Kinetics of Maternal Antibodies against Rubella and Varicella in Infants

Running title: maternal antibodies against rubella and varicella

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Key words: maternal antibodies, rubella, varicella, vaccination

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Word count: 2853

Acknowledgement: The authors would like to thank all participating women and their lovely children. We would also like to thank Mrs Aline Bontenakel for performing the blood sampling in the infants.

Abstract

Kinetics of maternal rubella and varicella antibodies in 213 mother-infant pairs are described in a longitudinal study in Belgium.

Blood samples are taken at 7 time points (week 36 of pregnancy, birth (cord), 1, 3, 6, 9, and 12 months), and analyzed for anti-rubella IgG and anti-varicella IgG by enzyme linked immunosorbent assay (ELISA). A generalised exponential model is used to analyse maternal antibody decay in infants.

Model based, the mean duration of passive immunity is 2.1 months for rubella and 2.4 months for varicella.

Infants are susceptible at young age for rubella, a disease with high vaccination coverage, as well as for varicella, an endemic disease in western Europe.

Introduction

Congenital rubella syndrome (CRS) is a severe complication of rubella infection during pregnancy. Vaccination against rubella has been performed since several decades and the prevalence of CRS has fortunately decreased enormously. In Belgium, rubella vaccination is recommended since 1972 for adolescent girls (monovalent vaccine), and since 1994 for both boys and girls through Measles Mumps Rubella (MMR) vaccine administration at the age of 15 months, with a second dose at 11 years of age. The age of the first dose was lowered in 2002 to 12 months [1]. MMR vaccination of susceptible women at fertile age is recommended.

Varicella is endemic in Belgium. Vaccines are available on the market but recommended only for risk groups. Seroprevalence data show 96.8% seropositivity at childbearing age for varicella antibodies [2].

The amount of antibodies against infectious diseases is subject to many epidemiological factors, e.g. vaccination programmes, spread of the disease and natural boosting, time since infection. Yet, the maternal antibody titer is of critical importance to the neonatal titer at birth [3] . Timing of administration of the first dose of a MMR vaccine should take into account the presence of maternal antibodies, since it is known that they can hamper humoral responses to vaccination, in particular for attenuated vaccines [4, 5].

In the present paper the duration of the presence of maternal antibodies against rubella and varicella is studied in a prospective cohort of woman-infant pairs, describing on the one hand a disease with high vaccination coverage and on the other hand an endemic disease in the population. Results on persistence of measles maternal antibodies have been published elsewhere [6] .

Methodology

- *Study design*

A prospective multi-centre study was conducted in Antwerp, Belgium, in accordance with the Helsinki Declaration, ICH-GCP and procedures established by Belgian law. Healthy pregnant women aged 18 to 40 years and their healthy offspring were included starting April 2006 and follow-up lasted until November 2008. Informed consent was obtained. Exclusion criteria were impaired immunology in mother or child, administration of immunoglobulins or blood products during the study period, preterm delivery (<36 weeks) and low birth weight (<2400 g). A questionnaire was completed on demographics, validated vaccination history and medical history. Growth parameters, breastfeeding, day-care attendance, immunization data, and medical histories for all household members were registered at each visit. Venous whole blood was collected during pregnancy (10 ml at week 36), at birth (10 ml cord blood), in all infants (2 ml) at month 1 (27-34 days), month 3 (84-99 days) and month 12 (358-372 days) and randomly at either month 6 (175-189 days) or month 9 (267-282 days). All samples except for cord blood, were collected during home visits. Samples were centrifuged at 2000 rpm within 8 hours after sampling and stored at -20°C.

- *Serology*

Rubella quantitative measurement of Immunoglobulin G (IgG) was analyzed with ELISA ETI-RUBEK-G PLUS® (Diasorin, Germany). The protective cut-off value is 10 IU/mL, according to international standards [7].

