using the same data, this current analysis focuses on exposure to antibody-stimulating proteins and polysaccharides in vaccines and their association with neuropsychological outcomes. Participant selection, data collection, and other methodology have been documented and published in detail elsewhere.[6–8]

Participant selection

Children between the ages of 7 and 10 years from four Managed Care Organizations (MCO) that were part of CDC's Vaccine Safety Datalink (VSD) were enrolled in the study. Children were born between 1 January 1993 and 30 March 1997. Low birth weight children (<2500 g), children with certain conditions (e.g., encephalitis, meningitis, or lead poisoning) that could influence neuropsychological outcomes, and children on certain medications (e.g., antidepressants or anticonvulsants) were excluded from the study.[6,8] Only one singleton child per family was eligible for inclusion. Eligible children were MCO members from birth, were continuously enrolled through their first year of life, and received all their first year vaccines at the MCO. All parents provided written informed consent for enrolling their children, and the original study was approved by the Institutional Review Boards (IRBs) of CDC and participating MCOs. The current analysis was considered exempt from human subject research review.

Vaccination history and antigen calculation

Children's immunization histories were determined from computerized immunization records, paper medical records, and immunization records provided by parents. The number of antigens in each vaccine was estimated based on published data according to vaccine type, manufacturer, and year of receipt.[9] Whereas most vaccines received by study children contained from 1 to 69 antigens, the whole cell pertussis component of diphtheria, tetanus, pertussis (DTP) vaccine, and DTP-Hib vaccines contained 3000 antigens. In contrast, the acellular pertussis component of DTaP vaccines contained only two to four antigens, depending on the manufacturer. The diphtheria and tetanus components added only one antigen each, whereas the Hib component added two (Appendix 1).

Neuropsychological outcomes assessment

Trained assessors evaluated each child in-person using a standardized test battery covering 42 neuropsychological outcomes in a controlled clinic environment.[6] These measures were selected on the basis of a previous CDC-led screening study utilizing healthcare databases and recommendations from an external panel of independent consultants.[6,10,11] For this analysis, 27 of the 42 measures were organized into eight theoretical domains on the basis of expert opinion and previous study on this data by Barile et al. (2011): general intellectual function, speech and language, verbal memory, fine motor coordination, attention and executive function, tics, visual spatial ability, and behavior regulation. This analysis included similar domain-specific measures except for one additional measure of attention and executive function (Gordon Diagnostic System Vigilance Task Errors).[7] Measures on stuttering were not included in this analysis, as the previous study found the data on stuttering to have poor reliability.[7] The selected measures included subscales from the Wechsler Abbreviated Scale of Intelligence,[12] Woodcock–Johnson III,[13] Boston Naming Test,[14] Developmental Neuropsychological Assessment,[15] Clinical Evaluation of Language Fundamentals—Third Edition,[16] California Verbal Learning Test—Children's Version,[17] Wechsler Intelligence Scale for Children—revised,[18] Gordon Diagnostic System (GDS),[19] Conners' Rating Scales—Revised,[20] and the Behavioral Rating Inventory of Executive Functioning,[21] finger

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tapping test,[22] and observations for phonic and motor tics. All the measures were scored on continuous scales except for tics (motor and phonic), which were dichotomous (yes/no). Only measurements by trained assessors were included in the analysis except for hyperactivity, inattentiveness, and behavior regulation where parental ratings were included.

Children, household, and maternal characteristics

Information on the child's birth date, sex, birth weight, APGAR score, anemia, pica, medication use, and thimerosal exposure from vaccines up to 7 months of age was obtained from medical and immunization records. In a face-to-face interview with the mother, using a standardized questionnaire, additional information was collected on study children (e.g., race, computer exposure, daycare, breastfeeding, child's ADHD medication use 12 h prior to neuropsychological assessments, and prenatal exposure to lead), household characteristics (e.g., poverty level, siblings, language spoken, and single parenthood), and maternal education. The Kaufman Brief Intelligence Test was administered to the mothers to measure their IQ scores. The Home Observation for Measurement of Environment inventory was used to assess the home environment.[23,24] Information on maternal history of neuropsychological conditions and children's prenatal exposure to thimerosal from maternal vaccines was obtained from maternal medical records.

