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Effect of Pre-pregnancy Pertussis Vaccination in Young Infants

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Short summary: Post-partum Tdap vaccination increases significantly maternal antibody concentrations at consecutive delivery, and blunting of the later born siblings' immune responses occurs, similar to the effect of Tdap vaccination during pregnancy.

Abstract

Background

Maternal pertussis antibodies can hamper infant immune responses to pertussis vaccines. The effect of offering a maternal tetanus, diphtheria, acellular pertussis (Tdap) booster between 2 consecutive pregnancies is investigated.

Methods

A prospective study was conducted (Belgium, 2008-2014) on kinetics of maternal pertussis antibodies in unvaccinated women and their infants (group A, N=86) and in siblings (group B, N=58), born after the woman received a Tdap vaccination. Anti-Pertussis Toxin (PT), anti-Filamentous Haemaglutinin (FHA) and anti-Pertactin (Prn) antibodies were measured in maternal blood before and after vaccination and at both deliveries, in cord blood from both siblings and before and after a priming series of infant pertussis vaccines.

Results

All pertussis antibodies were significantly higher in group B siblings at birth, even with growing time interval since maternal vaccination. Blunting of the infant pertussis vaccine response was detected in group B siblings.

We estimated a maximum time interval between repeat Tdap doses in adult women in order to have a beneficial effect for the consecutive infant.

Conclusions

Pre-pregnancy Tdap vaccination increases significantly maternal antibody concentrations at consecutive delivery. However, similarly to the effect of Tdap vaccination during pregnancy, blunting of the infant immune responses occurs after a pre-pregnancy immunisation.

40 Key words: maternal antibodies; pertussis; vaccination; pre-pregnancy; blunting

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Background

Despite universal infant vaccination programs against *Bordetella pertussis*, there is an increase in reported whooping cough cases, particularly in industrialized countries. Most outbreaks occur in adolescents and young adults [1] and they are representing a source of infection for unvaccinated infants and newborns. Highest incidence of pertussis disease is recorded in very young children who have not yet been (fully) vaccinated and/ or did not receive sufficient maternal antibodies [2].

At first, cocoon vaccination was recommended to protect young infants from disease, but cost effectiveness studies, as well as difficulties to reach high coverages among all persons in contact with young infants, discouraged the strategy [3]. Since 2012, maternal vaccination during pregnancy is a recommended strategy in an increasing number of (industrialized) countries to protect young infants from disease. Many countries advise to immunize at every pregnancy, since antibodies are rapidly waning after an adult booster dose [4], and a high concentration of maternal antibodies is needed for transfer of antibodies from mother to fetus during pregnancy [5]. For pertussis, a correlate of protection is not well defined, yet high concentrations of anti-Pertactin (Prn) and mainly anti-Pertussis Toxin (PT) antibodies, are related to protection [6]. After birth, maternal antibodies decrease rapidly in the offspring, mostly within two months [7].

The present paper reports results of a prospective study on the transfer and persistence of maternal antibodies in newborns, whenever a tetanus, diphtheria and pertussis (Tdap) booster vaccination is given to women between two consecutive pregnancies. Previously, we reported an interim analysis on a subsample of this cohort study [8]. The primary aim of the study is to evaluate whether a pre-pregnancy booster helps to increase antibody concentrations to pertussis in neonates, thereby closing the susceptibility gap. A secondary aim is to evaluate possible blunting of the infant immune responses to pertussis containing vaccines after a pre-pregnancy maternal booster vaccination, similar to blunting of the infant immune responses after Tdap administration in pregnancy [9]. In addition, modelling the anti-

PT IgG antibody concentrations enables drafting possible recommendations regarding the timing of consecutive maternal booster doses.

Methods

Study design

A prospective multicenter study was conducted in Antwerp, Belgium, between 2008 and 2014, in accordance with the Helsinki Declaration, ICH-GCP and procedures established by Belgian law. Ethical approval was obtained (University Hospital of Antwerp). Women participating in a study on maternal antibody kinetics were recruited [10-12]. Informed consent was obtained from the women and from both parents of the participating children. For the women, the exclusion criteria were pertussis vaccination within ten years prior to study participation, immunological disorders and recent administration of immunoglobulins prior to vaccination. Exclusion criteria for the children were low birth weight (<2400 g), prematurity (<36 weeks gestation) and immunological disorders. Serum samples were taken from all women at delivery (10 mL), from the cord (10 mL), and from the infant at 1 month of age, 6 or 9 months of age (randomly) and 12 months of age (2 cc) (Group A). Time points were chosen based on waning of maternal measles antibodies [10].

Women were then offered a tetanus, diphtheria, acellular pertussis (Tdap) vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) after the first delivery. One month after vaccination, a blood sample (10 mL) was taken. At consecutive delivery, blood was again taken from the woman and the cord and later on from the sibling at 1 month of age, 6 or 9 months (randomly and equal to the older sibling) and 12 months of age (Group B).

All infants were vaccinated within the standard Belgian vaccination schedule with the hexavalent aP containing vaccine Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium) at 8, 12 and 16 weeks.

An extended questionnaire collected information on obstetrical risk factors, demographics, vaccination history, and general medical history. Growth parameters, breastfeeding data, immunization data, and medical history were collected at each visit.

Study vaccines

Licensed Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) was used to immunize women in the deltoid muscle. Boostrix® contains 5 Lf of tetanus toxoid (TT), 2.5 Lf of diphtheria toxoid (DT), 8 µg of inactivated PT, 8 µg of FHA and 2.5 µg of Prn. Infants were vaccinated with the hexavalent vaccine Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium), containing 25 Lf of DT, 10 Lf of TT, 25 µg PT, 25 µg FHA and 8 µg Prn, inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

Laboratory

All serum samples were centrifuged at 2000 rpm within 24 hours after withdrawal, and stored between -20°C and -40°C. Serum leftovers from women and infants in group A were selected for analysis in view of the inclusion of their siblings (group B siblings). An in-house ELISA was used to test all samples for anti-PT, anti-FHA and anti-Prn IgG antibodies at GSK Biologicals, Belgium. The limit of detection of the assay was 5 EU/mL for all three antibodies. For women, a booster response was defined as a post-vaccination antibody concentration ≥ 20 EU/mL with a pre-vaccination antibody concentration <5 EU/mL, a post-vaccination rise of at least 4 times the pre-vaccination antibody concentration in subjects with a pre-vaccination antibody concentration ≥5 EU/mL and <20 EU/mL; or at least twice the pre-vaccination antibody concentration in subjects with a pre-vaccination antibody concentration ≥20 EU/mL. The pre-vaccination value was the maternal sample taken at the first delivery.

Statistics

Antibody geometric mean concentrations (GMCs) with 95% CI were calculated. Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests. The analysis was performed using SPSS statistical software version 23.0.

Non-linear mixed effect models (NLMM) were employed to model the dynamics of anti-PT antibodies in both mothers and infants. Anti-PT antibodies were chosen because they, as well as anti-Prn antibodies, correlate with protection [13]. The model building procedure is motivated in the Appendix. The models were fitted using Monolix software [14]. The anti-PT antibody values less than 5 EU/mL are treated as left-censored data. Results of the NLMM are expressed as medians and interquartile range (IQR), because of possible asymmetry for the quantities under study as well as for ease of interpretation. Figure 1 in the Appendix shows a presumed visualization of the dynamics of the anti-PT antibody levels in the infants, indicating the different slopes and rates, used in the NLMM model.

