Search of antimeasles antibodies in HIV-infected children after basic immunization

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Abstract

Objective: to determine the presence of antimeasles antibodies in children perinatally infected with HIV and properly immunized.

Methods: a retrospective cohort study conducted in Belo Horizonte by the Universidade Federal de Minas Gerais, between 1995 and 1996. Twenty one children perinatally infected with HIV and 29 immunocompetent noninfected children were included in the study. Information about measles vaccination was obtained from patients’ immunization charts. The presence of neutralizing antibodies against the measles was determined by the plaque reduction neutralization test and IgM was measured by ELISA. The level of significance was set at 5% in all the performed statistical analyses.

Results: median age was 44.5 months for HIV-infected patients and 62.0 months for noninfected children (P=0.64). Both groups received on average two doses of antimeasles vaccine. All HIV-seronegative patients presented antimeasles antibody titers greater than 50 mIU/ml, whereas 57.1% of infected children presented titers above this value (P=0.0001). The geometric mean titer of neutralizing antibodies was significantly lower in the group of HIV-infected children (433.5 mIU/ml) than in noninfected children (1,668.1 mIU/ml), P=0.001. All patients in both groups were negative for antimeasles IgM.

Conclusion: in the present study, HIV-infected children showed a lower seroprevalence of antimeasles antibody after immunization than noninfected children. These results emphasize the risk of acquisition of measles virus and the need to evaluate alternatives to the vaccination of HIV-infected children in an attempt to maximize the protection against the measles in this group of patients.


Introduction

The vaccine against measles is made up of live, attenuated virus and is thus contraindicated for immunodepressed patients considering the risk for replication of the virus. However, the severity of measles in children infected by the human immunodeficiency virus (HIV) resulted in the routine indication of measles vaccine for these patients. According to the recommendations of the Advisory Committee in Immunization Practices (ACIP), the use of live, attenuated virus vaccines against measles is contraindicated only in...
children presenting severe immunologic involvement; more specifically, presenting a CD4 count lower than 15.00% of total lymphocytes. Between 1989 and 1991, there were 19 fatal cases of measles in the United States, out of which 11 were HIV-infected children. In Zairian hospitals, the mortality rate due to measles in HIV-infected children can be as high as 40.00%.²

Up until 1996, there were no studies reporting increased side-effects of measles vaccination in HIV-infected patients.³⁻⁸ The report of a fatal case of measles pneumonitis caused by the vaccinal strain of the measles virus renewed the discussion on the risks of measles vaccination for HIV seropositive patients.⁹ Others reported that the vaccine virus was not found in cultures of mononuclear and polymorphonuclear peripheral blood cells or in serum of HIV-infected patients immunized with the measles, mumps, and rubella (MMR) vaccine. These patients also did not present increased levels of p24 antigen after the immunization, which suggests that there was no stimulation of HIV replication as a result of the use of the MMR vaccine.¹⁰

The immunologic response to the vaccine can be incomplete and transient, and thus it is recommended that specific immunoglobulins be administered to children exposed to measles and with symptomatic HIV infection, even if they were previously submitted to proper vaccination.¹¹⁻¹²

The transfer of maternal antibodies can be seriously affected by the presence of HIV infection. Other authors have suggested that children born to HIV-infected mothers be submitted to measles serology as soon as possible; in this sense, the vaccine against measles should be anticipated in children who receive poor transfer of maternal antibodies.¹² The same authors also suggested that HIV-infected children be immunized against measles as early as possible, due to the transient characteristic of the acquired immunity transmitted by the mother and in order to avoid any immune damage.²⁻¹²

Currently, the study of the protection against measles following active immunization of children with perinatally acquired HIV infection is invested with more importance due to the reappearance of measles in Brazil and in other countries worldwide. Routine vaccination and mass vaccination campaigns caused the Brazilian measles incidence rates to drop significantly to 0.05 cases per 100,000 inhabitants in 1995. However, since the 1992 campaign, only 32.00% of Brazilian cities have maintained an adequate routine vaccination coverage of children aged less than one year. Taking into account the 5.00% rate of failure in the first vaccination, the country has accumulated an approximate total of four million children susceptible to measles in the past few years. As a result, there have been outbreaks of the disease in 1996 and 1997. According to the Brazilian Ministry of Health, this epidemiological situation can only be solved with more intense mass vaccination campaigns, with high vaccination coverages, and with the identification of susceptible areas within different communities — in which case specific control strategies should be carried out.¹³⁻¹⁴

We designed this study with the objective of verifying the presence of measles antibodies in children with perinatally acquired HIV and properly immunized. The study of the seroprevalence of measles antibodies can help to design a more adequate immunization routine for HIV-infected children, in which the immunologic potentials are maximized either by the use of new immunobiological products or by the modification of the basic routine (decreasing age range for administration of the vaccines, administering additional dosages, or, still, avoiding the rapid appearance of a clinical status of immunodepression).