Varicella quantitative measurement was analyzed with ELISA Enzygnost® Anti VZV IgG (Siemens, Germany). The assay was calibrated against the WHO International Standard for Varicella Zoster Immunoglobulin (50 IU), and quantitative results are

reported in milli-International Units per milliliter. If corrected Optical Density (OD) was > 0.2, the sample was considered positive, if corrected OD was < 0.1, the sample was negative. A sample with a corrected OD between 0.1 and 0.2 was inconclusive. Quantitative results were calculated with the aid of the α-method using kit dependent parameters. If corrected OD < 0.1, quantitative results could not be trusted. The estimated 'protective' cut-off value was set on 100mIU/mL based on the optical density measurements. Values between 50 and 100 mIU/mL were considered inconclusive. An exact protective cut-off value for varicella is not known using the described ELISA.

The laboratory analyses were performed at the Programme of Virology, Scientific Institute of Public Health, Belgium.

- *Statistical analyses*

Statistical methodology consisted of three non-linear decay models: exponential decay, generalized exponential decay and a Gompertz model. All three models were fitted assuming a lognormal distribution for the antibody level. We extended the models with covariates: duration of breastfeeding, parity, childcare and the (log) antibody level of the mother at week 36 of pregnancy, for both the measurements at birth and the decay with time. We used the Akaike Information Criterion (AIC) [8] to select the best model, i.e. the model with minimum AIC. For each model, covariates were eliminated according to a stepwise procedure based on the likelihood ratio test. Non-reproducible results are taken into account by left censoring. The *generalized exponential model* fitted best to relate the maternal antibody level of the infant to time and other potentially influential factors (based on AIC) and was selected to analyse the results. Detailed statistical information is provided in appendix. We used SAS® (v9.2, SAS Institute Inc, USA) for the statistical analysis.

Results

1. Demographic results

Initially, 221 women and 227 children participated in the study. Four woman-child pairs were excluded because neither infant samples nor cord samples were available. Another 4 woman-child pairs were excluded because the infant(s) met exclusion criteria. The analysis was performed on data of 213 women and 216 children. During the study period, 16 infants were lost to follow up at different time points, mainly because of the burden of the number of blood drawings. These children were included in the analysis until the moment of dropout (Table 1). At 9 and 12 months of age, results from children who had a proven episode of varicella (N= 17) were excluded from the analysis as well as results from children who received MMR vaccination (N=3).

2. Disease history and serological results of the women

A. Rubella

166 women had written proof of immunization with a rubella (containing) vaccine; 43 women reported a past natural infection of whom 12 women also received a vaccination (documented). Four women did not receive any vaccination nor had the disease. No one received more than one dose of MMR.

Three percent (N=6) of all women had antibody levels below the protective cut-off value by childbearing age: 1 woman reporting a natural infection, 4 with documented vaccination history and one woman with unknown status of vaccination. No significant difference was found in GMT between women who experienced natural rubella infection and women who had been vaccinated (p= 0.8; unpaired student's t-test). GMT for all women against rubella was 25 IU/mL (Table 2). [7]

B. Varicella

Antibodies against varicella were present in 98% of participating women. 11% reported an episode of zoster in the past. No woman reported zoster without having a history of varicella. GMT for all women against varicella was 625 mIU/mL (Table 2). GMT in women who had a history of zoster did not differ significantly from those who did not report shingles (p=0.2; unpaired student's t-test).

3. Serological results of the infants

Antibody levels in cord blood strongly correlated with maternal titers during pregnancy. Table 2 shows GMT at different time points in infants and mothers. At three months of age 66% of the infants had lost maternal antibodies. At the age of 6 months, only 4% of the children still had protective antibody titers against rubella and 12% had positive antibody titers against varicella. At 9 and 12 months none of the children had detectable maternal antibodies for either disease.

4. Model based results

Figures 1 and 2 depict the individual profiles of the antibody level (AL) decay per child for rubella and varicella respectively, according to the generalised exponential model.

For rubella, 50% of the children had lost protection at 2.1 months of age; at 3 months, 69% had lost protection (<10 IU/ml)(Figure 1).

According to the model, 50% of the children had lost varicella protection through maternal antibodies at 2.4 months of age, when assuming 100mIU/mL as the threshold (Figure 2). At three months of age the model predicts 54% of the infants to lose maternal antibodies to varicella. The discrepancy between the result of the

model and the observed proportion positive for varicella antibodies (41%, Table 2) at three months of age, was indicative of the influence of the left censored observations on the overall fit of the model (see appendix).