Finally, a robust simple linear regression model was fitted to investigate the association between the antibody levels in women at delivery and in the cord.

All the rates mentioned in the manuscript are exponential decay or growth rates expressed in months, unless indicated differently. A significance level of 5% was used for all analyses. Blunting of vaccine immune responses was defined as a significantly lower GMC of pertussis specific IgG antibodies in group B siblings.

Results

General characteristics of the study population

In total, 86 women received a Tdap vaccine after a first pregnancy. All women had been vaccinated against pertussis during childhood with whole cell pertussis (wP) containing vaccines and received no documented pertussis booster for at least 10 years prior to the study booster. Of the 86 vaccinated women, 58 women became pregnant again and delivered within the study period.

The median interval between the first delivery and the Tdap vaccination was 16.07 months (min-max: 8.46- 43.31). The median interval between the Tdap booster and the consecutive delivery was 16.8 months (min-max: 6.20-56.49). Three women had a negative pregnancy test at the time of vaccination but were pregnant 1 month later, despite contraceptive advice. The mean age of the women was 29.97 years at first, and 32.04 years at consecutive delivery. Mean duration of both pregnancies was comparable. No significant difference in birth weight and length of both siblings was seen, nor gender ratio differences (Table 1).

Insert Table 1 here.

Laboratory results

a) Maternal results

Table 2 summarizes the GMC of IgG antibody concentrations to PT, FHA and Prn in all women at delivery of the first born infants (Group A) (= pre-vaccination sample of the mother), 1 month post-booster vaccination, and at the moment of delivery of the Group B siblings.

Insert Table 2 here.

At baseline, 93% of the women had detectable anti-FHA IgG antibodies and 79% had detectable anti-Prn IgG antibodies, while 52% had anti-PT IgG antibody concentrations > 5 EU/mL. 16% of participating

women were seronegative for both anti-PT and anti-Prn antibodies at baseline. One month after vaccination, 97.7% of the women showed a booster response to FHA and Prn while 90.7% showed a booster response to PT. All but one woman responded with a rise in all pertussis-specific antibodies, and there was no difference in magnitude of the response for anti-PT responses, nor for anti-Prn responses, between seronegative women and seropositive women pre-vaccination. At the next delivery, the mean maternal antibody levels for all 3 antigens had declined significantly ($p<0.001$) compared to one month after vaccination, but were still significantly higher compared to baseline concentrations for all antibodies ($p<0.001$). In 8 women, the anti-PT antibody concentration dropped below the threshold of 5 EU/mL at the consecutive delivery.

b) Cord results

Table 3 shows the transplacental transport ratio in both cohorts of infants. In general, GMC in cord blood exceeds GMC in the mother at delivery for both siblings. Ratios did not significantly differ at consecutive pregnancies.

Insert Table 3 here.

c) Infant results

Group B siblings have significantly higher antibody levels to all pertussis specific antigens at birth compared to group A infants, lasting up to the age of 1 month, before the start of the infant vaccination program. After three doses of primary vaccination, all antibody concentrations are consistently lower in group B siblings (month 6). For anti-FHA and anti-Prn, the IgG concentrations are significantly lower at the ages of 9 and 12 months. For anti-PT on the other hand, the antibody concentrations are only significantly lower at the age of 12 months (Table 2).

Modelling results

a) Maternal results

Based on the NLMM, the median time for anti-PT antibodies in women to decrease by 50% (i.e. the half-life) is 15.87 months (IQR: 14.86 – 16.74). The age of the mother at vaccination had no significant effect on this half-life.

Since there is no correlate of protection for pertussis, it is unclear how high the maternal antibody concentration at delivery should be in order to protect the offspring. Figure 2 shows the median time point (with IQR) whenever antibody concentrations in women fall below a pre-specified level (from 5 - 25 EU/mL) after a booster vaccination. After a median time of 55.56 months (IQR: 51.76 – 59.33) post-vaccination, the anti-PT IgG antibody levels in women decline below the threshold value of 5 EU/mL, and after a median time of 30.24 months (IQR: 27.77 – 33.25), the anti-PT IgG antibody levels have reached the threshold of 15 EU/mL. For comparison, the GMC of maternal antibodies at delivery of the group B siblings was 13,5 IU/mL, and this titer corresponded with possibly enough maternal antibodies in the cord to protect young infants.

Insert Figure 1 here

b) Infant results

The results of the NLMM confirm that siblings in group B have a significantly higher antibody concentration at birth (i.e. A_0 in Figure 1)) compared to group A infants. After birth and before the start of the infant vaccination program, maternal antibody levels decrease very fast in both groups (Figure 3): the median time for anti-PT antibody levels in group A infants to fall below 5 EU/mL, is 1.21 months (IQR: 1.08 – 1.36), while it takes about 2.21 months (IQR: 2.09 – 2.39) to drop below 5 EU/mL in group B siblings. The half-life of maternal anti-PT antibody levels in infants is approximately 5 weeks (33 days and 30 days for children group A and B, respectively).

Infants in group B have a significantly lower increase rate (denoted by γ in Figure 1) of anti-PT IgG antibody levels after priming with 3 vaccine doses. The time point *h*, at which we observe the highest antibody concentration before waning of antibodies, is estimated to be at 6.82 months (IQR: 6.63 – 7.01). The last scheduled vaccination was administered at 16 weeks of age, but the effect of 3 subsequent primary doses made the antibody levels still increase approximately 2.82 months after the last dose.

We included gender and birthweight of the infants, centered around its mean, in the NLMM: boys were born with significantly higher anti-PT antibody concentrations compared to girls in both groups. Time point *h* in boys is significantly lower (5.55 months (IQR: 5.33 – 5.77)), compared to the estimated value of 7.06 months [6.84 – 7.28] for girls. Newborns with higher birth weight tend to have higher estimated decay rates β albeit this small difference is not clinically relevant.

c) Correlation between maternal and infant antibody levels

The robust simple linear regression model shows a significant positive association between the anti-PT IgG antibody levels in women at delivery and in the cord. The predicted anti-PT IgG antibody levels in the cord of the infant whose mother had anti-PT IgG antibody levels at delivery of 5, 10, 15, 20 and 25 EU/mL, are respectively: 10.43 IU/mL [9.31 – 11.55], 18.80 [17.04 – 20.56], 26.53 [23.87 – 29.19], 33.88 [30.12 – 37.64] and 40.96 [35.97 – 45.95] EU/mL.

In order to evaluate the possible interval between several booster doses of Tdap in 1 woman, the correlation of maternal antibodies at delivery, with titers in cord and the loss of these maternal antibodies in the young infant over time (as a measure for protection from disease) could offer insights in the need for repeat booster, also considering possible interference of infant immune responses on vaccines. If, at delivery, the anti-PT antibody titer in the mother is 15 EU/mL, it takes roughly 2.5 months in infants for anti-PT antibody levels to decline below 5 EU/mL and 1 month to fall below 10 EU/mL (Figure 2).

