Clinical aspects and complete blood counts in children exposed to HIV-1: comparison between infected patients and seroreverters

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Abstract

Objective: to analyze the evolution of clinical and hematological aspects of children exposed to the vertical transmission of HIV-1, comparing infected patients with uninfected ones or seroreverters.

Methods: prospective, descriptive, longitudinal study. We analyzed 79 children born from HIV-1 infected mothers, under clinical follow up from March, 1996 until November, 1997, at the Immunodeficiency division of the Hospital de Clínicas da Unicamp (State University Hospital of Campinas).

Results: failure to thrive was observed in both groups, but was greater among seroreverters. Among the infected children, 23 mothers did not use AZT during pregnancy, 16 of them (61.5%) had been breastfed, four were classified into clinical category N, seven into A and fifteen into B. Clinical manifestations in patients younger than one year were seen in 18 infected children (69.2%). Anemia was observed in 73.1% of the infected group and in 41.5% of the seroreverters (P<0.008). The comparison between the groups showed that the most common hematologic alterations in the infected children was microcytosis and hypochromia (P<0.05), lymphopenia between 15 and 18 months (P<0.05), monocytosis between 9 and 12 months (P<0.05) and a tendency towards high ferritin levels, with no statistical significance.

Conclusions: microcytic and hypochromic anemia were observed in both groups: iron deficiency in the uninfected children, and chronic disease anemia in the infected ones. The infected children presented with monocytosis and lymphopenia at an earlier stage.


Introduction

In Latin America and the Caribbean, the Acquired Immunodeficiency Syndrome, or AIDS, has spread more intensely among women and, consequently, there is an increased the rate of vertical transmission. In Brazil, between 1998 and 1999, 90.2% of the cases of AIDS in children aged less than 13 years were a result of vertical transmission.1 A study carried out in the state of São Paulo indicated that the rate of vertical transmission of the human immunodeficiency virus (HIV) was 16.0% and, in the population studied, there were 12.0% of mothers with advanced-stage disease and 39.0% of children being breastfed.2

The HIV type 1, or HIV-1, can be transmitted in utero, during labor, and postpartum through breastfeeding. Early diagnosis of the infection can be obtained by Protein Chain Reaction (PCR) of DNA from cord or peripheral blood lymphocytes in up to 30.0% of infected newborns, thus suggesting infection in utero.3 Infected newborn infants who were not breastfed (over 75.0%) were reported PCR-DNA negative at birth and, after two to four weeks of life, these infants were found infected suggesting transmission of the HIV-1 at delivery.4

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The infection by the HIV-1 and the resulting immunodeficiency syndrome can determine significant hematologic alterations in children not only during the advanced stage, but also during the stage of clinical latency and onset of symptoms. Hematologic abnormalities can be attributed to direct and indirect effects of the HIV-1 infection, to opportunistic infections, and to toxicity of the therapeutic agents.

In adults, during the primary infection stage, there may be symptoms of lymphopenia, followed by lymphocytosis and atypical lymphocytes, neutropenia, thrombocytopenia, and transient pancytopenia. During the asymptomatic stage, there is a gradual decrease in the CD(4+) T cell count, which initially may be masked by lymphocytosis due to an increased CD8(+) T cell count. Upon diagnosis of AIDS, patients present lymphopenia and, usually, pancytopenia. Anemia is usually normochytic normochromic; however, at times, it can be macrocytic even in the absence of treatment with AZT (zidovudine). Moreover, occasionally there may be cases of microangiopathic hemolytic anemia or of thrombotic thrombocytopenic purpura.6

The immune system can operate both as a substratum for HIV-1 replication and for defense. Consequently, the differences between the immune system of an adult host and that of a child host (which is still developing) contribute to the complex particularities of the pathogenesis of HIV infection in children.

The quantitative and qualitative laboratory standards, the clinical symptoms, the opportunistic infections, and the neoplasias caused by AIDS in pediatric patients differ from those in adult patients.

