

# HIV-I infection in perinatally exposed siblings and twins

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## Abstract

In a multicentre study on perinatal HIV-I infection including 1493 children born from 1471 pregnancies to 1415 infected mothers, 22 twin pairs and 56 sibships (115 children) were recorded. The frequency of twin pregnancies was 1.5 (22/1471) and 3.9% (56/1415) seropositive women had more than one at risk pregnancy. In 18 twin pairs with a known infection status nine of the 36 children (25%) were infected. Discordance in infection status was present in only one (5.5%) dizygous pair. A high relative risk of infection (23.1) in a twin was observed when the other was infected. Infection was unrelated to gestational age, mode of delivery, or birth weight. Infection status was defined in 41 sibships (84 children including one first born twin pair and one third born child). When the first born was infected, 11/26 (42.3%) second born children were also infected, whereas this happened in only 2/16 (12.5%) second or third born children when the first born was uninfected. Two out of nine first born (22.2%) and 5/21 (23.8%) second born children prospectively followed up from birth acquired the infection. Results of this study demonstrate that neither twin nor second pregnancies are at increased risk of mother to child HIV-I transmission. Overall data suggest that non-casual factors in mother and/or child influence perinatal infection.

Transmission from the mother to her offspring accounts for most cases of human immunodeficiency virus type I (HIV-I) infection in children.<sup>1,2</sup> Transmission occurs mostly in utero,<sup>3</sup> but intrapartum exposure to maternal blood and vaginal fluid<sup>4,5</sup> or breast feeding<sup>2,6</sup> may be additional routes. Results of large studies on efficiency of mother to child transmission have been recently reviewed.<sup>2</sup>

The risk of vertical transmission of HIV-I in twin or second pregnancies has not yet been defined. Reports on twins<sup>7-9</sup> are anecdotal and studies in siblings contain selection bias<sup>10,11</sup> or are based on very limited series.<sup>12,13</sup> Studies in twins may provide information on the biological background influencing perinatal transmission<sup>8</sup> and clinical outcome of infection. In addition, data are needed to counsel HIV-I infected women who wish a second pregnancy.<sup>10,12</sup> In the present study these issues were addressed

through the data emerging from the Italian Register for HIV infection in children, which was instituted in 1985 by the Italian Association of Paediatrics.<sup>1,14</sup>

## Methods

### DATA COLLECTION

Children with HIV-I infection or born to seropositive mothers at 75 participating centres were enrolled in our multicentre study. At the time of writing 1493 children born to HIV-I infected mothers were recorded (828 prospectively followed up from birth). Data were collected through registration and follow up forms as previously described.<sup>1</sup> Information requested included personal data, infection status and clinical condition, mode of delivery and mother's clinical condition at delivery, gestational age, birth weight, and type of feeding. Children were considered breast fed independently from duration. Siblings and twins born to infected mothers were noted. To determine zygosity in twins information on placenta, blood and tissue types, and physical features was requested. These criteria allowed us to establish dizygosity and make a reasonable presumption of monozygosity.<sup>7,15</sup>

### CASE DEFINITION

Infection and clinical status were classified according to the Centers for Disease Control recommendations (P-0, P-1, etc.).<sup>16</sup> The infection status was defined by presence/absence of HIV-I antibodies (evaluated by enzyme linked immunoassay and the western blot test<sup>17,18</sup>) after 15 months of age or, in younger subjects, by a convincing presence of viral markers (p24 antigenaemia,<sup>17</sup> proviral sequences detected by polymerase chain reaction,<sup>18</sup> or repeated viral antigen expression in peripheral blood cells<sup>19</sup>) and/or virus isolation from peripheral blood mononuclear cells.<sup>20</sup>

### STATISTICAL ANALYSIS

Data were processed through SPSSX package (SPSS Inc, Chicago, 1986). The relative risk of infection and its significance were calculated through Epi Info Statcalc program (Centers for Disease Control, Atlanta and World Health Organisation, Geneva). Student's *t* test or non-parametric Mann-Whitney tests were used to calculate the significance of differences of means or medians. Differences in frequencies were assessed by  $\chi^2$  or Fisher's exact test. Ages

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Table 1 Concordance in infection status according to zygosity in 18 twin pairs perinatally exposed to HIV-1

	Zygosity			Total (n=18)
	Monozygosity (n=5)	Dizygosity (n=8)	Uncertain (n=5)	
Both infected	1	1	2	4
Both uninfected	4	6	3	13
One infected	0	1	0	1
Concordance (%)	5/5 (100)	7/8 (87.5%)	5/5 (100)	17/18 (94.4%)

were reported as median and range. Gestational age and birth weight were expressed as mean (SD).