Several factors were investigated in the model: antibody level in the women at week 36 of pregnancy, duration of pregnancy, parity, breastfeeding, gender, weight at birth and day-care attendance. From these covariates, one was retained: the (log) antibody level of the mother at week 36 of pregnancy was shown to have a statistically significant positive impact on both the measurement at birth and the antibody decay in the infant for rubella and varicella (all p-values < 0.001).

Concerning rubella, the duration of breastfeeding was only a moderately significant variable (p-value 0.0435). However, when removing the factor from the model, AIC increased considerably, which shows the relevance of the covariate.

A fixed effects-analysis for each recruitment center was performed and showed no differences between the 5 recruitment centers.

Discussion

The present paper shows that the prevalence of antibodies against rubella and varicella at childbearing age is high, reflecting possibly a good coverage of the recommended rubella vaccination programme already in the 70ies and 80ies, and high endemicity of chickenpox in the country. Antibodies in neonates at birth strongly correlate with maternal values for both infectious diseases. However, they decline exponentially over time. The model based mean time to immunity loss was 2.1 months for rubella and 2.4 months for varicella. At 9 months of age, none of the infants still had positive antibody titers for either disease.

The added value of the present study is the longitudinal data collection in infants during the first year of life and the substantial number of mother-child pairs.

For *rubella,* the results confirm the trend of shortening protection of infants by passively acquired antibodies. In a study performed in 1979 (USA), 1 in 6 children was still protected at 11.9 ±0.24 months of age against rubella, even hampering immune response to rubella vaccination [9]. De Azevedo (1994, Brazil) [10] described a more rapid decline in maternal antibodies, leaving only 15% of infants seropositive at the age of 6 months. Nicoara et al (1999, Switzerland) [11] found 32% of children seropositive between 3-6 months and 5.2% at 9-12 months . Klinge et al (2000, Germany) [12] reported no seropositive children at 9 months of age and similar humoral MMR vaccine responses in children at 9 or 12 months of age, as a proof of absence of maternal protection. A more recent study of Leineweber in 2004 in Switzerland [13] showed that only 13% of children still had antibodies at the age of 6-9 months.

It is known from literature and practise that children over the age of 6 months become susceptible to *varicella.* Studies in the 1960ies already stated that maternal protection against varicella lasts shortly [14]. A Swiss study (2006) depicted a rapid loss of high levels of maternal antibodies in young children, leaving all children unprotected above the age of 6 months [15]. A recent French publication reported similar high titers of antibodies at birth and only 29.5% of children reached the 100 mIU/ml threshold between 3 and 6 months of age [16]. In the present study, susceptibility of young infants is well illustrated by the 17 varicella cases (7.8%) occurring from the age of 6 months on.

The main aim of rubella vaccination is to prevent CRS. Three percent of participating women in the present study appeared to be susceptible to rubella. The data of the present study are comparable to other European countries (Table 3) [17-25]. Any effort should be made to reach high immunisation coverage rate with rubella vaccine, since even with high coverage there still are susceptible women. However, rubella immunisation has an impact on the serological status of women at childbearing age, as described in an Australian study (1976-2000). An increase of lower titers of protective levels in women at childbearing age was found to be related with higher coverage of vaccination over time, which could be explained by the absence of circulation of natural infection. Although susceptibility remained 2-3%, the increase in low titers is of concern for the amount of passively transferred antibodies [17].
The duration of the presence of rubella antibodies after vaccination and natural infection was studied extensively [26, 27]. Davidkin et al [28] conducted a follow up study after administration of rubella containing vaccine in Finland. One in three children was low protected against rubella after 17 years; they had been vaccinated

with 1 vaccine dose at 12-18 months and 1 dose at 6 years of age. Longer protection was noticed after 2 doses at 6 and 11 years. Johnson et al reported that an early booster might lead to greater susceptibility at childbearing age. Levels of naturally acquired antibodies were more persistent than for vaccine induced antibodies [28]. Vaccine induced rubella antibodies decline over time to below the cut off, but there is evidence that such vaccinees do not develop viraemia when challenged by rubella. This could explain why, despite waning of vaccine induced antibodies, outbreaks are only described in unvaccinated populations [30, 31].