Statistical methods

We categorized exposure to the number of antigens from vaccines up to 2 years of age into three different exposure categories: total antigens up to 7 months ($\leq$214 days), 12 months ($\leq$366 days), and 24 months ($\leq$731 days). We defined threshold scores for neuropsychological outcomes on continuous scales as scores at or below mean $-$ 2 standard deviations (SD), except for GDS test for errors for which we defined the threshold score as mean + 2SD. We considered a 2SD from the mean as a threshold for the neuropsychological outcomes to be a similar effect as that used for significant concerns for intellectual disabilities as defined by intelligence tests (i.e. IQ $<$ 70 with mean of 100 and SD of 15). For dichotomous outcomes (e.g., tics), the threshold was defined as an affirmative rating ('yes') by the assessor. We calculated the mean (SD) scores as well as the frequency of scores below threshold for each test. To minimize the likelihood of type 1 errors from multiple testing,[6] we only assessed associations between antigens and domain-specific outcomes (below-threshold scores in any domain-specific tests: yes/no) as opposed to each test-specific scores. We used logistic regression adjusting for all covariates to evaluate associations between antigen exposures and outcomes in seven domains (excluding visual spatial ability because of the small number of outcomes and including motor and phonic tics separately). All analyses were conducted at 0.05 significance level. We estimated odds ratios (OR) with 95% confidence intervals (CI) per 1000 increase in antigens on a continuous scale. OR confidence limits that included 1 were considered not to be statistically significant. We also performed a comparison of antigen exposures above versus below the 10th percentile of the exposure distribution. To further validate findings from the regression analyses, structural equation modeling (SEM) techniques were also employed to reduce the potential influence of measurement error that may be unaddressed when using ordinary least squares regression.[7] Latent model adjusted associations were assessed for six domains that included multiple indicators (outcome measures) using SEM techniques. SEM did not use any cut-off points for the outcomes. Continuous outcomes were utilized to take advantage of the range of variability for each outcome and follow methods outlined in previous vaccine safety studies.[7]

Since the antigen content of whole cell pertussis-containing vaccines was much greater than other vaccines, additional analyses comparing receipt of 0, 1, 2, 3, or 4 or more pertussis-containing vaccines up to 7, 12, and 24 months and neuropsychological outcomes were carried out.

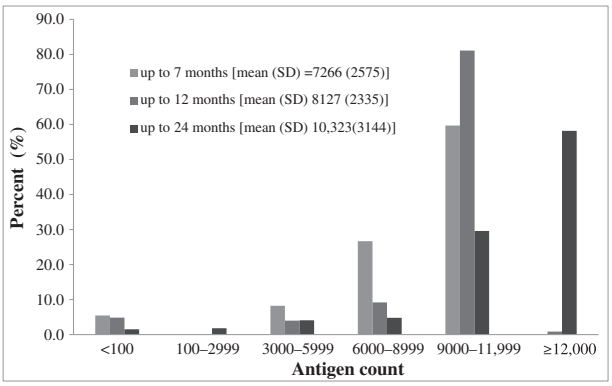
All regression analyses were conducted using SAS software (version 9.3, Cary, NC). SEM analyses were conducted using Mplus software (version 6.12, Los Angeles, CA).

RESULTS

A total of 3648 children were selected for recruitment, of whom 1107 (30.3%) underwent neuropsychological assessment. Children with missing vaccination records (n = 1), missing prenatal records (n = 5), and missing weight data (n = 7), and children who were found to have one or more of the exclusionary medical conditions during medical record abstraction (n = 47) were excluded from analysis, resulting in 1047 children in the final analysis.[6] The mean (SD) age of the enrolled children was 9.3 (1.1) years, about half were boys and half were White (Table 1). Children received 9, 10, and 14 vaccines on average by ages 7, 12, and 24 months, respectively (data not shown). These vaccines contained a mean of 7266 antigens by age

Table 1. Socio-demographic, pre and perinatal, and clinical characteristics of study children ($N = 1047$)

