Title page:

Pertussis vaccination during pregnancy: antibody persistence in infants.

Alba VILAJELIU[a,b], MD, MPH; Laia FERRER[c], MD; Jordina MUNRÓS[c], MD; Anna GONCÉ[c], MD, PhD; Marta LÓPEZ[c], MD; Josep COSTA[d], BS, PhD; José M BAYAS[a], MD, PhD; for the PERTU Working Group.

[a]Department of Preventive Medicine and Epidemiology. Hospital Clínic - Universitat de Barcelona, Barcelona, Spain

[b]ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain.

[c]Department of Maternal-Fetal Medicine. BCNatal - Barcelona Center of Maternal-Fetal and Neonatal Medicine. Hospital Clínic and Hospital Sant Joan de Déu. Universitat de Barcelona, Barcelona, Spain.

[d]Department of Microbiology, Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona, Spain.

Corresponding author:

Alba Vilajeliu, MD, MPH

Department of Preventive Medicine and Epidemiology, Hospital Clínic de Barcelona – Universitat de Barcelona. ISGlobal. Villarroel, 170 08036 Barcelona (Spain)

albavilajeliu@gmail.com Telephone: +34 932274089 Fax: +34 93 4510405

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Abstract

Maternal pertussis vaccination is associated with higher levels of pertussis antibodies at birth. We assessed the persistence of pertussis antibodies until primary vaccination in infants whose mothers received Tdap (tetanus, diphtheria, acellular pertussis) vaccine during pregnancy. Infants were born at the Hospital Clinic of Barcelona (Spain) in November 2014. Anti-PT IgG was determined by ELISA at delivery, between the first and second month of life, and estimated at 2 months of age. The study included 37 infants whose mothers received Tdap between 21 and 38 weeks of gestation. Infants presented a decline in GMC of anti-PT IgG between peripartum and follow-up levels, 52.7 (95% CI 34.7–80.2) versus 7.5 (95% CI 4.2–13.3) at 2 months of age ($p < 0.001$). The median half-life of maternal antibodies was 47 days. More than half (51.4%) the infants presented detectable anti-PT IgG before the start of primary infant vaccination.

Key Words: maternal vaccination; neonatal pertussis; antibody persistence; infant vaccination.

Main text

Introduction

Despite high coverage of pertussis infant vaccination, the disease has re-emerged in some industrialized countries, resulting in morbidity and mortality in young infants [1,2]. In Spain, an increase in pertussis incidence has been reported since 2010 reaching more than 7 cases per 100,000 inhabitants in 2014. The highest morbidity and mortality has been observed in those younger than 3 months [3].

During the last years, various countries have recommended to vaccinate pregnant women with a pertussis-containing vaccine aiming to protect infants during the first months of life. More specifically, in December 2012, the Centers for Disease Control and Prevention (CDC) issued interim recommendations indicating that women should be revaccinated during each pregnancy, with the optimal timing for Tdap vaccination being between 27 and 36 weeks of gestation [4]. The rationale for this public health measure is based on evidence of transplacental transfer of maternal antibodies [5–7], and the indirect effect of protecting the mother from an infectious disease, which is important as they are a frequent source of infection for infant pertussis cases [8].

Although there are no accepted pertussis antibody levels that provide protection against pertussis, high levels of pertussis antibodies in cord blood have been associated with clinical protection against pertussis [9] and a case-control study estimated a 93% vaccine effectiveness in protecting newborns and infants against this disease [10].

Little data is available on the decay of antibody concentrations in infants from maternal vaccination during pregnancy until primary infant pertussis vaccination. The objective of this study was to assess the persistence of pertussis antibodies (anti-PT IgG) between delivery and until primary infant vaccination in infants whose mothers received Tdap during pregnancy.

Material and Methods

Study characteristics

Prospective observational study of infants whose mothers received Tdap vaccination during pregnancy, and who delivered at Hospital Clinic of Barcelona (HCB) in November 2014 (PERTU-II Study). Inclusion criteria were maternal age ≥18 years, Tdap vaccination during the current pregnancy, and maternal signature of written informed consent. The venous umbilical cord blood samples were collected consecutively and an infant heel blood sample between the first and second month of life. In twin pregnancies, a blood sample was collected only from the firstborn. Pertussis antibody concentrations were determined at delivery, between the first and second month of life (before starting primary pertussis infant vaccination), and estimated at 2 months of age. We recruited a convenience sample, including only cases in which the results of peripartum and pre-immunisation were available.