Insert Figure 2 here

Discussion

At baseline, 48% of the women in the present long term follow up study, had undetectable anti-PT antibody levels, indicating the lack of maternal antibodies to be transferred and hence the lack of protection offered at birth. After a post-partum Tdap booster vaccination, good humoral responses were measured. Yet, similarly to other vaccination studies with Tdap in adults [15, 16], [17], a rapid drop in antibody concentrations is measured after vaccination: the half-life of anti-pertussis antibodies is estimated to be 15.90 months. However, median antibody concentrations remained significantly higher in women at a next delivery, even if the booster dose was offered more than 2 years before consecutive delivery. And significantly increased anti-pertussis antigen specific maternal antibody concentrations were still encountered in that offspring, born after the booster dose. In 13% (8/58) of the women, the anti-PT IgG had declined below the lower limit of 5 IU/mL by the time of the next delivery, indicating possible susceptibility to disease for the young infant, and underlining the importance of repetitive boosting with each new pregnancy.

Transplacental transport in children born before and after the pre-pregnancy booster vaccination was equally effective. Previously, adequate transport has been described in prospective cohorts [9, 18, 19]. Yet, this is the first study describing transplacental transport in siblings, and no effect of vaccination or parity was found on the adequacy of the transport. We would like to stress that our cohort consisted of healthy pregnant women with mostly healthy pregnancies, while transplacental transport can be influenced by placental dysfunction or disease. We confirm in the present study the recently published Swiss data that the presence of high titers of maternal antibodies during the entire pregnancy, or a long period thereof, results in adequate transport and high concentrations at birth [20].

Using a NLMM, we were able to calculate the impact of time lapse between repeat boosters in healthy adult women on transferred antibody levels towards the fetus. Based on these results, the time frame to

be considered between consecutive pertussis booster vaccines should be no longer than 30 months (roughly 2.5 years), in order to have median anti-pertussis antibody concentrations in pregnant women as high as 15 EU/mL at the moment of delivery. This level corresponds to anti-PT IgG antibody levels in cord blood of 26.53 EU/mL in the present study (IQR: 23.87 – 29.19). These results are based on the assumption that the estimated decay rate derived from our model is the same as the one we would obtain with a larger number of samples (See Appendix), and on the assumption that the positive effect that we found in group B siblings, is protective against disease. This is not a recommendation, yet a finding that has to be confirmed based on additional persistence studies, and possibly including information on antibody levels at multiple time points to inform antibody dynamics. In addition, similar calculations when vaccination is offered during pregnancy are needed and planned.

Infants born after a pre-pregnancy booster had significantly higher anti-PT GMC at birth that endured up to one month of age, thus closing potentially the susceptibility gap for infection. These results are in line with on the effect of Tdap vaccination during pregnancy [9, 18, 19]. The median half-life of anti-PT IgG antibodies in infants is relatively short (5 weeks) which is again in line with literature [21-23]. Since no correlate of protection is known for pertussis [13], it is not sure whether the elevated antibody concentrations in the second born cohort are sufficiently protective against disease, or whether the blunting later in infant life, after a primary series of three vaccines, is meaningful in terms of protection. Indeed, the effect of these maternal antibodies on the immune responses to the primary infant vaccination, is still detectable in group B siblings born after a pre-pregnancy booster dose. This so-called blunting effect has been described after maternal Tdap vaccination during pregnancy [9, 18, 19, 24], however this is the first study to describe the same effect after a pre-pregnancy (or post partum) booster dose. What we measure is in fact the effect of cocoon vaccination on offspring that is born later on. Effectiveness data are lacking whether the elevated titer of maternal antibodies is protecting young infants from disease.

At months 6 and 9, after primary vaccination, differences between both siblings are not always significant for all 3 tested antibody types, perhaps due to the small sample size. GMC in infants at 12 months of age, measured in previous studies [25], are comparable with GMC in children at 1 month of age after a pre-pregnancy booster, suggesting that GMC in group B siblings at birth would be compatible with clinical protection.

The present study shows that also countries with a recommendation for cocoon vaccination, or with repeated booster recommendations, should be aware that significantly higher antibody concentrations are transferred to the offspring, thereby leading to possible early life protection but also potential interference of the maternal antibodies with the infant's immune response. Data could be used for cost-benefit calculations for subsequent doses in women of childbearing age.

There are a few shortcomings in this study. There is a rather low number of infants born after the booster dose as a result of the difficulty to plan this upfront. Besides, the study was not powered to detect small differences in antibody levels between both siblings, with growing time interval between vaccination and delivery of a second born child. In addition, the time points of blood sampling were chosen according to the original study on measles antibody kinetics, and those were likely not the most optimal time points for pertussis responses. Nevertheless, pre- and post-vaccination samples were available and are always taken at the same time points. In order to understand better the relevance of the findings, measurement of the functionality of the antibodies, would be valuable, ideally comparing functionality of antibodies elicited during pregnancy, and as in this project, in a non-pregnant status.

To conclude, the present study is the first study describing the effects of a pre-pregnancy Tdap booster vaccine, comparing kinetics of pertussis antibodies between siblings born before and after the booster

297 dose. The maternal antibodies at birth are significantly higher in the second born siblings, even with a

298 growing time interval between booster and subsequent delivery.

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Footnote page

Funding

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Conflict of interest

Potential conflicts of interest: EL was member of an advisory board on Pertussis vaccines (GSK Biologicals, 2016, non-financial support). The University of Antwerp obtains grants from several vaccine manufacturers for the conduct of vaccine trials for which PVD is the investigator. NH is holder of the scientific chair of evidence-based vaccinology sponsored by an unrestricted gift from Pfizer and GSK.

Meetings

Preliminary data have been presented at the INMIS congress in November 2015 in The Gambia. And a first interim analysis has been published in: ‘Effect of a prepregnancy pertussis booster dose on maternal

antibody titers in young infants. Leuridan E, Hens N, Peeters N, de Witte L, Van der Meeren O, Van Damme P. Pediatr Infect Dis J. 2011 Jul;30(7):608-10.'

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Legends

Table 1: Demographic characteristics of the participating women and infants

Table 2: Geometric Mean Concentration (GMC) with 95% confidence interval (95% CI) for antibody concentrations against Filamentous Hemagglutinin (FHA), Pertactin (Prn) and Pertussis Toxin (PT) in women and infants at different time points, expressed in Elisa Units per milliliter (EU/mL).
There was not enough sample available anymore from all women at the delivery of the first born child.
Statistical test used: unpaired t-test.

Figure 1: The median time point (with their IQR) whenever the anti-PT IgG antibodies in women, after booster vaccination, decline below a pre-specified threshold (x-axis) between 5 and 25 EU/mL.

Figure 2: Decline of the maternal antibodies during the first months of life, in both groups of infants (group A infants expressed in red and group B siblings expressed in blue). The solid lines show the median time points (with their IQR in dotted lines, y-axis) whenever the maternal anti-PT IgG antibodies in infants start to decline below a pre-specified threshold (x-axis) between 5 and 10 EU/mL (when no infant vaccination is performed).

Table 3: Transplacental transport ratio for Pertussis Toxin, Filamentous haemagglutinin and pertactin antibodies in group A and group B infants. Statistical test used: unpaired t-test.