Variables	*N*	%
Age, years (mean ± standard deviation)	9.3 (1.1)	
Sex, male	509	48.6
Race/ethnicity		
White	509	48.6
Black	142	13.6
Hispanic	316	30.2
Others	80	7.6
Only English spoken in the household	656	62.7
Single parent	204	19.5
Daycare home or center	870	83.1
Child birth weight, grams		
2500–2999	120	11.5
3000–3999	768	73.4
4000+	159	15.2
Maternal age at birth, years		
<40	973	92.9
≥40	74	7.1
ADHD medication use	38	3.6
Maternal education level		
Less than high school diploma	46	4.4
High school diploma or equivalent	165	15.8
Some college	297	28.4
College graduate or higher	539	51.5



SD= Standard Deviation

Figure 1. Distribution of total cumulative antigen counts by age range among the study participants ($N = 1047$)

7 months, 8127 by 12 months, and 10 323 by age 24 months (Figure 1). Most children (59.7% and 81.1%, respectively) received less than 12 000 antigens up to 7 and 12 months and 12 000 or more (58.2%) up to 24 months (Figure 1).

The highest frequency (23.9%) of one or more domain-specific tests below their defined thresholds was for the behavior regulation domain, and the lowest (1.0%) was for visual spatial ability. Corresponding frequencies for other domains ranged from 2.4% for fine motor coordination to 12.9% for tics (Table 2).

Table 3 illustrates the results from domain-specific multivariate models for associations between cumulative antigen exposures up to 7, 12, and 24 months and scores below threshold for any test in the domain. These models adjusted for all socio-demographic (e.g., age, race, sex, and poverty level), family and household (e.g., language spoken, siblings and HOME index), pre and perinatal (e.g., birth weight, APGAR score, and pre and postnatal thimerosal exposure), clinical (e.g., anemia and medication use), and maternal (e.g., education and IQ score) characteristics. We found no significant associations between any of the domain-specific outcomes and increase in number of antigens (per 1000) from vaccines up to 7, 12, and 24 months (OR ranged from 0.93 to 1.12). The 10th percentiles of cumulative antigens exposures from vaccines up to 7, 12, and 24 months were 3021 (range: 0 to 9085), 6039 (range: 0 to 12 086), and 6155 (range: 0 to 21 120), respectively. Compared with children in the lowest 10th percentile of antigens received up to 24 months, children with higher antigen counts (above the 10th percentile) were less likely to have a below-threshold score for attention and executive function tests (adjusted OR = 0.51, 95% CI = 0.26, 0.99), indicating that higher antigen exposure was associated with better functioning on these measures. None of the other associations were statistically significant.

In the SEM analyses, each of the models fit well. (For all models: root mean square error of approximation = 0.04, comparative fit index = 0.92). Children with higher antigen counts up to 24 months were found to have significantly better attention and executive function outcomes (b for 1000 increase in antigen counts = 0.08 and $p = 0.02$) in latent model analysis (Table 4). No other statistically significant associations were detected.

We also performed analyses to assess the association between maximum number of antigens in a day and neuropsychological outcomes. There were no significant findings (data not shown). Additionally, there were no significant associations between number of pertussis-containing vaccines received and any of the outcomes (data not shown).

DISCUSSION

We found no evidence of an adverse association between the number of antigens received from vaccines in the first 2 years of life and any of the categories of neuropsychological outcomes evaluated. We believe our approach to quantitatively assess exposure to antigenic content of vaccine (i.e., the number of antibody-stimulating proteins and polysaccharides) provides a more granular approach in assessing immunological stimulation from vaccines

Table 2. Mean (standard deviation) and frequency of scores below the defined threshold for neuropsychological domain-specific tests among study children ($N = 1047$)