**Patients and methods**

**Groups**

From August 1995 to August 1996 we selected a total of 50 HIV-infected and noninfected patients who were divided into two study groups. The group of perinatal HIV infection included 21 patients followed-up at the Training and Reference Center Orestes Diniz (TRC). The second group of patients included 29 children who were being treated in different outpatient settings of the Hospital das Clínicas, teaching hospital of the Universidade Federal de Minas Gerais (HC-UFMG), and who fulfilled the study criteria. We carried out a retrospective, or historical, cohort study in which immunization against measles was the central element in the constitution of the cohort; we defined exposure according to HIV infection and the event measured was the presence of measles antibodies.

The group of HIV-infected children included only patients who were infected perinatally; more specifically, children born to mothers diagnosed with HIV infection or whose mother died of pathologies suggestive of HIV infection and with no report of other possible source of the transmission of HIV. The group of seropositives included patients greater than 15 months and with at least two enzyme linked immunosorbent assays (ELISA) positive for HIV, carried out at different times, and with at least one positive Western-Blot test; these tests were carried out before the study in routine follow-up procedures at the TRC.

Children in the control group (seronegatives) were outpatients of several different pediatric departments of the HC-UFMG, who were selected by our team of researchers when they presented at the central laboratory for the collection of blood. The criteria for inclusion in the control group were: patients greater than or equal to 15 months; no pathologies, conditions or even medications that may affect the immune response of patients (particularly anemia, malnutrition, repeated infections, neoplasias, use of corticoids or chemotherapeutics, and blood transfusion);
normal for age results of laboratory tests on immune response (complete hemogram, differential white blood cell count for CD4 and CD8, serum immunoglobulin, and beta2-microglobulin); serology negative for HIV infection; and consent of parents or guardians.

We carried out clinical screening of patients by interviewing the parents at the laboratory and collecting an additional blood sample for the examinations above in addition to the samples collected according to the routine of the services. We evaluated the vaccination of patients according to their immunization records, which also provided information regarding dosage of vaccines and the date of last immunization. The immunization records were also used as a criterion for inclusion in the study of patients of both groups.

We also established the criterion that patients of both groups had to have had two doses of vaccine against measles, or at least one vaccine before the first year of life (though the Ministry of Health - National Immunization Program determines that the second dose of the vaccine is mandatory for the conclusion of the basic vaccination scheme). In order to count the dosages administered, we computed the administration of MMR vaccines as measles vaccines. Children were immunized during routine protocols at public healthcare services or private medical offices prior to this study and without any interference by the authors.

None of the controls presented positive ELISA for HIV infection; only one patient was excluded for presenting CD4 count below normal for age and was later indicated to the TRC Orestes Diniz for investigation of immunodeficiency.

Informed consent from parents or guardians of patients was established as a criterion for inclusion in the study; the parents or guardians signed the informed consent forms prior to the collection of blood. The informed consent form and the protocol for collection of data were designed specifically for this investigation and were approved by both the Pediatrics Department of the School of Medicine at the Universidade Federal de Minas Gerais, and the Technical and Scientific Committee of the HC-UFMG.

**Measles serology**

Blood samples were centrifuged immediately after collection at the central laboratory of the HC-UFMG; serum samples were frozen at -20 °C so that all samples could be tested at the same time. The technicians who carried out the examinations at the laboratory of virological technology Biomanguinhos and at the laboratory of respiratory viruses of the FIOCRUZ, state or Rio de Janeiro, were blinded for samples being from infected or noninfected patients. Serum samples were labeled only with the patient’s initials and with the patient’s number in the study.

Plaque reduction neutralization titers was carried out according to the procedures described by Whitle et al.16 Samples were considered positive when presenting titers higher than 50 mIU/mL. The plaque reduction neutralization titers is especially useful for the examination of persistence of transplacental antibodies and of duration of immunity in individuals who were administered the vaccine, in other words, of susceptibility to the measles virus. The presence of specific IgM antibodies against measles was verified by human antibody-based capture enzyme immunoassay according to the protocol standardized by the Centers for Disease Control and Prevention (CDC), which is considered the gold standard for the diagnosis of recent infection by the measles virus.17,18

**Statistical analysis**

We employed Fisher’s test based on analysis of variance (ANOVA) for the comparison of the arithmetic averages; when necessary, we employed nonparametric methods for the comparison of medians (Kruskall-Wallis). The chi-squared test was used in the comparison of frequencies. When the chi-squared test was not adequate, we employed Fisher’s exact test for the analysis of results.19 Statistical analyses were processed using the EPI-Info version 6.04b. Comparison of titers of measles antibodies was carried out based on the geometric averages of the titers of individuals whose values were within the limits of detection by the laboratory method employed. Student’s t test was used in the comparison of geometric mean titers (GMT). We adopted a 5.00% significance level for all the statistical analyses. The size of the group of noninfected children (controls) was calculated in order to ensure at least an 80.00% statistical power in the comparison of titers of measles antibodies; in this sense, we estimated a relative risk of less than 0.5; in other words, we supposed that the seroprevalence of measles antibodies in infected patients would be approximately 50.00% lower than that estimated for the noninfected patients.