Possibly, the abnormalities such as anemia, lymphopenia, leukopenia, neutropenia, hyposegmentation of neutrophils, alterations in monocytes, thrombocytopenia, and myelodysplasia, previously described by Spivak et al., represent early and persistent abnormalities in HIV-1 vertically infected children. It is also possible that multiple factors contribute to the differences in fetal and newborn infant cell infection and ability to endure viral replication.

In this sense, our objective was to assess clinical and hematological characteristics (especially hemogram and serum ferritin) in children vertically exposed to the HIV-1 infection in children.

Patients and methods

We carried out a prospective longitudinal descriptive study. For the definition of HIV-1 infection and clinical classification of patients, we followed the criteria established by the Centers for Disease Control (CDC), and by the Brazilian Ministry of Health (National Revision and Definition of Cases of AIDS in Children). Patients who remained asymptomatic were classified as N, patients with mild symptoms as A, moderate symptoms as B, and severe symptoms as C. The division of these categories in subitems from 1 to 3 were used to describe immunological category. The total and relative CD(4+) T cell count according to age was used in this assessment. The category 1 identifies a relatively intact immune system; category 2, mild (fall in CD4(+) T cells < 20.0%); and category 3, severe immunosuppression (fall in CD4(+) T cells > 20.0%).

We examined 79 children who were being followed-up at the Immunodeficiency Outpatient Clinic of the Hospital de Clínicas, teaching hospital of the Universidade de Campinas (Unicamp); children were of both sexes and their mothers had confirmed HIV-1 infection during gestation. The follow-up period of the study was carried out from March 1996 to November 1997, and the population sample was divided into two groups: HIV-1 infected children with clinical symptoms and positive laboratory exams confirming the infection, and seroreverters children (noninfected).

In addition to the ELISA and Western-Blot, we also carried out PCR-DNA for diagnosis of HIV-1 infection.

The clinical and laboratory protocols of each patient were carried out within three-month intervals until the age of 18 months; subsequently, the protocols were carried out in six-month intervals. We also included children who had started treatment with antiretrovirals during the evolution of the infection. Our study protocol was fully approved by the ethics committee of the Hospital das Clínicas, Universidade de Campinas. Parents or guardians of patients were informed about our study and signed an informed consent form.

The anthropometric measures of patients were collected at the first outpatient appointment. Anthropometric data were analyzed according to Z scores (two standard deviations below the average) using the Siscres W system for analysis of anthropometric data (version 1.0) developed by Morcilly et al., which employs National Center for Health Statistics (NCHS) parameters.

Exam techniques and their respective reference values were the same reported by Silva et al.,5 The Pediatric AIDS Clinical Trials Group (PACTG) Protocol 076 consists in continuous administration of oral zidovudine between the 14th and 34th weeks of gestation, with subsequent administration of intravenous AZT at delivery and oral AZT for the newborn infant during the first six weeks of life, even without confirmation of diagnosis. The PACTG protocol also includes the recommendation to avoid breastfeeding.13

We implemented a data base using the EPI-Info software version 6.04-b. Statistical analyses were carried out using the Statistical Analysis System (SAS). Association between variables was verified using the chi-squared test; when this test was not appropriate, we applied Fisher’s exact test.

We applied a significance level of alpha < 5.0% for the elimination of the null hypothesis.
Results

Out of the total population of 79 children (26 HIV-infected and 53 seroreverters), there were 38 (48.1%) boys and 41 (51.9%) girls. Table 1 shows the characteristics of both groups according to sex, breastfeeding, administration of AZT during gestation (oral only), and medications used. Only one mother in the group of seroreverters was administered intravenous AZT during delivery, considering that until October 1997 the PACTG 076 had not been fully implemented at the obstetrics department at our services.

The age at beginning of follow-up ranged from 20 days to seven years and nine months and, at the end of the study, from eight months and 15 days to eight years and four months. The age median was two years and two months for infected children and nine months for noninfected, with age averages of, respectively, two years and seven months and one year and three months.

