ORIGINAL ARTICLE

Prevalence of anti-Varicella-Zoster virus antibodies in French infants below 15 months of age

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Short title: Prevalence of anti-VZV antibodies in infants

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Abstract

Varicella is a widespread disease of childhood resulting from primary infection with Varicella Zoster Virus (VZV). The objective of this study was to determine the kinetics of decline of maternal varicella antibodies between 0 and 15 months of age in French infants in order to estimate the duration of passively acquired maternal VZV IgG.

This prospective multicentre study was conducted between October 2005 and January 2007 in paediatric wards and/or paediatric emergency units of seven French hospitals scattered throughout the country. The level of anti-VZV IgG antibodies in serum was measured by the Time-Resolved Fluorescence Immunoassay (TRFIA) technique (threshold 150 mIU/ml: considered as protective).

345 infants were included. Seventy-seven percent of mothers reported a history of varicella. A rapid decline of the prevalence of anti-VZV antibodies was observed during the first few months of life with the mean antibody titre decreasing from 536 mIU/mL in the [0-1] month group to below the 150 mIU/mL threshold at already 3-4 months. The half-life of passively acquired maternal immunoglobulins was around 6 weeks.

Based on a large number of subjects, this study clearly demonstrated for the first time in France, high levels of passively acquired maternal antibodies during the neonatal period and allowed to estimate the duration of passively acquired maternal VZV IgG in French infants. After 4-5 months, infants had very low levels of maternal VZV IgG below the negative (150 mIU/ml) cut off of the VZV IgG TRFIA.
Keywords: Varicella, maternal antibodies, kinetics, vaccine

Abbreviations: VZV, varicella zoster virus
Introduction

Varicella (chickenpox) is a widespread disease of childhood resulting from a primary infection with Varicella Zoster Virus (VZV). In France, an age-specific prevalence study reported seroprevalence rates of about 50% by 4 years of age and 90% by 8 years (11). The disease is usually benign but may, however, lead to severe complications and occasionally death (7, 17).

In France, varicella vaccines are available since 2004 but are not yet recommended on routine basis (1).

When mothers experienced varicella or received VZV vaccination, infants are considered protected during the first months of life by passive transfer of anti-VZV maternal antibodies (2). The antibody titre in the newborn was shown to be proportional to the level in the mother (22). However, passive immunity declines rapidly and the exact duration and extent of protection remains uncertain. In other countries, some studies have shown that maternal antibodies were no longer detectable at 6 months (8) or even already at 4 months (19).

The objective of this study was to determine the kinetics of decline of maternal varicella antibodies between 0 and 15 months of age in French infants in order to estimate the duration of passively acquired maternal VZV IgG in French infants. This could be useful, in countries in which routine vaccination is recommended, to assess the optimal age for varicella vaccination in infants.

Material and Methods
This prospective multicentre study was conducted between October 2005 and January 2007 in paediatric wards and/or paediatric emergency units of seven French hospitals scattered throughout the country. Each centre had to consecutively include 6 infants in each of the 9 following age categories: 0-3 months, 4-6 months, 7, 8, 9, 10, 11, 12 months, 13-15 months.

Inclusion criteria

To be included, infants had to be between 0 and 15 months old. They had to be hospitalized or seen in an outpatient department with a scheduled blood sampling. Furthermore, they had to be born after at least 37 gestational weeks with a birth weight of at least 2800g. One or both parents had to sign the written inform consent.

Non-inclusion criteria

Infants with history of varicella or anti-VZV immunization were excluded as well as those reporting contact with a varicella infected subject within 3 weeks before inclusion. Infants with known or suspected immunodeficiency, history of immune globulins- or blood transfusion, or whose mother was transfused during pregnancy were also excluded.

Data collection

For each infant, the following information was collected: recruitment site (paediatric emergency unit, paediatric in- or out-patient department), date of birth, gender, birth weight, gestational age, maternal age, and maternal history of varicella or anti-varicella vaccination.