**Results**

**General characteristics of groups**

The symptoms of HIV infection were classified as mild or moderate in approximately 73.00% of patients, according to criteria of the classification system for human immunodeficiency virus infection in children less than 13 years of age.20 In turn, by taking immunosuppression alone into consideration (measured by the relative CD4 lymphocyte count) we verified that 90.90% of children presented moderate or severe immunosuppression (categories 2 and 3 in the classification system referred above). The use of antiretroviral drugs (AZT or DDI) was observed in 54.50% of patients at the moment of blood collection for serology. Only 27.30% of patients were being administered intravenous immunoglobulin, either simultaneously or not with antiretroviral drugs. Blood samples were collected
within a minimum interval of three weeks after administration of IV immunoglobulin in these patients. Table 1 shows the distribution of the 21 infected patients in relation to clinical and laboratory classification, to the use of antiretroviral drugs, IV immunoglobulin, and titers of measles antibodies. The small number of patients in this category rendered the results inconclusive (statistical power of less than 40.00%).

The age of children seropositive for HIV ranged from 15 months to 10 years, and of children in the control group, from 19 months to 13 years. Table 2 shows that the age median for the group of HIV-infected patients was 44.5 months, and for the group of noninfected patients, 62.0 months; this difference was not statistically significant (P = 0.64; Kruskall–Wallis).

The noninfected patients were selected out of several different departments of the Bias Fortes outpatient clinic of the HC-UFMG. Patients had been indicated for collection of blood for preoperative laboratory exams of elective surgeries in 51.61% of cases.

Table 1 - Distribution of HIV-infected and uninfected patients, according to neutralizing titers of measles, clinical and laboratory classification, use of antiretroviral drugs and intravenous immunoglobulin

<table>
<thead>
<tr>
<th>Neutralizing titer</th>
<th>Yes</th>
<th>No</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDC classification/94</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (1, 2 and 3)</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>B (1, 2 and 3)</td>
<td>5</td>
<td>5</td>
<td>*</td>
</tr>
<tr>
<td>C (2 and 3)</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Antiretroviral drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT/ DDI</td>
<td>6</td>
<td>5</td>
<td>1.00 †</td>
</tr>
<tr>
<td>Do not use</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>IV immunoglobulin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>2</td>
<td>1.00 †</td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square test is not appropriate: more than 20% of cases presented an expected value less than 5.
† Fisher’s exact test

Measles serology

The median for number of doses and for the interval since last measles vaccination were statistically similar between both groups (P = 0.062 and P = 0.98, respectively), as shown in Table 2. The two groups were also statistically similar in the stratification of patients according to the categories of dosage and intervals (P = 0.12 and P = 0.49, respectively). One control and five HIV-infected children had received only one dosage of the vaccine; all six of these patients were older than one year at the moment of the immunization against measles.

The GMT (Table 2) of antibodies against measles was significantly higher in the group of noninfected patients (P = 0.001). The calculation of the GMT included only the children whose titers were within the limits of detection by the method employed (50.0 to 6309.5 mIU/ml). Figure 1 shows the distribution of the 50 patients according to titers against measles and infection by the HIV. The figure shows that 90.00% of HIV-infected patients presented titers of antibodies lower than 1,000 mIU/ml; whereas the noninfected patients are heterogeneously distributed with a tendency to present higher values.

Table 2 - Distribution of 50 patients, according to HIV infection, age, number of doses and interval since last vaccination against measles and antibody titers against measles

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Infected (n=21)</th>
<th>Uninfected (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>57.6 ± 30.5</td>
<td>64.6 ± 36.1</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>44.5</td>
<td>62.0</td>
<td>0.64 †</td>
</tr>
<tr>
<td><strong>Interval in months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>29.4 ± 31.9</td>
<td>25.2 ± 18.3</td>
<td>0</td>
</tr>
<tr>
<td>Median</td>
<td>17.0</td>
<td>16.0</td>
<td>0.98 ‡</td>
</tr>
<tr>
<td><strong>Number of doses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>1.9 ± 0.7</td>
<td>2.3 ± 0.7</td>
<td>0.062 §</td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td><strong>GMT †</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mIU/ml) (n=12)</td>
<td>433.5</td>
<td>1,668.1</td>
<td>0.001 ‡</td>
</tr>
<tr>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Time, in months, between last vaccination against measles and blood sampling for serological tests.
† Geometric mean titer of patients with neutralizing antibody titers between 50 and 6,309.5 mIU/ml.
§ Kruskal–Wallis.
‡ Five patients with >6,309.5 mIU/ml were excluded.
† Student t test.
Search of antimeasles antibodies ... - Lindgren-Alves CR, et alii

Figure 1 - Distribution of HIV-infected and uninfected patients, according to neutralizing antibody titers against measles

(P = 0.55; Kruskall Wallis). None of the children in our population (infected or not) presented positive response to IgM antibodies against measles on ELISA; patients were thus considered seronegative for this immunoglobulin.