	Score	Scores below defined threshold*	
Domain and tests	Mean (SD)	*n*	%
General intellectual function			
WASI Vocabulary	53.8 (10.3)	25	2.4
WASI Similarities	54.9 (9.3)	34	3.3
WASI Block Design	52.2 (11.2)	5	0.5
WASI Matrix Reasoning	53.5 (9.7)	60	5.8
Woodcock–Johnson III Letter Word Identification	107.0 (11.7)	22	2.1
Scores below threshold for any test	–	115	11.0
Speech and language			
Boston Naming Test	39.6 (8.0)	39	3.7
NEPSY: Speeded naming	10.4 (2.7)	32	3.1
NEPSY: Comprehension of instructions	10.9 (2.8)	54	5.2
CELF: Formulated sentences	11.0 (2.7)	25	2.4
CELF: Recalling sentences	10.7 (3.1)	25	2.4
Scores below threshold for any test	–	130	12.4
Verbal memory			
CVLT-C: Free recall, no delay	54.0 (9.9)	40	3.8
CVLT-C: Free recall, short delay	0.4 (1.0)	52	5.0
CVLT-C: Free recall, long delay	0.5 (0.9)	37	3.5
CVLT-C: Cued recall, short delay	0.5 (1.0)	39	3.7
CVLT-C: Cued recall, long delay	0.5 (0.9)	36	3.5
Scores below threshold for any test	–	116	11.1
Fine motor coordination			
Finger Tapping: Dominant hand	38.8 (6.8)	13	1.3
Finger Tapping: Nondominant hand	34.5 (6.3)	16	1.6
Scores below threshold for any test	–	25	2.4
Attention and executive function			
Gordon Diagnostic Test: Correct response	40.5 (5.1)	53	5.1
Gordon Diagnostic Test: Errors	7.6 (13.3)	30	2.9
Wechsler Intelligence Scale (digit span): Forward recall	8.1 (1.9)	14	1.3
Wechsler Intelligence Scale (digit span): Backward recall	4.5 (1.6)	10	1.0
Scores below threshold for any test	–	87	8.3
Tics (assessor rating)			
Motor tics	–	93	8.9
Phonic tics	–	76	7.3
Tics (any)	–	135	12.9
Tics (both types)	–	34	3.3
Visual spatial ability			
Stanford Binet Copying	43.6 (5.3)	10	1.0
Behavior regulation			
CRS-R: Parent-rating hyperactivity	54.1 (10.9)	184	17.7
CRS-R: Parent-rating inattentiveness	52.1 (10.5)	153	14.7
BRIEF: Parent-rating behavior regulation	49.1 (10.5)	127	12.2
Scores below threshold for any test	–	249	23.9

*Threshold for any test defined as scores greater than mean – two standard deviation (except for Gordon Diagnostic Test: Errors, it was mean + two standard deviation) for tests with continuous scores; for tests with binary outcomes (yes/no), threshold defined as being assessed affirmatively (yes).
Abbreviations: WASI = Wechsler Abbreviated Scale of Intelligence; NEPSY = Developmental Neuropsychological Assessment; CELF = Clinical Evaluation of Language Fundamentals – third edition; CVLT = California Verbal Learning Test—Children's version; CRS-R, Conners' Rating Scales—Revised; BRIEF, Behavioral Rating Inventory of Executive Functioning.

than simple counting of the number of vaccines received. A similar measure of antigenic exposure was used in a recent VSD study that found no association between number of vaccine antigens received up to 24 months of life and development of autism spectrum disorder.[25]

Some parents' concern about the number of vaccines that their children receive is based on a belief that too many vaccines administered too early in life can overwhelm or weaken the immune system of young children. Biologically, however, infants are capable of generating adequate protective immune responses against most pathogens and to multiple vaccines at the time of birth.[1,26,27] Offit et al. conservatively estimated that an immuno-competent infant has the theoretical capacity to respond to nearly 10 000 vaccines (or 1 million antigens assuming 100 antigens per vaccine), and the continuous reproduction of immune system cells (e.g., B and T cells) ensures that

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Table 3. Adjusted association between neuropsychological outcomes (scores below defined threshold) at 7–10 years and total antigens received in the first 2 years of life ($N = 1047$)*