The initial clinical and immunological assessment indicated four N patients, out of which two were N1; seven A patients, out of which one A1, five A2, and one A3; and 15 B patients, out of which four B1, three B2, and eight B3.

Anthropometric examination and clinical symptoms

In the group of seroreverters, seven (13.2%) children presented weight Z score, and 11 (20.7%) height Z score, less than two standard deviations. During the first year of life, we observed the following characteristics in 30 of these children: anemic syndrome (n=8; 26.6%); low weight gain (n=5; 16.7%); asthmatiform syndrome with diagnosis of gastroesophageal reflux (n=3; 10.0%); allergy, nonspecific dermatitis, acute otitis media, upper airway infection (n=2; 6.7%). Other diagnosis such as protein-calorie malnutrition, scabies, oral or perineal candidiasis, tuberculosis, pneumonia, sinusitis, congenital cytomegalovirus (CMV) infection, toxoplasmosis, rubella, syphilis, and thrombocytopenia count were also observed.

In the group of infected children, only two (7.7%) presented weight and height Z scores less than two standard deviations for age. Symptoms of the disease were identified in 18 (69.2%) patients before age one year, and in seven after age one year; symptoms were not reported in one hospitalized children. The most frequent diagnoses were: lymphoproliferative syndrome (n=7; 38.9%); chronic pneumopathy (n=3; 16.6%); hepatosplenomegaly and anemic syndrome (n=2; 11.1%); strophulus, oral candidiasis, repeated pneumonia, lymphoid interstitial pneumonitis, splenomegaly, acute diarrhea, congenital CMV infection, and others.

Comparative analysis of red blood cell exams according to age

The periodicity of exams was not always ideal since patients occasionally missed appointments and collection of laboratory samples. We analyzed a total of 345 hemograms. For the statistical analysis, we selected only one exam according to each age range; consequently, the number of hemograms analyzed was reduced to 193.

Figures 1, 2, and 3 show the prevalence of anemia in HIV-1 infected patients and seroreverters; these figures also show the evolution of microcytosis, hypochromia, and macrocytosis. Anemia was more frequent in infected patients aged nine to 12 months (P<0.05); its incidence varied from 17.0 to 60.0% up to 30 months of age. In children aged 30 to 36 months, anemia affected 100.0% of infected patients (P<0.05). In subsequent ages, the incidence of anemia was higher in infected children, with the exception of ages four years and five years and six months. Both groups presented similar microcytosis up to age 48 months. Hypochromia, in turn, was more prevalent between 30 to 36-month old infected children (P<0.05). Macrocytosis was observed in 100.0% of children infected and aged zero to three months, and in five (56.0%) of noninfected children. The mothers of these children were administered AZT during gestation and the babies, during six weeks. Three were also administered SMX-TMP and one, isoniazide. Macrocytosis was again detected between nine and 12 months of age in 33.0% of infected patients; it was not, however, detected in seroreverters (P<0.05).

In sum, anemia was diagnosed in 73.1% of infected children and in 41.5% of seroreverters; this difference was statistically significant (P=0.008), but there was no association with immunological categories.

Table 1 - Characteristics of patients exposed to vertical transmission of HIV-1, infected and seroreverters, and prescribed treatment

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Infected n=26</th>
<th>Serorreverters n=53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>17 (65.4%)</td>
<td>24 (45.3%)</td>
</tr>
<tr>
<td>male</td>
<td>9 (34.6%)</td>
<td>29 (54.7%)</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>16 (61.5%)</td>
<td>8 (15.1%)</td>
</tr>
<tr>
<td>no</td>
<td>10 (38.5%)</td>
<td>45 (84.9%)</td>
</tr>
<tr>
<td>Use of AZT during gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>3 (11.5%)</td>
<td>10 (18.9%)</td>
</tr>
<tr>
<td>no</td>
<td>23 (88.5%)</td>
<td>43 (81.1%)</td>
</tr>
<tr>
<td>Zidovudine (AZT)</td>
<td>19 (73.1%)</td>
<td>10 (18.9%)</td>
</tr>
<tr>
<td>Didanosine (DDI)</td>
<td>17 (65.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SMX-TMP</td>
<td>23 (88.5%)</td>
<td>36 (67.8%)</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>13 (50.0%)</td>
<td>20 (37.7%)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>9 (34.6%)</td>
<td>6 (11.3%)</td>
</tr>
</tbody>
</table>