Maternal Tdap vaccination protocol in pregnancy in Catalonia (Spain)

In January 2014, the Department of Health of Catalonia (Spain) recommended vaccination of all pregnant women with one dose of Tdap from 20 weeks of gestation, ideally between 27-36 weeks, regardless of their vaccination history

[11]. The vaccine is administered intramuscularly into the deltoid muscle during a routine pregnancy check-up. The vaccine used is Triaxis (Sanofi Pasteur MSD, France), in some countries licensed as Adacel, which contains five antigens purified from *B. pertussis* (2.5 µg pertussis toxin, 5 µg filamentous hemagglutinin, 3 µg pertactin and 5 µg fimbriae 2/3), ≥ 20 IU tetanus toxoid and ≥ 20 IU diphtheria toxoid [12].

Laboratory methods

Levels of anti-PT IgG were determined using the Pertussis Toxin ELISA Testkit IgG/IgA TESTKIT, Sekisui Virotech GmbH, Germany, and expressed as international units (IU/ml). Absorbance readings were measured and quantified against an international reference serum, with known amounts of the respective antibodies (in 2008, preparation No. 6/140 was established as the First International Standard for Pertussis Antiserum (Human), expressed in IU/ml, using, as a reference, a pattern drawn up by the FDA: U.S. Human anti-pertussis reference sera lot 3 and lot 4 for IgG antibodies. IU were taken to be equivalent to Elisa Units (EU) referring to the widely used pattern of the FDA in studies carried out before 2008) [13–15].The lower limit of detection (LLOD) of anti-PT IgG was 5 IU/ml. Titers of ≥10 IU/ml were considered as an elevated cut-off [16]. All samples were analyzed by the HCB microbiology service.

Collection of variables

The following variables were collected from medical records: maternal date of birth, country of origin, parity, history of immune system disorders (autoimmune disease or HIV infection), date of last menstruation, date of administration of

Tdap vaccine during pregnancy, newborn date of birth, sex and birth weight and date of infant's heel blood sample.

Statistical analysis

In the univariate analysis absolute frequencies were used to describe categorical variables and means and standard deviation (SD) or 95% confidence intervals (CI) for quantitative variables with a normal distribution, and medians and interquartile range otherwise. Anti-PT IgG levels were described as geometric mean concentrations (GMC) and 95% CI for umbilical cord blood and infant sera. Values below the LLOD were considered to be half of the value of detection [6,7]. Antibody concentrations at two months of age were estimated by linear interpolation using pertussis antibody concentrations (anti-PT IgG) at delivery and between the first and second month of life.

For quantitative comparisons of antibody levels at delivery and at 2 months of age, the Wilcoxon test for paired data was used. Independent sample Mann-Whitney U and Kruskall-Wallis tests were used to assess differences in antibody levels between groups. The statistical analysis was performed using the STATA ® statistical package v12.1. Statistical significance was established as $p < 0.05$. The study was approved by the HCB Clinical Research Ethics Committee.

Results

The study included 37 infants whose mothers received Tdap vaccine between 21 and 38 weeks of gestation (3 at 21-26^{+6} [8.6%], 17 at 27-31^{+6} [48.6%], 13 at

32-36^{+6} [37.1%] and 2 at 37-40 [5.7%] weeks of gestation). In 2 participants, the date on Tdap vaccination was missing. The Tdap vaccine was administered between 1 and 19 weeks before delivery (median: 9.1 weeks). No infant was born before 37 weeks of gestation. The median days between delivery and collection of the infant blood sample (follow-up) was 45 days (Q1-Q3:42-48). The demographic and clinical characteristics of the study population are presented in Table 1.

Infants of Tdap-vaccinated women (n=37) showed a decline in GMC of anti-PT IgG between peripartum and follow-up levels of 52.7 IU/ml (95% CI 34.7– 80.2) in umbilical cord blood and an estimated 7.5 IU/ml (95% CI 4.2–13.3) at 2 months of age (Wilcoxon test paired samples, p <0.001). The magnitude of the transplacental transfer of anti-PT IgG (GMC) according to gestational age of Tdap vaccination is shown in Table 2. We also analyzed anti-PT IgG concentrations according to the time elapsed between Tdap administration and the gestational age at delivery, but no significant differences were observed (Mann-Whitney, p = 0.1964).

We examined whether these findings on Tdap vaccination were expected to persist over time. It was estimated that, at two months of age, 51.4% of infants would have detectable titers and 29.7% a high cut-off (≥ 10 IU/ml). We found that newborns of women vaccinated with Tdap during the third trimester (≥27 weeks of gestation) were expected to sustain the highest GMCs of anti-PT IgG over time, although the finding was not significant (Mann-Whitney, p=0.0842).