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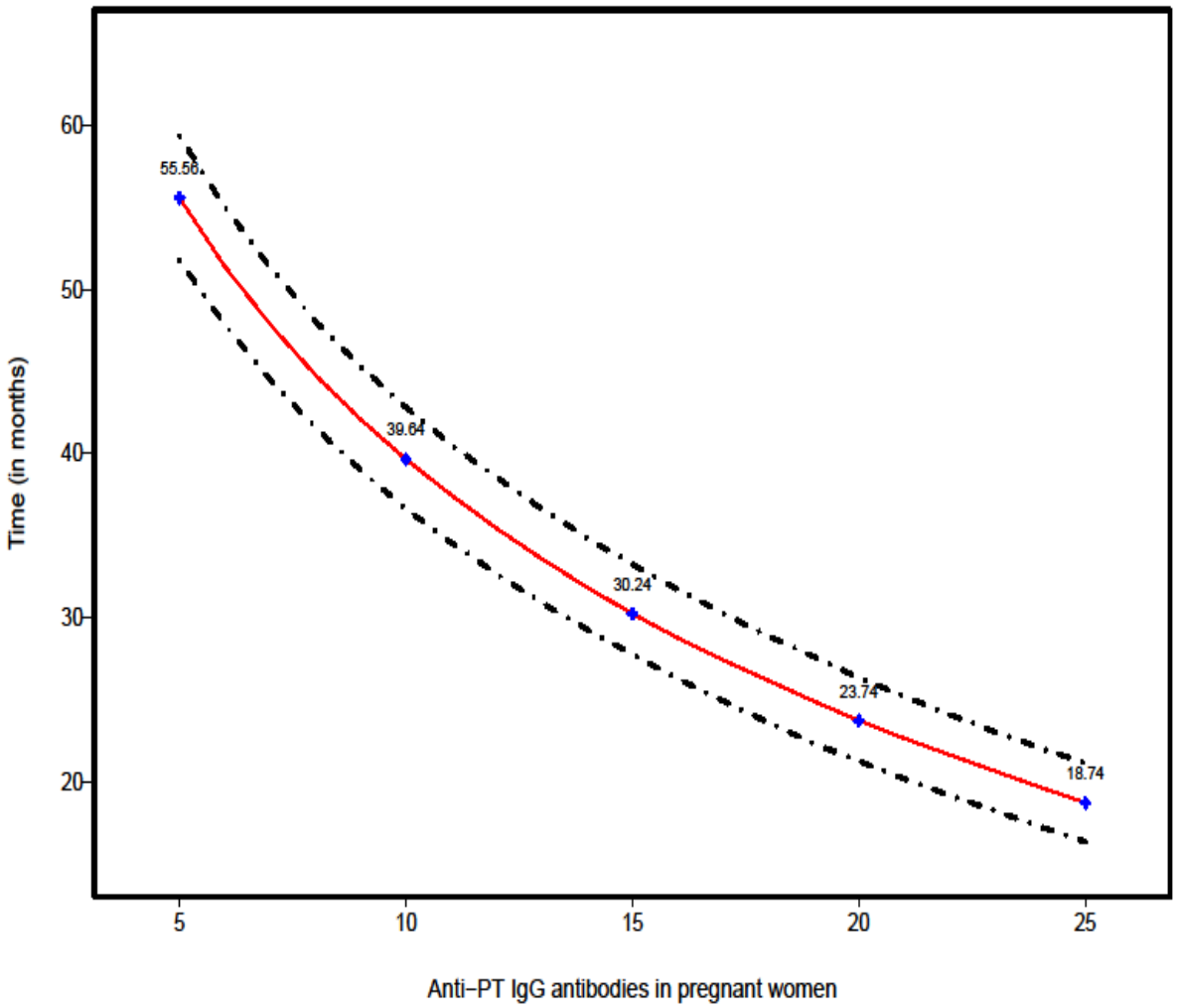

Figure 1: The median time point (with their IQR) whenever the anti-PT IgG antibodies in women, after booster vaccination, decline below a pre-specified threshold (x-axis) between 5 and 25 EU/mL.

Figure 2 Click here to download Figure Figure 2_revision.docx

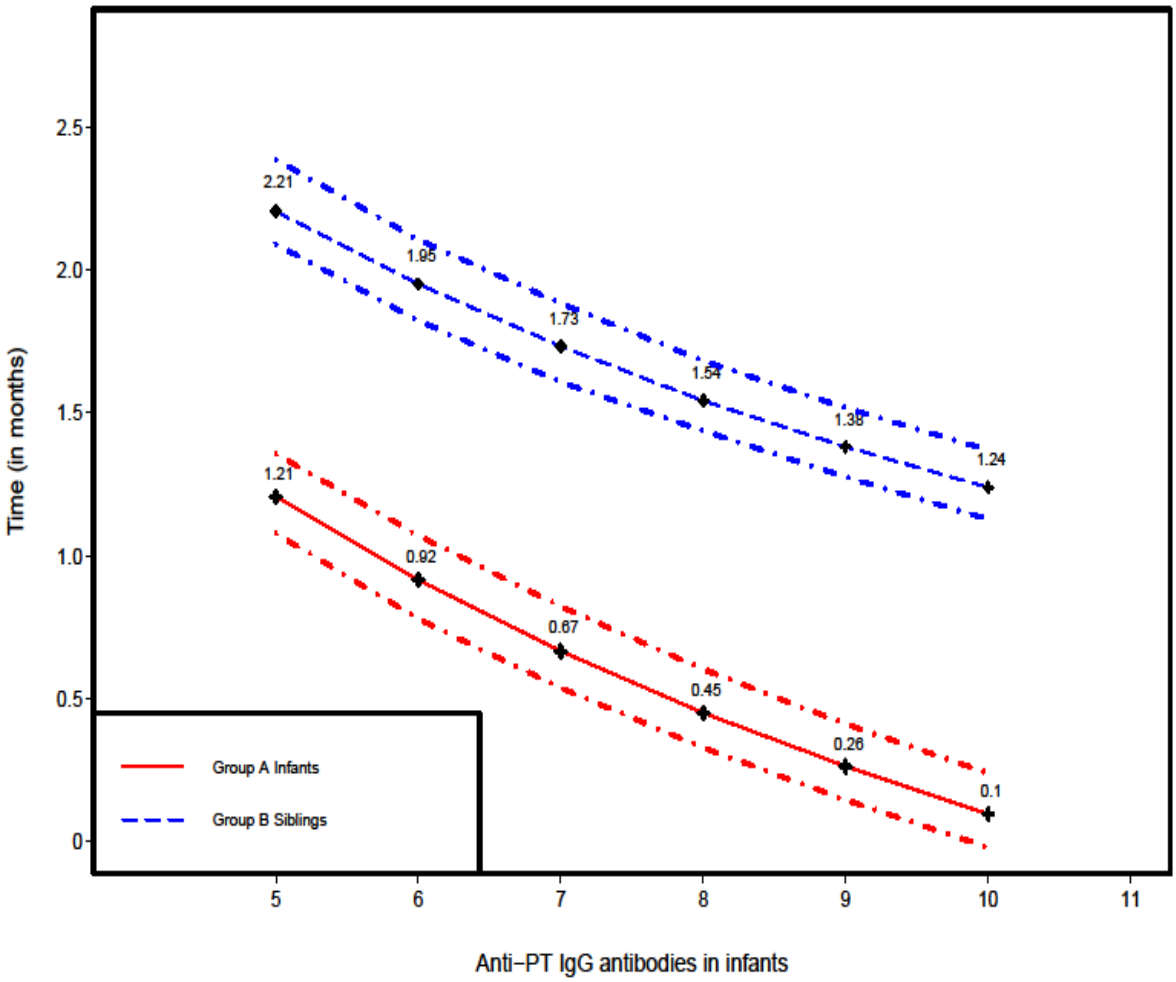

Figure 2: Decline of the maternal antibodies during the first months of life, in both groups of infants (group A infants expressed in red and group B siblings expressed in blue). The solid lines show the median time points (with their IQR in dotted lines, y-axis) whenever the maternal anti-PT IgG antibodies in infants start to decline below a pre-specified threshold (x-axis) between 5 and 10 EU/mL (when no infant vaccination is performed).