Levels of naturally acquired antibodies are more persistent than for vaccine induced antibodies. Estimated half life after rubella infection is 114 years according to Amanna et al, [27] and repetitive exposures and infections seem not absolutely required for maintaining long-term antiviral antibody responses.

In the present study, GMT does not differ between women who reported a natural infection and those who were vaccinated, but the naturally infected group is rather small.

Implications of the study results

In Europe, the first dose of MMR is administered usually at 12-15 months of age. Based on the presented results and literature, a susceptibility gap exists for rubella between the loss of maternal antibodies and the start of protection offered by vaccination. Most children attend day-care facilities starting from the age of 3- 4 months in Flanders, Belgium, and susceptible infants will match with infectious ones. Earlier vaccination could be considered to close the gap, however, humoral immune responses to live attenuated vaccines in young infants are known to be immature before 6-9 months of age [29, 30]. For measles , the results of this very cohort

showed also a broadening gap of susceptibility [6]. Authors argued to close this gap in case of possible contact with measles (epidemic, travel etc) by immunization with an additional dose at the age of 6 months. The main purpose of rubella vaccination however, is to provide women with rubella immunity lasting over the reproductive age and therefore, an early vaccination against rubella is strictly speaking not a priority. Moreover, rubella cases under the age of 12 months are rarely reported and without major complications [31] . Therefore, we do not recommend an earlier administration of a MMR vaccine for the rubella component, but strongly recommend vaccination of susceptible women, and timely vaccination of all infants, especially with the use of the combination vaccine MMR.

Varicella is a serious infection if acquired during pregnancy and in very young and premature children for whom the disease can be more pronounced [3]. Therefore post-partum vaccination of susceptible women (after screening) is a feasible, utile and even cost-effective measure to take [32] [33]. If universal varicella vaccination would be considered to be implemented, timing of the first dose of a varicella containing vaccine should take into account the here presented early loss of maternally derived protection. Few studies have been conducted with varicella vaccines administered at ages below 12 months. They show that vaccination at the age of 9-10 months elicits similar humoral immune responses compared to vaccination at 12 months of age [34, 35].

Study related remarks

A possible shortcoming of the study is the used ELISA test for varicella antibody testing. This test does not target specifically the envelop glycoproteins and achieves a 100% specificity with a 83% sensitivity after natural infection versus 78% sensitivity

post-vaccination [36]. The gold standard for varicella antibody measurement is FAMA (fluorescent antibody to membrane antigen test). However, since none of the participating women was vaccinated against varicella, the presently used test is supposed to detect sufficiently the naturally derived antibodies.

Finally, 11% of participating women appeared to have experienced a varicella zoster episode in the past. This finding is not surprising and in line with previous published data: in the UK, 10.5% of women have suffered from zoster before the age of 45 years [37] and in the USA 8-10% of women before the age of 40 [38].

Conclusion

Maternal antibodies wane rapidly for both rubella and varicella. One should be aware that children lose passive maternal protection before the age of 6 months in regions with high rubella vaccination coverage. However, immunity of fertile women is most important since CRS is to be prevented in newborns. Therefore, vaccination of susceptible adults and maintenance of high coverage of rubella vaccination programmes should precede. Additionally, the presented results underline the importance of timely administration of MMR vaccines.

In case a country starts a universal varicella vaccination program, the early loss of maternal antibodies should be taken into account. Additionally vaccination of susceptible women at childbearing age is to be recommended.

References:

[1] Vandermeulen C, Mathieu R, Geert LR, Pierre VD, Karel H. Long-term persistence of antibodies after one or two doses of MMR-vaccine. Vaccine 2007 Sep 17;25(37-38):6672-6.

[2] Nardone A, de Ory F, Carton M, Cohen D, van Damme P, Davidkin I, et al. The comparative sero-epidemiology of varicella zoster virus in 11 countries in the European region. Vaccine 2007 Nov 7;25(45):7866-72.

[3] van der Zwet WC, Vandenbroucke-Grauls CMJE, van Elburg RM, Cranendonk A, Zaaijer HL. Neonatal antibody titers against varicella-zoster virus in relation to gestational age, birth weight, and maternal titer. Pediatrics 2002;109(1):79-85.