Discussion

In our study, anti-PT IgG levels decreased significantly after delivery and the main factor for higher anti-PT IgG levels at 2 months of age was higher umbilical cord concentrations ($p<0.001$). These findings are consistent with the results of other studies which found that maternal vaccination during pregnancy is associated with significantly-higher levels of pertussis antibodies at birth [5–7,17,18]. In addition, Maertens et al showed that infants born to vaccinated women had significantly higher GMC at birth and during the first 2 months of life compared to the offspring of unvaccinated women, thereby closing the susceptibility gap for pertussis in infants [5–7,17,18].

Although not reaching significance, in our study infants of mothers vaccinated during the third trimester of pregnancy presented anti-PT IgG T levels higher than the lower limit of quantification at 2 months of age. Previously, Munoz et al showed that maternal vaccination of 33 women at 30-32^{6} weeks of gestation with Tdap resulted in high concentrations of pertussis-specific antibodies at 2 months of age [7]. Abu Raya et al suggested that vaccination of pregnant women with Tdap between 27-30^{+6} weeks was associated with the highest umbilical cord GMCs of anti-PT IgG compared with vaccination beyond 31 weeks gestation [17]. However, most recently Eberhardt et al (n=335) suggested that early-second trimester maternal Tdap immunisation significantly increase neonatal antibodies [19].

This study adds evidence to the definition of the half-life of anti-PT IgG in infancy in the Tdap era. It is reported that the mean half-life of transferred anti-

PT IgG is approximately 43 days [7], or 36 days [20]. Our results show that maternal antibodies wane with a half-life of 47 days, and that the ability of maternally-acquired anti-PT IgG to persist is short lived (51.4% with detectable anti-PT IgG titers at 2 months), meaning that starting infant vaccination at 2 months of age is of great importance.

Our study has some limitations: we did not collect information on previous doses of pertussis vaccination nor the personal history of pertussis disease, making it impossible to evaluate the possible effects of natural boosting on our findings. However, given the age and country of origin of the subjects studied, it is almost certain that the majority had received primary vaccination in childhood with pertussis whole cell vaccine. A higher sample size including premature infants would have been desirable. Likewise, other pertussis antigens potentially involved in protection against pertussis infection were not determined.

Conclusions

More than half the infants of mothers immunized during late pregnancy presented pertussis antibodies (anti-PT IgG) before the start of primary infant vaccination.

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Table 1: Demographic characteristics of women receiving tetanus, diphtheria, and acellular pertussis (Tdap) vaccination during pregnancy and their newborns (n=37).

Variable	Total
	N (%)
Maternal age (years)	
Age group 1, 20-24	2 (5.4 %)
Age group 2, 25-29	4 (10.8 %)
Age group 3, 30-34	16 (43.2 %)
Age group 4, 35-39	12 (32.4 %)
Age group 5, ≥40	3 (8.1 %)
mean (SD), years	33.8 (4.6)
Country of birth	
Spain	28 (75.7%)
Rest of Europe	2 (5.4%)
Africa	1 (2.7%)
America	4 (10.8%)
Asia	2 (5.4%)
Parity	
≥ 1	15 (40.5%)
Maternal history of immune system disorders	
Yes	5 (13.5%)
Twin pregnancy	
Yes	1 (2.7%)
Weeks of gestation at birth	
median (Q1- Q3), weeks ^{days}	40^{+1} (39^{+1}- 40^{+5})
Type of delivery	
Vaginal	22 (59.5%)
Cesarean	15 (40.5%)
Sex of newborn	
Male	18 (48.7%)
Birth weight	
mean (SD), grams	3,159.5 (SD 498.4)

Table 2: Geometric mean concentrations (GMCs) of immunoglobulin (Ig) G to pertussis toxin (PT) during the peripartum period and 2 months after delivery (estimated) stratified by sequential time frames of administration of tetanus, diphtheria, and acelullar pertussis (Tdap) vaccine during pregnancy.

	Total	**Peripartum**	**Pre-immunisation**	
Variable	**N (%)**	**GMC (95% CI)****	**GMC (95% CI)****	**p-value*****
Weeks of gestation at Tdap vaccination*				
20-26 weeks	3 (8.6%)	29.9 (11.2-79.5)	2.5 (-)	0.317
27-31 weeks	17 (48.6%)	52.5 (29.4-93.8)	6.8 (2.9-15.6)	0.001
32-36 weeks	13 (37.1%)	62.5 (27.3-143.6)	8.7 (4.0-19.0)	0.009
≥37 weeks	2 (5.7%)	83.7 (-)	31.1 (-)	0.180
Total	37	52.7 (34.7-80.2)	7.5 (4.2-13.3)	<0.001

*In 2 cases the date of Tdap administration was missing

**The GMCs between weeks of gestation groups were compared using Kruskall-Wallis test (peripartum, p-value 0.779; pre-immunisation, p-value 0.756)

***The GMCs between peripartum and pre-immunisation were compared using Wilcoxon test for paired data