Table 1

Demographic characteristics women			
Mean age at vaccination in years (SEM)	30.53 (0.36)		
Median interval between delivery first child and vaccination in months (IQR)	16.07 (11.90)		
Mean interval between vaccination – blood sampling in month (SEM)	1.00 (0.01)		
Median interval between vaccination and delivery child 2 in months (IQR)	16.80 (15.48)		
	Delivery infant A	**Delivery sibling B**	**p-value**
Mean age of the mother at delivery in years (SEM)	29.97 (0.37)	32.04 (0.41)	Not of interest
Gestational age at birth in weeks (SEM)	39.43 (0.13)	39.25 (0.17)	0.395
Mean duration of breastfeeding in months (SEM)	4.43 (0.31)	4.72 (0.40)	0.555
Demographic characteristics infants	**Group A infants**	**Group B siblings**	**p-value**
Gender, No. Male (%)/ Female (%)	39 (45.88)/ 46 (54.12)	30 (51.72)/ 28 (48.28)	0.383
Mean age at blood sample month 1 in months (SEM)	1.01 (0.01)	1.02 (0.01)	0.758
Mean age at blood sample month 6 in months (SEM)	6.01 (0.03)	6.56 (0.11)	0.256
Mean age at blood sample month 9 in months (SEM)	9.00 (0.02)	9.19 (0.11)	0.120
Mean age at blood sample month 12 in months (SEM)	12.04 (0.02)	12.46 (0.10)	<0.001
Mean age at hexavalent vaccine dose 1 in months (SEM)	2.25 (0.04)	2.13 (0.10)	0.532
Mean age at hexavalent vaccine dose 2 in months (SEM)	3.43 (0.05)	3.49 (0.10)	0.572
Mean age at hexavalent vaccine dose 3 in months (SEM)	4.62 (0.07)	4.73 (0.12)	0.450
Mean interval between hexavalent vaccine dose 3 and blood sample month 6 in months (SEM)	1.26 (0.11)	2.16 (0.10)	0.918
Mean interval between hexavalent vaccine dose 3 and blood sample month 9 in months (SEM)	4.42 (0.10)	4.39 (0.30)	0.984
Mean interval between hexavalent vaccine dose 3 and blood sample month 12 in months (SEM)	7.42 (0.07)	7.82 (0.13)	0.007

Table 1: Demographic characteristics of the participating women and infants. Statistical test used: unpaired t-test and chi-square test.

Table 2

GMC (95% CI) in women	Anti-FHA (EU/mL)		Anti-Prn (EU/mL)		Anti-PT (EU/mL)	
DELIVERY CHILD 1	21.50 (16.96-27.22)		15.84 (11.97-20.96)		6.47 (5.16-8.11)	
N samples	84		85		85	
ONE MONTH AFTER VACCINATION	770.75 (638.43-930.49)		658.88 (503.92-861.49)		69.97 (55.87-87.62)	
N samples	86		86		86	
DELIVERY CHILD 2	149.59 (118.22-189.28)		147.91 (100.37-217.96)		13.43 (10.10-17.85)	
N samples	55		55		55	
GMC (95% CI) in infants	**Anti-FHA (EU/mL)**		**Anti-Prn (EU/mL)**		**Anti-PT (EU/mL)**	
	GROUP A	**GROUP B**	**GROUP A**	**GROUP B**	**GROUP A**	**GROUP B**
CORD	32.16 (22.49-45.99)	239.85 (187.93-306.12)	22.60 (15.66-32.63)	253.74 (175.35-367.17)	9.68 (6.86-13.65)	22.61 (17.18-29.74)
p-value	<0.001		<0.001		<0.001	
N samples	46	55	48	55	48	55
MONTH 1	16.70 (10.84-25.73)	133.63 (105.38-169.44)	10.89 (7.04-16.86)	143.49 (101.31-203.25)	5.46 (3.91-7.63)	11.84 (8.81-15.92)
p-value	<0.001		<0.001		0.001	
N samples	34	53	35	53	35	53
MONTH 6	206.42 (116.36-366.17)	92.40 (67.79-125.96)	70.34 (41.63-118.85)	25.27 (14.45-44.18)	59.63 (34.25-103.83)	36.31 (27.17-48.53)
p-value	0.039		0.016		0.175	
N samples	15	11	15	11	15	11
MONTH 9	84.28 (64.82-109.57)	32.58 (22.19-47.85)	36.89 (25.72-52.90)	12.19 (6.97-21.33)	23.76 (17.33-32.58)	16.23 (11.29-23.31)
p-value	<0.001		0.002		0.154	
N samples	20	10	19	10	19	10
MONTH 12	57.75 (45.28-73.65)	27.99 (21.02-37.28)	12.31 (8.79-17.24)	7.05 (5.52-9.01)	10.87 (8.50-13.90)	6.39 (5.07-8.04)
p-value	0.001		0.009		0.003	
N samples	33	47	32	47	32	47

Table 2: Geometric Mean Concentration (GMC) with 95% confidence interval (95% CI) for antibody concentrations against Filamentous Hemagglutinin (FHA), Pertactin (Prn) and Pertussis Toxin (PT) in women and infants at different time points, expressed in Elisa Units per milliliter (EU/mL).There was not enough sample available anymore from all women at the delivery of the first born child. Statistical test used: unpaired t-test.

Mean ratio (SEM)	Transplacental transport ratio		p-value
	Group A infants	**Group B infants**	
Pertussis Toxin IgG	1.96 (0.18)	1.76 (0.08)	0.300
Filamentous Hemagglutinin IgG	1.78 (0.09)	1.74 (0.07)	0.899
Pertactin IgG	1.84 (0.13)	1.62 (0.08)	0.147

Table 3: Transplacental transport ratio for Pertussis Toxin, Filamentous haemagglutinin and pertactin antibodies in group A and group B infants. Statistical test used: unpaired t-test.

Effect of Pre-pregnancy Pertussis Vaccination in Young Infants
Appendix

Model building for data in infants:

Dynamics of antibodies in infants:

There are maximum four data points of anti-PT IgG antibody in each infant: at delivery (cord blood sample), 1 month after birth, 6 or 9 months after birth and 12 months after birth. Based on the recommended vaccination schedule in Belgium, infants were vaccinated at the ages of 8, 12 and 16 weeks. We propose to use the model with three ODEs (see Figure 1).