Antibody level measurement
Blood samples were collected during the infants’ clinical visit or hospitalization. Additional 0.5 ml of blood was collected in a dry tube and centrifuged 10 to 15 min at 3000rpm. After centrifugation, serum was extracted and stored at -20°C. At the end of the inclusion period, all serum samples were centralized at the Virus Reference Department at the Health Protection Agency, London, UK, for assessment of anti-VZV antibody titre. The level of anti-VZV IgG antibodies in serum was measured by the Time-Resolved Fluorescence Immunoassay (TRFIA) technique. The VZV TRFIA is a quantitative VZV IgG assay calibrated against British Standard VZV antibody (15, 16) and VZV IgG levels were interpreted as VZV IgG negative (VZV IgG less than 100 mIU/ml), VZV IgG equivocal (VZV IgG 100 mIU/ml to less than 150 mIU/ml) and VZV IgG protected (VZV IgG 150 mIU/ml or greater). For the calculation of the Mean anti-VZV antibody titres (Geometric Mean Titres, GMT), all results of antibody titres were taken into account.

Statistical analysis

GMT were given for each one-month interval from birth to 15 months of age. The antibody titre was compared according to infant gender, birth weight, gestational age, and maternal previous history of varicella. Qualitative variables were compared using the Chi-2 test and quantitative variables using the Student t-test or the non parametric Cochran Mantel-Haenszel test. A p value below 0.05 was considered as statistically significant.

Sample size estimation was based on the results from Linder et al. (14) who observed that 24% of 2 month-old babies carried anti-VZV maternal antibodies. The minimum sample size to detect a prevalence of 24% with a precision of 5% (alpha=0.05) was 281. This number was increased to 378 to compensate for possible recruitment anomalies, thus representing 54 infants per centre and...
42 for each age category. Statistical analysis was performed by MAPI-NAXIS, Lyon, France using SAS® V8 software.

A birth weight between 2600g and 2800g was considered as a minor protocol deviation and such infants were thus included in the analysis.

Ethical consideration
This study complies with the declaration of Helsinki and written inform consent was obtained from at least one of both parents (or from the infant’s legal representative) and the study protocol was approved by the local ethics committee. According to the French legislation, inform consent from one of both parents is sufficient if the study protocol presents no risk for the child.

Results
Population characteristics
353 infants were initially included. Eight were excluded because of missing information (5 had no information on antibody titre, 2 had received anti-VZV vaccination and one had no case report form. Thus, **345 infants enrolled from paediatric in-patient departments (48%), paediatric emergency units (34%) or paediatric out-patient departments (18%) were analysed.** Median age at inclusion was 8.7 months (range 0.03-15.6). Median gestational age was 39 weeks and median birth weight was 3330g. These two variables did not vary according to age (p=0.71 and 0.67, respectively). Fifty-two percent of infants were males, but this proportion decreased significantly according to age, from 67% in the [0-3] month age category to 36% in the [12-15] month group (p=0.04).
Mothers’ median age was 29 years (range 17-46). Among all mothers, 264/345 (76.5%) reported to have had varicella whereas 45 (13%) did not report to have developed the disease nor received vaccination. Thirty-six women (10.4%) could not provide the information.

Anti-VZV antibody titres

The mean anti-VZV antibody titres according to infants’ age are presented in Figure 1. For each one-month class, the antibody levels are given as geometric mean titres (GMT) together with the 95% confidence interval. A significant difference was observed according to age with the mean antibody titre decreasing from 536 mIU/mL in the [0-1] month group to below the 150 mIU/mL threshold at already 3-4 months (133 mIU/mL, Cochran Mantel Haenszel test gave p<0.001). Above 6 months, the GMT remained almost constant between 18 and 30 mIU/mL. Table 1 gives for each 3-month age category the proportion of infants with an anti-VZV antibody titre above the 150 mIU/mL threshold. This proportion decreased significantly from 83% in infants between 0 and 3 months of age to 29.5% in infants 3 to 6 months old. In the [6-9] month category, only one infant (1.1%) had a GMT above the threshold. In the [9-12] and [12-15] age categories, this proportion increased slightly to 3.5% and 2.2%, respectively.

Looking in more details in the first few months after birth, it appears that this percentage reached 95% in the [0-1] month category, but decreased to 75% in [1-3] months, to 50% in [3-4] months and 0% at 4 months (data not shown). Our data indicate that the half-life time of the anti-VZV antibodies is approximately 6 weeks.