[4] Glezen WP. Effect of maternal antibodies on the infant immune response 3. Vaccine 2003;21(24):3389-92.

[5] Gans H, DeHovitz R, Forghani B, Beeler J, Maldonado Y, Arvin AM. Measles and mumps vaccination as a model to investigate the developing immune system: passive and active immunity during the first year of life. Vaccine 2003;21(24):3398-405.

[6] Leuridan E. HN, Hutse V., Ieven M., Aerts M., Van Damme P. Early Waning of Maternal Measles Antibodies in the Era of Global Measles Vaccination. BMJ (Clinical research ed 2010(340):c1626.

[7] Skendzel LP. Rubella immunity. Defining the level of protective antibody. Am J Clin Pathol 1996 Aug;106(2):170-4.

[8] Akaike. Information theory and an extension of the maximum likelihood principle. Budapest: Akademia Kiado, 1973.

[9] Sato H, Albrecht P, Reynolds DW, Stagno S, Ennis FA. Transfer of measles, mumps, and rubella antibodies from mother to infant. Its effect on measles, mumps, and rubella immunization. AmJDisChild 1979;133(12):1240-3.

[10] De Azevedo Neto RS, Silveira AS, Nokes DJ, Yang HM, Passos SD, Cardoso MR, et al. Rubella seroepidemiology in a non-immunized population of Sao Paulo State, Brazil. Epidemiology and infection 1994 Aug;113(1):161-73.

[11] Nicoara C, Zach K, Trachsel D, Germann D, Matter L. Decay of passively acquired maternal antibodies against measles, mumps, and rubella viruses. ClinDiagnLab Immunol 1999;6(6):868-71.

[12] Klinge J, Lugauer S, Korn K, Heininger U, Stehr K. Comparison of immunogenicity and reactogenicity of a measles, mumps and rubella (MMR) vaccine in German children vaccinated at 9-11, 12-14 or 15-17 months of age. Vaccine 2000;18(27):3134-40.

[13] Leineweber B, Grote V, Schaad UB, Heininger U. Transplacentally acquired immunoglobulin G antibodies against measles, mumps, rubelia and varicella-zoster virus in preterm and full term newborns. Pediatric Infectious Disease Journal 2004;23(4):361-3.

[14] Brunell PA. Placental transfer of varicella-zoster antibody. Pediatrics 1966 Dec;38(6):1034-8.

[15] Heininger U, Desgrandchamps D, Schaad UB. Seroprevalence of Varicella-Zoster virus IgG antibodies in Swiss children during the first 16 months of age. Vaccine 2006 Apr 12;24(16):3258-60.

[16] Pinquier D, Gagneur A, Balu L, Brissaud O, Gras Le Guen C, Hau-Rainsard I, et al. Prevalence of anti-varicella-zoster virus antibodies in French infants under 15 months of age. Clin Vaccine Immunol 2009 Apr;16(4):484-7.

[17] Francis BH, Thomas AK, McCarty CA. The impact of rubella immunization on the serological status of women of childbearing age: a retrospective longitudinal study in Melbourne, Australia. Am J Public Health 2003 Aug;93(8):1274-6.

[18] Kakoulidou M, Forsgren M, Lewensohn-Fuchs I, Johansen K. Serum levels of rubella-specific antibodies in Swedish women following three decades of vaccination programmes. Vaccine 2009 Nov 29.

[19] Tookey PA, Cortina-Borja M, Peckham CS. Rubella susceptibility among pregnant women in North London, 1996-1999. Journal of public health medicine 2002 Sep;24(3):211-6.

[20] Karakoc GB, Altintas DU, Kilinc B, Karabay A, Mungan NO, Yilmaz M, et al. Seroprevalence of rubella in school girls and pregnant women 71. European Journal of Epidemiology 2002;18(1):81-4.