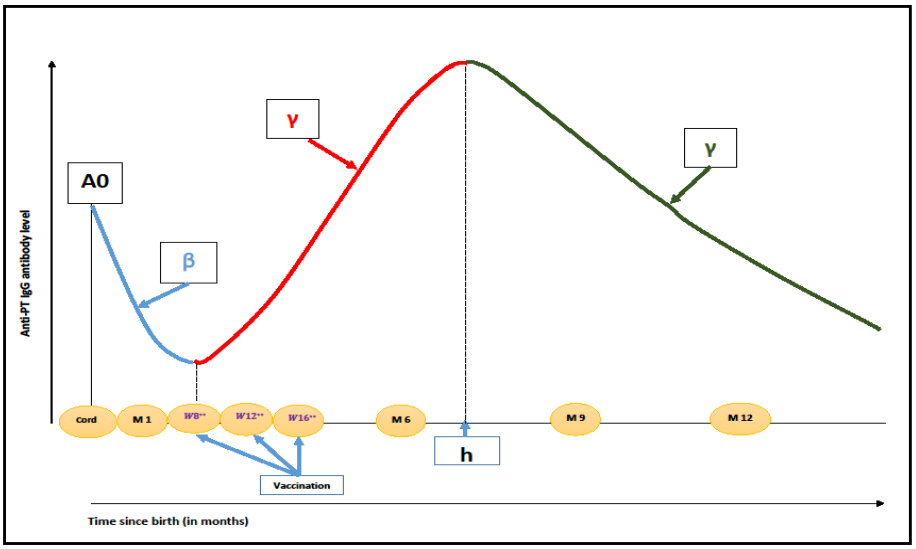

Figure 1: Illustrated presumed dynamics of anti-pertussis antibody levels in infants: A_0 is the antibody level at birth. After birth, the antibody levels decrease with decay rate β. After the administration of the first aP containing vaccine at week 8, the antibodies start to increase up to time point h at an overall rate γ. It is hypothesized that $h > 4$ since the infants are vaccinated at week 8, 12 and 16. After time point h, antibodies decrease again at rate α. Cord, M1, M6, M9 and M12 indicate the sampling time points. $W8^{**}$, $W12^{**}$ and $W16^{**}$ indicate the vaccination time points.

The dynamic change in anti-pertussis antibodies is written down as follows:

$$t \leq 2: \frac{dA}{dt} = -\beta A$$

$$2 < t \leq h: \frac{dA}{dt} = \gamma A$$

$$t > h: \frac{dA}{dt} = -\alpha A$$

This ODE system assumes that anti-PT IgG antibodies firstly decrease with a decay β right after birth till the 1st vaccination at month two. After the 1st vaccination, antibodies increase with an overall rate of γ which is accounted for the general increase due to three consecutive vaccination doses. Since there are not enough data points to model the increasing rate after each vaccination dose, only the overall rate γ is expressed and modelled. After the vaccination at month four, antibodies remain increasing, till time point h, and start decrease again with decay α.

Model building and selection:

Model selection is performed based on *AIC* obtained from models without any covariate fit in Monolix software. The anti-PT IgG antibody level at T_0(corresponding to cord sample) is A_0. Since there are maximum four measurements per subjects, we allow maximum four random effects. We let h fixed, and let $A_{0,i}$, β_i, γ_i and α_i be random. The individual parameters are assumed to have log-normal distribution.

To perform model selection, we fit different models with several scenarios: models with only 1 random effect, models with two random effects, models with three random effects and model with four random effects. Some models did not achieve convergence in Monolix software. Among all models whose convergence was achieved, the model with four random effects had the lowest *AIC* . As a result, we used this model to continue our analysis, where covariates were taken into account.

First of all, we consider the order of the infants (covariate *child2: child2 = 0* if the child from group A infants and *child2 = 1* if the child from group B siblings) as a categorical variable with group A infants as reference group . The model where *child2* is assumed to affect the estimate of all parameters did not converge in Monolix. The results presented in the manuscript, hence, were obtained from the model where *child2* was assumed to affect the estimate of four parameters ($A_{0,i}$, β_i, γ_i, α_i). Directly after, the effect of gender (covariate *gender*: *gender = 1* if male and *gender = 0* if female where baby girl is the reference group), birthweight (covariate *bweight* centered around its mean *bweight_mean*) of infants (in gram) were additionally taken into account.

Specification for final models:

- Model with only one covariate (*child2*):

Let denote A_0, β, γ and α the four population parameters. Individual parameters are assumed to have log-normal distribution, it follows:

$$log(A_{0,i}) = log(A_0) + \rho_{A_0} child2_i + \eta_{A_{0,i}}$$

$$log(\beta_i) = log(\beta) + \rho_{\beta} child2_i + \eta_{\beta,i}$$

$$log(\gamma_i) = log(\gamma) + \rho_{\gamma} child2_i + \eta_{\gamma,i}$$

$$log(\alpha_i) = log(\alpha) + \rho_{\alpha} child2_i + \eta_{\alpha,i}$$

Where:

$$\eta_{A_{0,i}} \sim N(0, {\delta_{A_0}}^2),$$

$$\eta_{\beta,i} \sim N(0, {\delta_{\beta}}^2),$$

$$\eta_{\gamma_i} \sim N(0, {\delta_\gamma}^2)$$

$$\eta_{\alpha_i} \sim N(0, {\delta_\alpha}^2),$$

We use anti-PT IgG antibody levels on the log10 scale and assume an additive residual error model, that is:

$$log_{10}(A_{obs,ij}) = log_{10}(A_{pred,ij}) + \varepsilon_{ij}$$

where $A_{obs,ij}$ and $A_{pred,ij}$ are the observed and predicted anti-PT IgG antibodies for the child *i* at time point *j*. The residual error is assumed to have a normal distribution with mean 0, that is:

$$\varepsilon_{ij} \sim N(0, \delta^2),$$

- Model with three covariates (*child2*, *gender*, *bweigth*):

Initially, we fit the models where only *gender* or *bweight* was considered as covariate separately. In the next step, only statistically significant relationship of *gender* and *bweight* was retained in the final model with three covariates since the central inference lies on the effect of *child2*.

The individual parameters in the final model were assumed to have log-normal distribution:

$$log(A_{0,i}) = log(A_0) + \rho_{child2,A_0}\, child2_i + \rho_{gender,A_0}\, gender_i + \eta_{A_{0,i}}$$

$$log(\beta_i) = log(\beta) + \rho_{child2,\beta} child2_i + \rho_{bweight,\beta}(bweight_i - bweight\_mean) + \eta_{\beta,i}$$

$$log(\gamma_i) = log(\gamma) + \rho_{child2,\gamma} child2_i + \eta_{\gamma,i}$$

$$log(\alpha_i) = log(\alpha) + \rho_{child2,\alpha} child2_i + \rho_{gender,\alpha} gender_i + \eta_{\alpha,i}$$

Moreover, *gender* is assumed to affect the estimate of *h.*

- Left-censored observations:

There are anti-PT IgG antibody values less than the limit of detection (5 IU/mL). These data will be treated as left censored during the estimation procedure in Monolix software.

Model diagnosis:

We perform model diagnosis for the final model by the means of the SAEM (Stochastic Approximation Expectation Maximization) convergence plot and residual plots (residuals v.s time and residuals v.sprediction).

Furthermore, the scatter plot of the observed antibody levels and predicted values are presented to partially evaluate the prediction ability of the model (see Figure 2).