Discussion

Our study was designed as a retrospective, or historical, cohort study in which the central variable (vaccination) was not controlled by the researchers. In this sense, the researchers selected the children with perinatally acquired HIV infection who were being followed-up at the TRC Orestes Diniz; due to operational reasons, it was impossible to prospectively follow-up a cohort of patients from birth in order to correctly indicate, administer, and record their vaccination.

All noninfected children presented titers of antibodies higher than 50 mIU/ml, which is the lower limit of detection by the method employed. In the case of HIV-infected patients, 12 (57.1%) out of the 21 children presented titers higher than 50 mIU/ml. The serum neutralizing antibody titers were not influenced by the number of dosages or by the interval since the last vaccination and blood collection, as shown in Tables 2 and 3. The prevalence of seropositive measles patients in our population was comparable to that reported in the literature, in which the results range from 50.00% to 59.40% in studies that applied similar methods.

Despite the fact that the presence of any value of detectable measles antibody titers can be considered protective, the data available in the literature suggest that people with very low titers may not be completely protected. Measles itself or without exanthema can occur in individuals with serum neutralizing antibody titers lower than 120 mIU/ml.

Peter, in a review article, described that 5.00% or less of children adequately immunized failed to respond to vaccination against measles. According to Krugman, approximately 15.00% of children adequately immunized can present undetectable titers of hemagglutination-inhibiting (HAI) antibody, and reimmunization of these children induced a classic booster response. However, Markowitz et al. reported the existence of secondary failure in the response to measles vaccines, which is described by the loss of antibodies after seroconversion. The magnitude and epidemiological relevance of this response, however, still require further studies. Despite these findings, the prevalence of HIV-infected children with titers higher than 50 mIU/ml was significantly lower than that of controls. This finding certainly is not simply a result of primary or secondary failure, or of fall in titers of antibodies, but rather of the action of the HIV on the several mechanisms of immune response.

In our study, the GMT of patients with serum neutralizing antibody titers higher than 50 mIU/ml was also significantly lower in children seropositive for the HIV (433.5 x 1668.1 mIU/ml), which can be attributed to the decreased production of antibodies as a response to the vaccine, to the more accelerated loss of antibodies produced, or both. Similar results were described in the literature, but the authors were also not able to describe the baseline immune mechanism.

The low prevalence of measles antibodies in HIV-infected children corroborates the concern with measles outbreaks in susceptible individuals due to primary or

Table 3 - Distribution of 50 patients, according to antibody titers against measles, HIV infection, number of doses and interval since last vaccination against measles

<table>
<thead>
<tr>
<th>Antibody titers against measles</th>
<th>Infected (n=21)</th>
<th>Uninfected (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 50 mIU/ml</td>
<td>12</td>
<td>29</td>
<td>0.0001*</td>
</tr>
<tr>
<td>&lt; 50 mIU/ml</td>
<td>9</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of doses</th>
<th>Mean±standard deviation</th>
<th>Median</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.2±0.7</td>
<td>2.0</td>
<td>0.19†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interval in months</th>
<th>Mean±standard deviation</th>
<th>Median</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.3±25.4</td>
<td>16.0</td>
<td>0.45†</td>
</tr>
</tbody>
</table>

* Fisher’s exact test
† Kruskal-Wallis
secondary failure of the immunization. In these cases, even immunized patients are capable of transmitting the wild-type virus. These situations may compromise the attempts to eliminate measles due to the highly transmissible characteristic of the wild-type virus even though the number of susceptible individuals may be relatively low.

We concluded that despite the fact that the children with perinatally acquired HIV infection were adequately immunized against measles, these patients presented a lower prevalence of antibodies against measles and a decreased magnitude of immune response, when compared to children of similar age groups and without involvement of the immune system.

Our findings indicate the need for an improved monitoring of children who were perinatally infected with the HIV virus due to the risk for measles. Special measures may involve alterations in the vaccination calendar, such as anticipation of the first dose of the vaccine against measles and/or introduction of an additional reinforcement; use of more immunogenic products; and, finally, prevention of the perinatal transmission of the HIV and reduction in immunologic damage by systematic administration of antiretrovirals during pregnancy, delivery, and the neonatal period.

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References


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