[21] Wysokinska T, Janaszek W, Bucholc B, Gorska P, Gniadek G, Slusarczyk J, et al. The prevalence of anti-rubella antibodies in women of childbearing age in Poland. Vaccine 2004;22(15-16):1899-902.
[22] Goncalves G, Nascimento MS, Reu C, Cutts FT. Levels of rubella antibody among vaccinated and unvaccinated Portuguese mothers and their newborns. Vaccine 2006 Nov 30;24(49-50):7142-7.
[23] Sasmaz T, Kurt AO, Ozturk C, Bugdayci R, Oner S. Rubella seroprevalence in women in the reproductive period, Mersin, Turkey. Vaccine 2007 Jan 15;25(5):912-7.
[24] Gioula G FA, Exindari M, Atmatzidis G, Chatzidimitriou D, Melidou A, Kuriazopoulou-Dalaina V. Rubella immunity and vaccination coverage of the population of northern Greece in 2006. Eurosurveillance 2007;12(11).
[25] Seneze C, Haus-Cheymol R, Hanslik T. [Rubella seroprevalence in 234 military young women aged between 19 and 31 years]. Presse Med 2008 Dec;37(12):1717-22.
[26] Plotkin S OW, Offit P. Vaccines: Elsevier, 2008.
[27] Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med 2007 Nov 8;357(19):1903-15.
[28] Johnson C, Tookey P. Rubella immunisation: serological status and attitudes of pregnant women. Health Visit 1988 Feb;61(2):43-5.
[29] Gans HA, Lew-Yasukawa L, Beeler J, DeHovitz R, Maldonado Y, Arvin AM. Immune responses of 6, 9 and 12 month old infants immunized with measles or mumps vaccine and the effects of passive antibodies on these responses. Pediatric Research 1999;45(4):161A-A.
[30] Siegrist CA. Vaccination in the neonatal period and early infancy. Intern Rev Immunol 2000;19:195-219.
[31] http://www.euvac.net/graphics/euvac/pdf/rubella_report_2008.pdf, consulted 15 May 2010
[32] Pinot de Moira A, Edmunds WJ, Breuer J. The cost-effectiveness of antenatal varicella screening with post-partum vaccination of susceptibles. Vaccine 2006 Feb 27;24(9):1298-307.
[33] Alfonsi V, Montomoli E, Manini I, Alberini I, Gentile C, Rota MC, et al. Susceptibility to varicella in childbearing age women, Central Italy: is there a need for vaccinating this population group? Vaccine 2007 Aug 10;25(32):6086-8.
[34] Dhillon S, Curran MP. Live attenuated measles, mumps, rubella, and varicella zoster virus vaccine (Priorix-Tetra). Paediatric drugs 2008;10(5):337-47.
[35] Hadinegoro SR, Hindra IS, Han HH, Gatchalian S, Bock HL. Reactogenicity and immunogenicity of a live-attenuated refrigerator-stable varicella vaccine (OKA strain) in healthy seronegative subjects age 10 months to 12 years. The Southeast Asian journal of tropical medicine and public health 2009 Sep;40(5):991-9.
[36] Sauerbrei A, Wutzler P. Serological detection of varicella-zoster virus-specific immunoglobulin G by an enzyme-linked immunosorbent assay using glycoprotein antigen. Journal of clinical microbiology 2006 Sep;44(9):3094-7.
[37] Chapman RS, Cross KW, Fleming DM. The incidence of shingles and its implications for vaccination policy. Vaccine 2003 Jun 2;21(19-20):2541-7.
[38] Insinga RP, Itzler RF, Pellissier JM, Saddier P, Nikas AA. The incidence of herpes zoster in a United States administrative database. Journal of general internal medicine 2005 Aug;20(8):748-53.

Table 1: demographic results and properties of women and infants

*Table2: serological results of both women and infants according to age *positive sample>= 10IU/mL; **positive sample >=100 mIU/mL*

Figure1: Individual profiles for Rubella antibody level (AL) decay (Y axis= antibody level in IU/mL) in infants over time (x-axis= age in months), red line= cut-off at 10 IU/mL, black line= predicted mean curve)

Figure 2: Individual profiles Varicella antibody level (AL) decay (Y axis= antibody level in mIU/mL) in infants over time (x-axis= age in months), red line= cut-off at 100 mIU/mL, black line= predicted mean curve

Table 3: seroprevalence data of rubella antibodies in adult populations across Europe (literature data)