	Up to 7 months		Up to 12 months		Up to 24 months	
	Adjusted odds ratio (95% confidence interval)					
Domain	Per 1000 increase in antigens	Compared with lower 10th percentile	Per 1000 increase in antigens	Compared with lower 10th percentile	Per 1000 increase in antigens	Compared with lower 10th percentile
General intellectual function	1.00 (0.90, 1.10)	1.18 (0.57, 2.43)	1.03 (0.92, 1.14)	1.31 (0.61, 2.82)	1.02 (0.95, 1.10)	0.97 (0.47, 2.00)
Speech and language	0.95 (0.87, 1.04)	0.63 (0.34, 1.19)	0.99 (0.90, 1.09)	1.02 (0.51, 2.03)	0.95 (0.89, 1.02)	0.85 (0.43, 1.67)
Verbal memory	0.98 (0.90, 1.07)	0.99 (0.49, 1.99)	1.02 (0.93, 1.12)	1.29 (0.62, 2.69)	0.98 (0.92, 1.05)	0.98 (0.50, 1.93)
Fine motor coordination	1.06 (0.87, 1.28)	1.11 (0.24, 5.10)	1.02 (0.84, 1.24)	0.91 (0.23, 3.57)	1.03 (0.89, 1.19)	1.23 (0.31, 4.88)
Attention and executive function	0.96 (0.87, 1.06)	1.15 (0.53, 2.48)	0.93 (0.85, 1.02)	0.60 (0.31, 1.20)	0.94 (0.87, 1.02)	**0.51 (0.26, 0.99)**
Tics (any)	1.04 (0.95, 1.15)	1.28 (0.62, 2.66)	1.08 (0.98, 1.20)	1.09 (0.54, 2.20)	1.07 (0.99, 1.15)	1.66 (0.75, 3.68)
Motor tics	1.00 (0.90, 1.12)	1.36 (0.55, 3.32)	1.07 (0.94, 1.21)	1.24 (0.50, 3.09)	1.08 (0.98, 1.18)	2.15 (0.73, 6.35)
Phonic tics	1.10 (0.97, 1.25)	1.21 (0.49, 2.98)	1.12 (0.97, 1.28)	1.07 (0.45, 2.55)	1.08 (0.98, 1.19)	2.22 (0.75, 6.56)
Behavior regulation	0.99 (0.92, 1.06)	0.96 (0.56, 1.67)	1.01 (0.94, 1.09)	1.34 (0.76, 2.34)	1.01 (0.96, 1.07)	1.32 (0.74, 2.33)

*Adjusted for age, sex, race, poverty level, Home Observation for Measurement of Environment index, having siblings, language spoken in the household, single parenthood, computer exposure, daycare attendance, birth weight, breastfeeding duration, APGAR score, maternal age at birth, prenatal thimerosal and lead exposure, thimerosal exposure up to 7 months of age, anemia, antibiotic use, pica, ADHD medication use, maternal education, maternal IQ score, maternal history of neuropsychological conditions, and Managed Care Organization sites.

Table 4. Latent models adjusted associations between neuropsychological outcomes at 7–10 years and total vaccine antigens received in the first 2 years of life ($N = 1047$)*,†

	Up to 7 months			Up to 12 months			Up to 24 months		
Domain	*B*	SE	*p*	*b*	SE	*p*	*b*	SE	*p*
General intellectual function	0.06	0.12	0.61	−0.11	0.11	0.34	−0.07	0.08	0.38
Speech and language	0.14	0.07	0.07	0.10	0.07	0.15	0.08	0.06	0.14
Verbal memory	0.10	0.10	0.33	0.10	0.11	0.37	0.12	0.08	0.13
Fine motor coordination	−0.01	0.07	0.92	0.05	0.07	0.46	0.02	0.06	0.74
Attention and executive function	0.02	0.04	0.63	0.06	0.04	0.20	**0.08**	**0.03**	**0.02**
Behavior regulation	−0.08	0.07	0.24	−0.05	0.07	0.50	−0.06	0.05	0.26

*Per 1000 increase in number of antigens.
†Adjusted for age, sex, race, poverty level, Home Observation for Measurement of Environment index, having siblings, language spoken in the household, single parenthood, computer exposure, daycare attendance, birth weight, breastfeeding duration, APGAR score, maternal age at birth, prenatal thimerosal and lead exposure, thimerosal exposure up to 7 months of age, anemia, antibiotic use, pica, ADHD medication use, maternal education, maternal IQ score, maternal history of neuropsychological conditions, and Managed Care Organization sites.