In infants below 6 months of age, the proportion of those with antibody titres above 150 mIU/mL was significantly higher when the mother reported a clinical history of varicella (64.8% versus
33.3%, p=0.038). Conversely, no difference was observed in infants above 6 months of age according to maternal history of varicella (data not shown).

Discussion

Based on a large number of subjects, this study clearly demonstrated high levels of passively acquired maternal antibodies during the neonatal period and allowed us to estimate, for the first time in France, the duration of passively acquired maternal VZV IgG in full term infants. A rapid decline of anti-VZV maternal antibodies was observed during the first few months of life with a substantial decrease already between [0-1] and [1-2] month groups (Figure 1). The percentage of infants with anti-VZV antibody titre above the threshold considered as protective decreased drastically from 83% at [0-3] months to 1% at [6-9] months and after 4 months, most infants seemed to be no longer protected by maternal anti-VZV antibodies (Table 1). These results are in accordance with those reported in the literature (Table 2). It is noteworthy that in the Leineweber study on preterm and full term infants (12), anti-VZV antibody persistence was only tested at 1 to 3 months and 6 to 12 months making it impossible to assess antibody levels between 4 and 6 months. In preterm infants, 56% had antibody persistence at 1-3 months versus 5% only (1/21) at 6-12 months. In full term infants, 13% had antibody persistence at 6-12 months. This decline of maternal antibody titre over the first months of life has been already documented for other infectious diseases (6, 12, 13). The half life of anti-VZV antibodies calculated in our study (6 weeks) is in accordance with results from a previous study reporting a half-life of passively transferred anti-VZV IgG of 45 days (21).

Our study has some limitations. In order to describe the actual maternal antibodies kinetics, it would have been more accurate to follow a cohort of newborn infants up to 15 months of age and
to repeatedly assess the anti-VZV antibody titre along this period. However, repeated blood
sampling in infants could induce ethical consideration and our data provide a reasonable
estimation of the proportion of infants considered as protected by maternal antibodies during the
first months of life. **Nevertheless, there are no reliable correlates of protection provided by**
passively acquired VZV IgG and breakthrough infection can occur. The 150 mIU/mL
threshold for protection used in the VZV TRFIA is based on correlation with the Merck
glycoprotein EIA and recommendations of the USA Advisory Committee on Immunization
Practices (15).

**Although it was shown to be cost-effective** (4, 5), anti-VZV vaccination is not recommended on
routine basis in France. In this context, our study reflects passive transfer of anti-VZV maternal
antibodies produced after a clinical episode of varicella rather than after anti-VZV vaccination.
The absence in our study of mothers with history of varicella vaccination did not allow us to
compare the antibody titre in infants whose mothers had history of varicella infection with that in
infants whose mothers had varicella vaccination only.

In our study, the proportion of infants with anti-VZV antibody titre below the threshold
considered as protective at [0-1] month (5%) suggested that a small part of French mothers in
childbearing age are probably not immunized against varicella and are susceptible to contract
infection during pregnancy or around birth. This is in line with data regarding seroprevalence of
varicella in the French population (11). In another study conducted in France at the end of 2005,
1.2% of 486 included pregnant women were VZV seronegative (20). Congenital varicella
syndrome is rare but the risk is approximately 2% when infection occurs at 13-19 weeks of
gestation. Congenital infection results in a wide clinical spectrum, which may include low birth
weight, and multiple congenital abnormalities leading in a certain number of cases to early death
in the first years of life. Maternal varicella occurring within 5 days before and 2 days after birth is
also associated with severe neonatal varicella with high case fatality for the newborn (18).
Vaccination of women in childbearing age without clinical history of varicella is a way to
decrease the morbidity associated with VZV. Since 2007, the “Haut Conseil de la Santé
Publique” has recommended in France the vaccination of women with no history of varicella
infection and willing to become pregnant (1).