n = total of patients
Comparative analysis of white blood cell exams

Figures 4, 5, and 6 show the leukocyte, neutrophils, and lymphocyte counts.

In the group of seroreverters, we observed leukocytosis from ages six to 15 months; leukopenia after age six months; neutropenia, lymphopenia, and atypical lymphocytes (low prevalence) up to age 18 months. Atypical lymphocytes were observed in a total 33.0% of patient between ages six to nine months. Considering the totality of exams carried out, 22.6% of seroreverters presented lymphopenia. Eosinophilia was identified in one patient before the age of three months, in another before age six months, and in five before age four years. Monocytosis was detected in 56.0% of children before age three months, in 26.0% to 28.0% of children aged up to 12 months, and remained between 20.0% to 40.0% in children aged up to 42 months.

In the group of HIV-1 infected children, there were no cases of leukocytosis. Leukopenia presented a low incidence...
up to age 18 months, which remained low after age 48 months. Atypical lymphocytes were significantly more prevalent in the group of infected patients (83.0%) in comparison to that of seroreverters (33.0%) (P<0.05) aged six to nine months. We identified a prevalence of lymphopenia in infected patients after age 12 months, with the incidence increasing to 75.0% between ages 15 to 18 months (P<0.05). This trend was also identified in the remaining age ranges. Considering the totality of hemograms, 38.5% of HIV-1 infected patients presented lymphopenia; whereas 22.6% of seroreverters presented this condition (P=0.140). There was no association between lymphopenia and the clinical and immunological classifications (P=0.297).

Eosinophilia occurred in one patient before age six months and only occasionally after age five years. Monocytosis occurred in 50.0% to 67.0% of children up to the age of nine months, and in 83.0% of those aged nine to 12 months; the difference between these two age groups

![Figure 3](image_url) - Evolution of anemia, microcytosis, hypochromia and macrocytosis in children infected by HIV-1 and seroreverters, aged between 54 and 96 months

![Figure 4](image_url) - Evolution of leukopenia, neutropenia, lymphopenia and atypical lymphocyte count in children infected by HIV-1 and seroreverters, aged between 0 and 18 months (the asterisks indicate the age groups in which statistically significant differences were observed)
Comparative analysis of platelet exams

In the group of noninfected patients, or seroreverters, thrombocytosis was identified in 33.0% of children aged less than three months and in those aged three to six months. Up to the age of two years, it ranged from 14.0% to 27.0%. Thrombocytopenia was diagnosed in one patient in the age group of three to six months, in that of nine to 12 months, and in that of 12 to 15 months.

In the group of infected patients, thrombocytosis affected 33.0% of children before age three months, one children between ages six to nine months, and one between ages 36 to 42 months. Thrombocytopenia was diagnosed in one patient aged six years and another aged seven years.

was statistically significant (P<0.05). Up to the age of 42 months, monocyte was present in 30.0% to 40.0% of patients in both groups; subsequently, this incidence decreased.
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Comparative analysis of serum ferritin, iron, and total iron binding capacity (TIBC)

In the group of seroreverters, we carried out 68 exams of serum ferritin whose results indicated 11 (16.2%) patients with levels below normal, 52 (76.5%) with normal levels, and five (7.3%) with elevated levels. Serum iron was obtained from 13 patients, out of which two (15.4%) presented reduced levels, 10 (77.0%) presented normal levels, and one presented elevated levels (patient was being administered ferrous sulfate). Out of the nine samples examined for TIBC, three (33.3%) presented normal levels and six (66.7%) presented elevated levels.