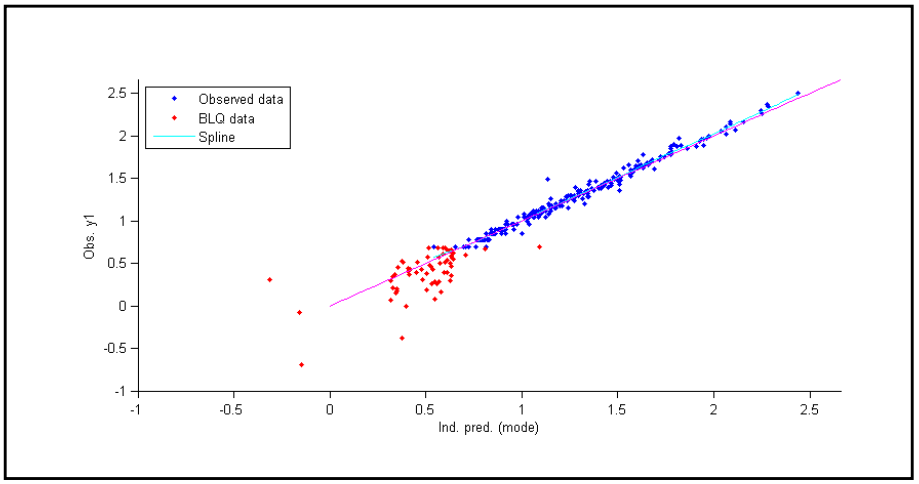

Figure 2: Scatter plot of the observed anti-PT IgG antibodies with respect to the predicted anti-PT IgG antibodies using the individual parameters (model in infants with only one covariate c*hild2*, output from Monolixsoftware).

Important results:

In the model with three covariates, it is shown that boys were born with significantly higher anti-PT IgG concentrations compared to girls in both groups of infants. The median anti-PT IgG antibody levels at birth in group A infants is respectively 8.51 EU/mL (IQR: 5.96 – 11.06) for girls and 13.3 EU/mL (IQR: 9.18 – 17.42) for boys; and in group B siblings 18.20 EU/mL (IQR: 17.63 – 18.77) for girls and 28.40 EU/mL (IQR: 27.55 – 29.25) for boys,respectively.

Model building for data in pregnant women:

Dynamics of antibodies in pregnant women:

There were three blood sampling occasions in each pregnant woman: at the 1st delivery, 1 month after booster vaccination and at the delivery of the 2nd child.

We denoted the antibody level at T_0(1st delivery) as A_0. In theory, antibody levels decrease over time until the women received a booster vaccination. From that moment, antibodies are assumed to immediately increase. Since we do not have enough data to observe the whole process, what we can model was the process illustrated in Figure 3. In this presumed dynamics, the solid line (in black) illustrates the theoretical change of antibodies. The dashed-dotted line (in red) is the curve based on the collected data. It is assumed that the decay rates after the peak (time point h) were the same in solid line and dashed line. However, it is a strong assumption and the conclusion based on the result of α should be interpreted with caution.

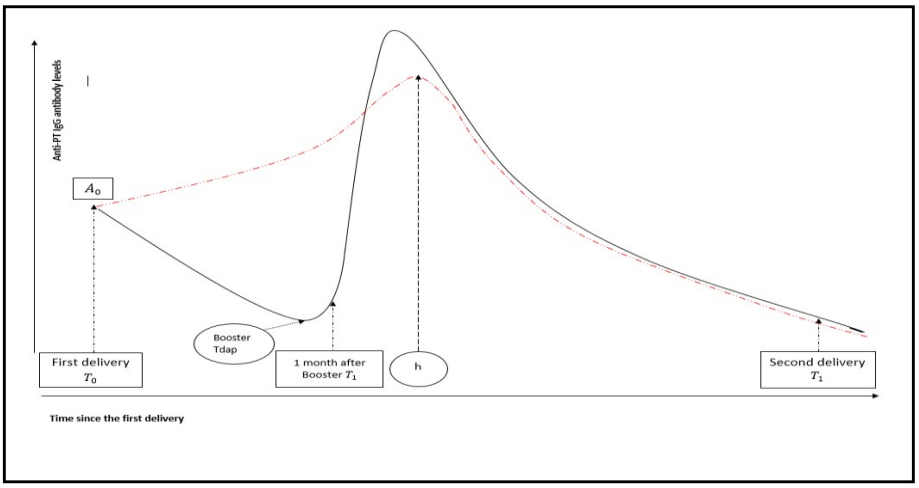

Figure 3: Illustrated presumed dynamics of antibody levels in pregnant women. Solid line (in black): The theoretical dynamics of antibodies, where it is assumed to decrease over time and then immediately increase after pregnant women received the booster vaccination. Dashed-dotted line (in red): The hypothesized dynamics of antibodies based on the collected data where it is assumed that antibodies increase between T_0 and T_1 with the rate ω then decrease between T_1 and T_2 with the decay α.

The ODEs for the evolution of antibodies in pregnant women are written as follows:

$$t \leq h: \frac{dA}{dt} = \omega A,$$

$$t > h: \frac{dA}{dt} = -\alpha A.$$

It is noticed that some notations used in this section, though might be the same as those in the infants' model, in fact carry their own meaning and values.

Model building and selection:

We fit non-linear mixed effects model using Monolix software. Model selection was performed based on *AIC* (smaller is better). Since there were maximum three measurements per subjects, we allowed maximum three random effects. We let h fixed, and let A_0,, ω and α be random (model A). This model , however, did not converge in Monolix. Based on the SAEM convergence plot, there seemed to be a negative correlation between h and ω. To further explore this notice, we extracted the sampled values of h and ω during SAEM in Monolix. The scatter plot (in Figure 4) shows a curvature relationship between the two variables.

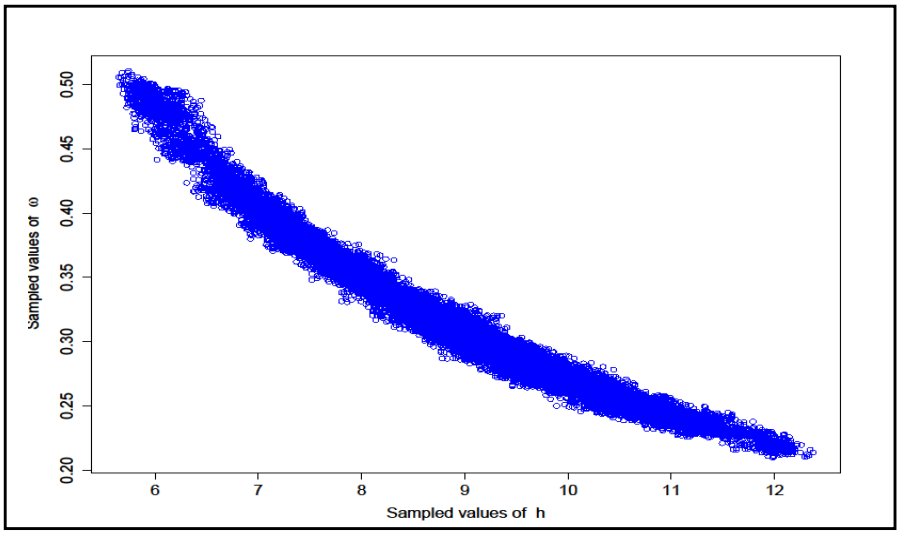

Figure 4: The scatter plot between sampled values of *h* and sampled values of ω from running model A in Monolix software.

We then applied the transformation to stabilize the sampling procedure in Monolix: $\omega = \frac{h}{e^{\gamma}}$ As a result, instead of estimating ω directly, we estimate γ.