Beside congenital and neonatal VZV, the burden of disease is important in young infants. Data
extracted from the French Medical Information System (PMSI-MCO=Programme de
Médicalisation des Systèmes d’Information Médecine Chirurgie Obstétrique) which covers
all public and private hospitals in the country indicate that 21 179 hospitals stays were
related to varicella between 1997 and 2002 representing an average of 3500 hospitalisations
a year (3). Among all cases, 7058 (33%) had complications and 159 (0.8%) died, 14 of them
before the age of 1 year. More recently, a national surveillance network based on 165
paediatric wards in hospitals located throughout France reported 1575 hospitalisations
related to varicella between March 2003 and July 2005, including 38 (2.4%) requiring
intensive care. This latter survey showed that complications, especially cutaneous
superinfections, were the major reasons for hospitalizations due to VZV infection and that these
complications steadily increased between 3 months and one year of age (9). VZV disease could
be less complicated before 3 months due to protection conferred by maternal VZV antibodies. In
an infant vaccine perspective, precise knowledge of maternal antibodies kinetics is important to
properly estimate the optimal age of vaccination. Indeed, when injecting live attenuated vaccines,
presence of maternal IgG antibodies may neutralize vaccine viruses hereby inhibiting the
vaccine-specific immune response. Therefore, another way to decrease the burden of VZV
disease in infants is to reduce the gap of immunity between the disappearance of transmitted
maternal antibodies and the age of the initiation of active immunization for countries with routine VZV vaccination program.

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References


Table 1: Median anti-VZV antibody titre and proportion of newborn infants with anti-VZV antibody titre above the 150 mIU/mL threshold according to infant age

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>[0-3]</th>
<th>[3-6]</th>
<th>[6-9]</th>
<th>[9-12]</th>
<th>≥12</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>48 (13.9)</td>
<td>44 (12.8)</td>
<td>94 (27.2)</td>
<td>114 (33.0)</td>
<td>45 (13.0)</td>
</tr>
<tr>
<td>Mean anti-VZV antibody titre (mIU/mL) [95% CI]</td>
<td>333.8 [253-440]</td>
<td>83.6 [63.4-110]</td>
<td>25.9 [22.5-29.8]</td>
<td>21.6 [19.1-24.5]</td>
<td>22.1 [16.9-28.7]</td>
</tr>
<tr>
<td>% of infants with antibody titre ≥ 150 mIU/mL [95%CI]</td>
<td>83.3 [69.8-92.5]</td>
<td>29.5 [16.8-45.2]</td>
<td>1.1 [0-5.8]</td>
<td>3.5 [1.0-8.7]</td>
<td>2.2 [0.1-11.8]</td>
</tr>
</tbody>
</table>
Table 2: Different studies having reported the anti-VZV maternal antibodies decay and the age at anti-VZV antibodies disappearance in the infant

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study period</th>
<th>Birth</th>
<th>Number of infants</th>
<th>Assay used to measure anti-VZV Ab</th>
<th>Age at anti-VZV Ab disappearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>France</td>
<td>2005-2006</td>
<td>Full term</td>
<td>345</td>
<td>TRFIA</td>
<td>4 months</td>
</tr>
<tr>
<td>Osaki (19)</td>
<td>Japan</td>
<td>1980</td>
<td>Full term</td>
<td>24</td>
<td>NT</td>
<td>4 months</td>
</tr>
<tr>
<td>Heininger (10)</td>
<td>Switzerland</td>
<td>1994-1999</td>
<td>Full term</td>
<td>240</td>
<td>ELISA</td>
<td>4-6 months</td>
</tr>
<tr>
<td>Linder (14)</td>
<td>Israel</td>
<td>1997</td>
<td>Preterm</td>
<td>120</td>
<td>IFAMA</td>
<td>2-6 months</td>
</tr>
<tr>
<td>Leineweber (12)</td>
<td>Switzerland</td>
<td>1999-2000</td>
<td>Preterm</td>
<td>66</td>
<td>ELISA</td>
<td>&lt; 6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td>&gt; 6 months</td>
</tr>
<tr>
<td>Van der Zwet (22)</td>
<td>Netherlands</td>
<td>1995-2000</td>
<td>Preterm</td>
<td>27</td>
<td>VIDAS</td>
<td>3 months</td>
</tr>
</tbody>
</table>

Figure 1: Anti-VZV antibody titres in 345 French infants between 0 and 15 months of age. Antibody levels are given as Geometric Mean Titres (GMT). Vertical bars indicate 95% confidence intervals.
Infant age (months) vs. Anti-VZV antibody titre (mIU/mL)