the capacity is more or less sustained.[1] Concerns about the number of vaccines and their effects on children's immune system might lead to vaccine refusals or delay in childhood immunizations.[1–3] However, using the same data as in our analysis, Smith and Woods have found no association with timely immunization during childhood and any of the adverse neuropsychological outcomes.[28]

Previous studies based on this dataset have reported a slightly increased risk of tics with increased thimerosal exposures from vaccines,[6,10] especially among boys.[7] In this study, we adjusted for prenatal and postnatal thimerosal exposure from vaccines up to 7 months of age from medical and immunization records, and we did not find any increased risk of tics with cumulative antigen exposures. Instead, we found that children with antigen counts above the 10th percentile did better in attention and executive function tests compared with children with antigen counts below the 10th percentile. Similar association of better attention and executive functioning with higher vaccine antigen exposure was observed in the latent model analysis results. However, this association does not imply a protective effect of higher antigen exposure. It should be noted that our domain-specific measures for attention and executive

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function included Gordon Diagnostic System Vigilance Task Errors, whereas the previous analyses did not include it.[7] Further investigation is needed to determine whether this finding is a true association or due to chance from multiple testing or due to some unknown confounding. Although domain-specific analyses reduced the likelihood of errors resulting from multiple testing and the latent constructs better account for measurement error, it also limited our ability to assess the effects of antigen exposure on each test separately.

This study builds on previously peer-reviewed methodology and improved statistical methods that adjust for a wide array of covariates and reduce the likelihood of type I error,[6,7] and evaluates the effects of antigens using multiple exposure categories. Measurements of both outcomes (using neuropsychological tests) and exposures (immunization history from multiple sources) were thorough and comprehensive. Use of number of vaccine antigens as primary exposure is also an enhanced feature of this study. However, there are several limitations.[6–8] Our analysis assumed that the levels of immune response were similar for all antigens, which we realize is an over-simplification. Differential level of immunologic stimulation by different types of vaccine-specific antigens was not accounted for in our analysis. Enrollment of less than one third (30%) of the selected eligible participants might have resulted in selection bias, and since some information was from self-report, there could also be some recall issues leading to inadequate adjustment of confounding factors. The direction and magnitude of such biases could not be ascertained but should be considered while interpreting the findings. Also, multiple exclusion criteria based on medical conditions (e.g., encephalitis) restricted enrollment of high risk children that limits the generalizability of the findings.[7,8] The relevance of our findings, based on children immunized according to the 1990's immunization schedule, to today's immunization schedule could also be questioned. Whole cell pertussis-containing vaccines are no longer used in the United States. The introduction of newly developed vaccines and the replacement of whole cell pertussis-containing vaccines (~3000 antigens) with acellular pertussis-containing vaccines (2–4 antigens) have significantly reduced the antigen load in the current immunization schedule compared with the 1990's schedule; the maximum number of antigens that a child could be exposed to by age 2 years in 2012 is 315 compared with several thousand in the late 1990s. Thus, the level of antigenic exposure from vaccines received by the children in our study was greater than the current vaccination schedule and provides relevant data for the current schedule. To draw further relevance to the antigen exposure from vaccines under current schedule, we also estimated the adjusted odds ratios for every 25-antigen increase;[25] the findings were similar, and we did not find any significant association with any of the outcomes (data not shown).

In conclusion, we did not find any statistical evidence of an association between adverse neuropsychological outcomes later in childhood with level of antigen exposure from vaccines in the first 2 years of life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

KEY POINTS

- The number of antigens present in vaccines varies, and the number of antigens can be used for better assessment of level of immunologic stimulation rather than simply counting the number of vaccines received.
- The introduction of newly developed vaccines and the replacement of whole cell pertussis-containing vaccines (~3000 antigens) with acellular pertussis-containing vaccines (2–4 antigens) have significantly reduced the antigen load in the current immunization schedule compared with the 1990's schedule.
- There is no association between numbers of antigens received from early childhood vaccines and development of neuropsychological outcomes later in the childhood.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix 1. Number of antibody-stimulating protein and polysaccharide antigens in vaccines*

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