The four-parameter model (A_0, γ, α, and h) with three random effects ($A_{0,i}$, γ_i, α_i) were fit. Based on the SAEM convergence plot produced from Monolix software, the convergence of γ seems to be very unstable. Hence, a two-random-effect model ($A_{0,i}$, α_i) was fit. The convergence of this model was well achieved.

To perform model selection, we run two models with only 1 random effect: $A_{0,i}$ or α_i separately. The *AIC* obtained from these two models are higher compared to the *AIC* of model with two random effects ($A_{0,i}$, α_i). As a result, the final model is the model with two random effects ($A_{0,i}$, α_i).

Specification for final model:
Let denote A_0 and α the two population parameters. The age at boostrix (centered around its mean: *age_mean*) is assumed to affect the estimate of all four parameters. We assume $A_{0,i}$ and α_i have log-normal distribution:

$$log(A_{0,i}) = \log(A_0) + \beta_{A_0}(age_i - age\_mean) + \eta_{A_{0,i}}$$

$$log(\alpha_i) = \log(\alpha) + \beta_{\alpha}(age_i - age\_mean) + \eta_{\alpha,i}$$

Where

$$\eta_{A_{0,i}} \sim N(0, {\delta_{A_0}}^2),$$

$$\eta_{\alpha_i} \sim N(0, {\delta_{\alpha}}^2),$$

We use anti-PT IgG antibody levels on the log10 scale and assume an additive residual error model, that is:

$$log_{10}(A_{obs,ij}) = log_{10}(A_{pred,ij}) + \varepsilon_{ij}$$

where $A_{obs,ij}$ and $A_{pred,ij}$ are the observed and predicted anti-PT IgG antibodies for women *i* at time *j*. The residual error is assumed to have a normal distribution with mean 0, that is:

$$\varepsilon_{ij} \sim N(0, \delta^2),$$

There are antibody values less than the limit of detection (5 IU/mL). These data will be treated as left censored during the estimation procedure in Monolix software.

Model diagnosis:
We perform model diagnosis for the final model by the means of the SAEM convergence plot and residual plots (residuals v.s time and residuals v.s prediction).

Moreover, the scatter plot of the observed values and predicted values are assessed to partially evaluate the prediction ability of the model (see Figure 5).

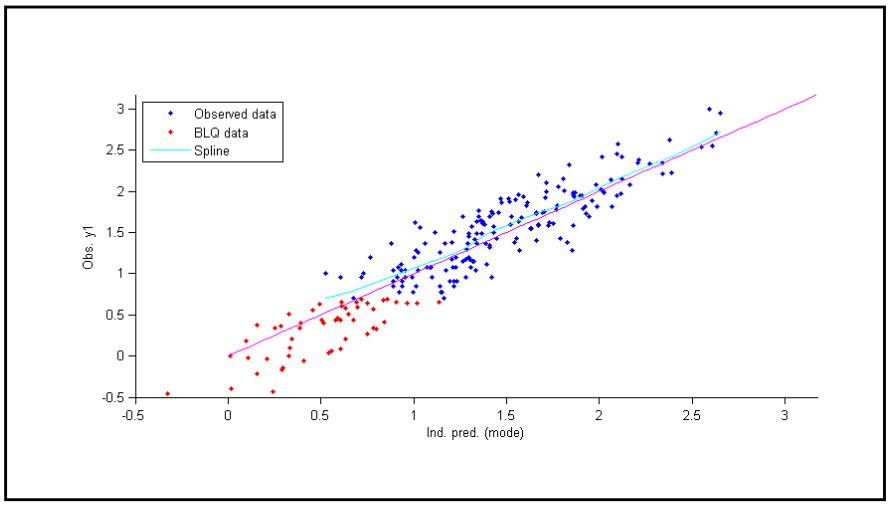

Figure 5: Scatter plot of the observed anti-PT IgG antibody levels with respect to the predicted anti-PT IgG antibody levels using the individual parameters (model in pregnant women, output from Monolix software).

Robust linear regression model to investigate the association between the anti-PT IgG antibodies in pregnant women at delivery and that in Cords of their infants:

Figure 6 shows the scatter plot between anti-PT IgG antibodies in Cords of infants and that in pregnant women at delivery. It is expected to see a linear trend between the two measurements.

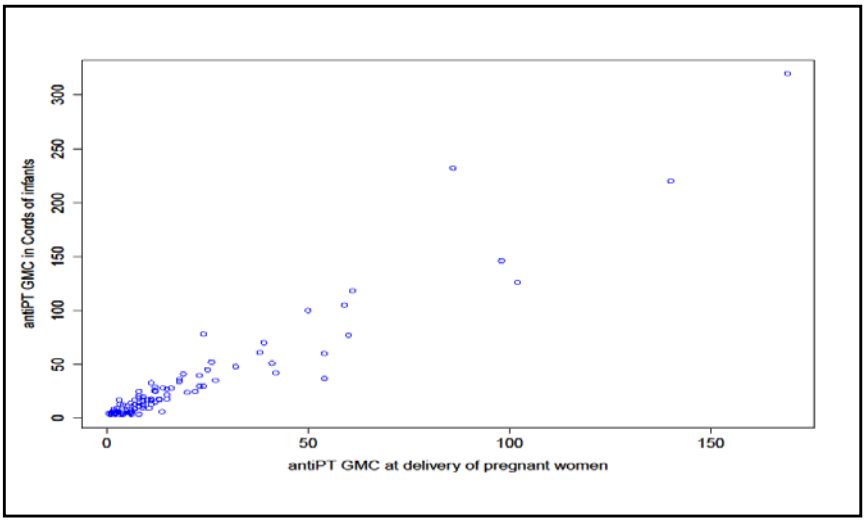

Figure 6: Scatter plot between anti-PT IgG antibodies in Cords of infants and that in pregnant women at delivery. Left censored observations were replaced by the individual predicted mode values obtained from the final NLMM models for data in infants and data in pregnant women.

Model building and selection:
The response variable is *antiCord* (anti-PT IgG antibodies in Cords of infants). Initially, a full model with four covariates: anti-PT IgG antibodies of pregnant women at delivery (*antiPTMum* covariate), the order of the child (*child2*), the gender (*gender*) and birthweight of the child (*bweight* covariate) along with the interaction between *child2* and *antiPTMum* was considered. However, this model did not satisify two important assumptions, namely, the normality and the homocesdasticity of the residuals. Hence, it is decided to transform the response variable and the *antiPTMum* covariate into natural log scale. The motivation to choose this transformation is that it helps to satisfy the assumptions of linear model, and among other potential transformations, it serves the purpose of easier interpretation. The results from model on transformed data shown that the effects of *gender*, *bweight*, *child2* and the interaction between *antiPTMum* and *child2* were not significant. Since the central interest lies in the association between the antibody levles in pregnant women at delivery and that in Cords of their infants, the final model is the simple linear regression model where only *antiPTMum* is considered ascovariate.

Specification for final model:
The final model could be written down as follows:

$$\log(antiCord_i) = \log(antiPTMum_i) + \varepsilon_i,$$

where $i = 1, 2, \ldots, 97$.

The assumption of homocesdasticity of the residuals is not satisfied. Hence, a robust linear regression model was fit using *rlm* function in the MASS package. The result from this robust linear regression was used for making inference.