In the group of infected children, five (17.9%) presented reduced serum ferritin levels, 16 (57.1%) normal levels, and seven (25.0%), elevated levels. There was no statistically significant difference between the two groups. Serum iron levels were below normal in seven (50.0%) patients, and normal in the remaining patients. Out of the eight samples examined for TIBC, four (50.0%) presented elevated levels, and the remaining were normal.

Discussion

The status of chronic malnutrition, diagnosed by greater involvement of the height factor in seroreverters, indicates that the nutritional support offered only to the group of infected children (basic foods and milk) influenced the anthropometric measures in this study. In the analysis of infection and nutrition, the social factor appeared as a determinant marker of malnutrition and early manifestação of the HIV-1 infection. The poor quality of life, in addition to the clinical status of the parent, the costs and complexity of treatment, unemployment, and orphanhood rendered the nutritional deficiencies even more striking.

The significant difference between the two groups in relation to breastfeeding variable indicates that nursing by HIV-1 infected mothers increased the risk for perinatal transmission. Also, out of the 15 mothers who were administered AZT during gestation, only one was administered intravenous AZT during delivery; thirteen babies were treated during the first six weeks of life. The failure to fully implement the PACTG 076 favored the elevated rate (20.0%) of vertical transmission of HIV-1.

In the United States and France, the impact of the PACTG 076, in 1994, allowed for a dramatic decrease in perinatal transmission of HIV. In Brazil, the services that have already adequately implemented this protocol have reported an important impact on the prevention of this type of transmission of the HIV-1; such is the case of the STDs and AIDS Municipal Center in the city of Porto Alegre, state of Rio Grande do Sul, which reported a 2.6% perinatal transmission rate between 1999 and 2000.

In both HIV-1 infected patients and seroreverters we observed microcytic and hypochromic anemia after the third month of life. Comparison of the infected and noninfected groups at nine and 30 months indicated a statistically significant difference (P<0.05); the comparison of total patients with greater incidence of anemia between infected (73.1%) and seroreverters (41.5%) also indicated a statistically significant difference.

There are several particularities that should be taken into consideration in the assessment of these results. First, in children nonexposed to the HIV-1, the hemoglobin levels fall from 18 g/dl at birth to 14 g/dl at two weeks of life. The iron released is stored and gradually reutilized when the total circulating levels of hemoglobin mass starts to increase with growth. Between the ages of four and 12 months, the total body iron increases twofold and an external source of iron is necessary. If that source is not provided, as in the population of this study, there can be early cases of anemia.

According to the literature, the predominant types of anemia in infected children are hypochromic anemia (40.0%) and microcytic anemia (56.0%), and there are also cases of anisocytosis and poikilocytosis. Also, the low body iron levels in HIV-1 infected mothers, the use of antiretrovirals, the HIV-1 infection itself in children, the loss of appetite, the diet poor in iron, the opportunistic bacterial infections, parvovirus B19, Micobacterium avium intracelulare, and others can contribute to the severity and persistence of anemia.

We observed 100.0% of macrocytosis at age 3 months in infected children; these children, however, did not present anemia. There was a significant difference (P<0.05) in relation to this abnormality in children aged nine to 12 months, which could be related to prophylactic (up to six weeks of life) or therapeutic (at age nine months) use of AZT associated with prophylaxis with SMX-TMP for Pneumocystis carinii.

The etiology of anemia in infected children is still not well-established. We identified four patients with anemia of chronic disease and two with iron deficiency anemia. However, 50.0% of infected patients and 37.7% of seroreverters were being administered ferrous sulfate, thus explaining why low serum iron was detected in only seven infected and two noninfected patients. Castaldo et al. detected iron deficiency in 48.0% of children with HIV-1 infection. In the cases of anemia of chronic disease, erythropoiesis is limited by hypoferrerna. Despite the fact that erythropoietin levels are elevated, there is a failure in bone marrow response due to the alterations in function of cytokines IL-1, IL-3, M-CSF, G-CSF, GM-CSF, TNF-R1, and TNF-RII (TNF receptors).