## Results

### TWINS

Twenty two twin pairs (22 boys and 22 girls) were recorded with a frequency of 1.5 (22 out of the 1471 total pregnancies). There were 1415 HIV-1 infected women. Twenty out of 22 mothers were symptomless at delivery.

Four twin pairs still had an indeterminate infection status (P=0). In 18 pairs the infection status was known, including 10 pairs prospectively followed up from birth.

In the group of twins with known infection status (18 boys and 18 girls aged 16.3 months at the last follow up, range 3.1–60.7 months), nine of the (25%) children were infected. Sex and/or blood and/or HLA typing differed in eight pairs. A reasonable presumption of monozygosity was made in five pairs, whereas zygosity was uncertain in five pairs. Concordance in infection status according to zygosity is reported in table 1. The relative risk of infection in one twin when the other was infected was 23.1 (95% confidence interval 3.3 to 162.1;  $p < 0.00001$ ). Infected and uninfected pairs differed neither in gestational age (36.6 (3.1) compared with 35.1 (2.1) weeks) nor frequency of vaginal delivery (2/4, 50% compared with 7/13, 54%). Frequency of infected pairs among those born at a gestational age less than 36 weeks (1/5, 20%) was comparable with that observed in pairs born at or after 36 weeks (3/12, 25%). Birth weight was similar in infected (2167 (336) g) and uninfected (2144 (373) g) twins. In contrast, the proportion of breast fed subjects was significantly higher in infected than in uninfected twins (5/9, 56% compared with 1/27, 4%;  $p = 0.002$ ).

Age at last check in infected children was 26 months (range 6–60 months). All HIV-1 infected children (two P-1 pairs, two P-2 pairs, and one additional P-2 patient) were surviving.

In one of 10 (10%) pairs prospectively followed up from birth both twins were HIV-1 positive. Six pairs (60%) were born by caesarean section, with a frequency comparable with that observed in the whole group. None had been breast fed.

### SIBLINGS

Fifty six out of 1415 (3.9%) seropositive women had more than one pregnancy and 115 children (68 boys and 47 girls) were born (including one first born twin pair, one second born twin pair, and one third born child). The presence of

HIV-1 related clinical manifestations was recorded in four primiparous women and in five women at second delivery.

Infection status was defined in 41 sibships—that is, 84 children inclusive of one first born twin pair and one third born child. This group included 22 boy and 20 girl first born children aged at the last follow up 38.1 months (range 4.9–93.9) and 30 boy and 12 girl second or third born children aged 16.3 months (range 3.1–60.7). Nine first and 21 second or third born children had been prospectively followed up from birth.

Considering all sibships with known infection status, 26/42 (61.9%) first born and 13/42 (30.9%) second or third born children were infected. On the other hand, when subjects prospectively followed up from birth were taken into account, the presence of infection was reported in an equal proportion of first (2/9, 22.2%) and second or third (5/21, 23.8%) children.

The frequencies of sibships that were concordant or discordant in infection status is reported in table 2. The large majority of discordant sibships included an infected first born and an uninfected second born child. Considering these results from a different point of view (table 3), it was observed that the frequency of infected second born children was significantly higher when the first born was infected than when the first child was uninfected. A 3.1 (95% confidence interval 0.8 to 12.4) relative risk of infection in second born was observed (when the first born was infected) with an at limit significance ( $p = 0.054$ ). Information on gestational age, birth weight, type of delivery, and feeding was available in 78 children. Results were comparable in first and second born infected or uninfected children (table 4). The overall frequency of breast feeding, however, was significantly higher in infected (17/36, 47.2%) than in uninfected (9/42, 21.4%;  $p = 0.03$ ) siblings.

The frequency of subjects classified as P-2 was comparable in first born (21/26, 80.7%) and second born (10/13, 76.9%) infected children,

Table 2 Concordance in infection status in 41 sibships perinatally exposed to HIV-1

Infection status	Pairs (%)	
Both infected	11 (26.8)	} Concordance 24/41 (58.5)
Both uninfected	13 (31.7)*†	
First born infected and second born uninfected	15 (36.5)	} Discordance 17/41 (41.4)
First born uninfected and second born infected	2 (4.8)	

\*The first born twin pair (both uninfected) were considered as one first born child.