The literature suggests that anemia can be related to the cytopathic effect of HIV-1 on erythrocytes, to the alterations in bone marrow stromal cells, to the presence of anterythrocyte antibodies or not, and to the infection of hematopoietic progenitor cells by the HIV-1.

Anemia is very common in HIV-1 infected adults, affecting approximately 30.0% of patients during the early
asymptomatic years and 80.0% to 90.0% of patients with progression of the HIV disease. It is considered an anemia of chronic disease, with predominance of normochromic normocytic anemia. Other alterations such as ataxia and koilonychia have been reported in the majority of patients.25

Atypical lymphocytes were identified before age one year in the infected children, with a significant difference between ages six and nine months; lymphopenia, in turn, was more frequent between ages 15 and 18 months. In the case of HIV-1 infected patients, the main immune deficiency was the continuous fall in CD4(+) T cell count. Normal newborn infants present a percentage of CD4(+) T cell count much higher than that of adults, with a prevalence of naive (CD45RA+) cells in comparison to the memory (CD45RO+) cells. With age, the percentage and absolute count of CD4(+) T cells in newborn infants becomes more approximate to those of adults.

The fall in CD4(+) T cells in cases of vertical HIV-1 infection is usually much faster than in adults; this fall is also correlated to clinical disease. The thymus will contribute to the reconstitution of T cells to its exhaustion, which is expressed by peripheral lymphopenia.26 Some children present a rapid fall in CD4(+) T lymphocytes, which results in early diagnosis of AIDS, whereas other children may remain healthy and free of symptoms for years.

It is understood that the prolonged increase in the count of HIV-1 RNA copies at the beginning of infection may be caused by a much more significant pool of CD4(+) T cells, which are more permissive to the infection by HIV-1, and which results in atypia and decrease in lymphocytes. In addition to the loss of CD4(+) T cells, there is also an increase in CD8 T cells. Though a more simplified description of what goes on, there is some level of immune activation that occurs as a result of the hypergammaglobulins and excess circulating lymphokines.27

The etiology of leukopenia and neutropenia observed in our population of infected children can be attributed to suppression of hematopoiesis, as reported by Scadden,28 or, still, to the use of myelotoxic drugs, to opportunistic infections, and to the presence of antineutrophil antibodies.21,29

We considered eosinophilia, which affected both groups occasionally, as a result of intestinal parasitosis and sensitization to the medication. In the group of infected patients, eosinophilia and increase in IgE levels may have occurred according to the change in cytokine pattern from TH1 to TH2.

Monocytosis in the HIV-1 infected patients was significantly different between the ages of nine and 12 months, thus indicating that the HIV-1 also employs strategies of immune exploitation and evasion in order to survive and reproduce, similarly to other intracellular pathogens.30

Thrombocytosis was also identified on occasion in both groups and was attributed to loss of bone marrow control after the infections, to iron or vitamin E deficiency,31 and to use of AZT. Thrombocytopenia was rare in infected patients probably since it is frequently found in the advanced stages of infection.9

Our findings allow us to posit that the use of AZT by the dyad mother-baby contributed to decreasing the vertical transmission rate, and that there was an association between breastfeeding and evolution to infection.

Altogether, our results allow for the following conclusions: 1) the weight and height deficits were more significant in seroreverters; 2) the clinical manifestations of HIV-1 infection occurred prior to one year of age in 69.5% of patients; 3) there was a significant difference in the abnormalities detected in the group of HIV-1 infected patients, such as those of anemia, hypochromia, macrocytosis, atypical lymphocytes, lymphopenia, and monocytosis; 4) the most prevalent type of anemia in both groups was microcytic and hypochromic anemia. There was an association between prevalence of anemia and development of the infection; the most frequent etiology in the group of infected patients was anemia of chronic disease.

References


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