†A third born child was uninfected.

Table 3 Frequency of HIV-1 infection in second and third children according to the infection status in first born children

First born child (n=42)	Second or third born child (%) (n=42)
Infected (n=26)	Infected: 11 (42.3)* Uninfected: 15
Uninfected (n=16)†	Infected: 2 (12.5)* Uninfected: 14‡

\* $p = 0.047$ .

†Including one first born twin pair.

‡Including one third born child.

Table 4 Perinatal data in first, second, and third born children infected or uninfected with HIV-I

	Gestational age (weeks)	No (%) vaginal delivery	Birth weight (g)	No (%) breast fed
Infected:				
First children (n=23)	38.8 (1.8)	20 (86.9)	2979 (574)	12 (52.1)
Second or third children (n=13)	38.8 (2.1)	10 (76.9)	2926 (586)	5 (38.4)
Uninfected:				
First children (n=15)	37.7 (4.3)	14 (87.5)	2910 (564)	3 (20)
Second or third children (n=27)	38.2 (2.6)	23 (85.1)	2973 (478)	6 (22.2)

and no difference was observed as to age at onset of symptoms (15 months, range 1–65 months and 13.5 months, range 1–65 months, respectively). Considering the 11 infected sibling pairs, clinical outcome overlapped in seven (64%) instances (one P-1 pair and six P-2 pairs).

### Discussion

Results of the present study clearly demonstrate that twins and second born children of HIV-I infected mothers are not at an increased risk of vertical transmission of the infection. In our country an investigation on 334 first born children identified at birth has recently shown that 19.1% of perinatally exposed subjects are actually infected (C Gabiano *et al.*, unpublished). Comparable or even lower figures in twins and second born children were observed in the present study when, to avoid selection bias, children followed up from birth were considered.

To our knowledge no study has yet determined the risk of perinatal HIV-I infection in twins. Available data on second born children are unreliable because they are based on few cases<sup>12 13</sup> or on second children of women who had previously given birth to an infected infant.<sup>10 11</sup> Our study provides figures for counselling seropositive mothers who wish a subsequent pregnancy. It is noteworthy that, despite information and prevention campaigns, some seropositive women plan a second pregnancy.<sup>10 12</sup>

Up to now discordant infection status had been documented in one monozygotic<sup>7</sup> and two dizygotic<sup>8 9</sup> twin pairs born to seropositive mothers. However, our results showed an overall concordance. This finding is not surprising, as twins share factors that have been suggested to influence mother to child HIV-I transmission in utero or at birth: poor clinical condition of the mother<sup>21</sup> with subsequent high circulating virus load during pregnancy,<sup>20</sup> quality of maternal antibody response to HIV-I,<sup>22–24</sup> length of pregnancy,<sup>22</sup> and type of delivery.<sup>6</sup> In addition, other intrauterine viral infections affecting only one twin have been documented but they are considered exceptional.<sup>25 26</sup>

Results in the twins and siblings that we studied provide additional information. Vaginal delivery did not increase the proportion of infected children, and consequently caesarean section cannot be considered a tool for preventing infection.<sup>6</sup> In contrast to a previous report,<sup>22</sup> prematurity did not increase the risk of becoming infected. Notably, the group of twins allowed us

to consider a significant number of children born before 36 weeks' gestation. Birth weight was similar in infected and uninfected children. Together with data on gestational age, this finding suggests that HIV-I infection affects neither length of pregnancy nor fetal growth. On the other hand, an increased frequency of breast fed children was observed in infected subjects. This finding is interesting but a selection bias cannot be ruled out. In fact, very few children identified at birth had been breast fed, as in our country most centres counsel bottle feeding for at risk infants. The small number of mothers who were symptomatic at delivery prevented us from considering the role of this variable in favouring vertical transmission. However, it is noteworthy that an equally low proportion of symptomatic women was recorded at first and second delivery suggesting that progression of clinical manifestations after pregnancy is not the rule.<sup>27</sup>

Our results lead us to speculate that some non-casual factor(s) in the mother and/or the child are crucial in favouring or preventing perinatal HIV-I infection. The role of maternal antibodies to discrete domains of gp120 has been shown.<sup>23 24</sup> The role of polymorphisms in HIV-I disease progression has been suggested,<sup>28</sup> but whether they also influence susceptibility to infection remains to be defined.

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