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Frequency of differential placental transfer to twins of maternal antiretroviral medications

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Condensation

In twins born to women taking anti-HIV medications, cord blood concentrations differed between the 2 twins by more than 50% for at least one drug in nearly one third of twin pairs, including a monochorionic diamniotic pair.

Short title: Antiretroviral placental transfer in twins

Keywords: twins, pregnancy, placental transfer, HIV, antiretrovirals

Acknowledgements

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Word count: abstract 245 words; manuscript 2478 words
Abstract

Background

Placental passage of drugs in twins is poorly understood, and is unknown regarding antiretrovirals (ARVs). In the event of large differences in the exposure of 2 twins to the same maternal therapy, this could have a clinical impact in terms of prevention of perinatal HIV transmission or adverse effects.

Objective

To describe the frequency of differential transplacental passage of antiretrovirals between twins.

Study Design

The study was performed retrospectively, on data from women included in a multicenter perinatal HIV cohort study. All twin pairs for which the mother received antiretroviral therapy and for which drug concentrations in both of the umbilical cords after cord clamping at delivery were studied. We considered that a difference in concentrations of more than 50% between twins was a substantial difference (ratios below 0.67 or above 1.50).

Results

We analyzed 29 twin pairs, 27 dichorionic and 2 monochorionic diamniotic. Cord blood concentrations differed between the 2 twins by more than 50% for at least one ARV in 9 twin pairs, 8 dichorionic and 1 monochorionic. Discordant concentrations were observed in one or more cases for several nucleoside reverse transcriptase inhibitors (tenofovir, emtricitabine, lamivudine, zidovudine) and protease inhibitors (atazanavir, lopinavir, saquinavir et ritonavir); within individual twin pairs placental transfer was discordant for one or more ARVs, but identical for others.

Conclusion

Concentrations differed in nearly one third of twin pairs. This may be due to interindividual genetic variability of placental transporters between dizygotic twins as well as physiological differences between twins.
Introduction

Placental passage of drugs has received little attention in twin pregnancies. Preclinical placental transfer studies are performed either in animals whose placentas differ from the human hemochorial placenta, or from experimental models (1,2), including the ex vivo human cotyledon perfusion model (2,3,4). Because pregnant women are usually excluded from clinical trials, clinical data is obtained only if umbilical cord blood is studied at delivery. Since twins account for only 2-3% of deliveries, there are few opportunities to include them in such studies.

In women with HIV infection, antiretrovirals (ARVs) are required to treat the mother and prevent vertical transmission to the child (5). Transplacental passage may be beneficial as pre-exposure prophylaxis for the fetus and the future neonate. However, in utero drug exposure of the fetus may cause adverse effects (6). Increased risks of malformations have been reported for the heart (7) in the case of zidovudine, central nervous system with efavirenz (7,8) and neural tube defects with dolutegravir (9,10). Exposure to zidovudine and other nucleoside reverse transcriptase inhibitors during gestation may cause mitochondrial toxicities, didanosine was associated with an increased risk of cancer (11), and efavirenz was associated with an increased risk of microcephaly (12). Thus, differential drug exposure may have a clinical impact for twins in terms of prevention and toxicities. It is not known whether both twins receive the same or differential drug exposure in case of maternal medication.

Our objective was to describe the frequency of discordant blood concentrations of antiretroviral drugs in twin pairs.

Methods

This is a descriptive, retrospective study of mother-child pairs of twins from five centers in the Ile de France region between 2001 and 2017 who received drug monitoring in the pharmacotoxicology laboratory of Bichat-Claude Bernard Hospital, Paris. All patients were included with their informed consent in the French Perinatal HIV Cohort Study (Enquête Périnatale Française, EPF), as approved by the Cochin Hospital Institutional Review Board and the French computer database watchdog commission (Commission Nationale de
The EPF is an observational study, therefore HIV and pregnancy care were determined in each center.

Cord blood samples were taken after delivery for both fetuses by venipuncture, in order to prevent contamination by amniotic fluid or maternal blood, after cord clamping. Samples were placed in heparinized tubes. Antiretroviral drug concentrations were determined using ultra high performance liquid chromatography coupled to a tandem mass spectrometer (13). The lower limit of quantification was 10ng/mL (LOQ<10ng/mL) for emtricitabine, lamivudine, tenofovir, zidovudine and glucuronide; 20ng/mL for atazanavir (LOQ<20ng/mL); 30ng/mL for lopinavir (LOQ<30ng/mL); and was 10ng/mL for ritonavir and saquinavir (LOQ<10ng/mL). The maximum reported differences between measured and theoretical concentrations in a set of external proficiency testing samples using the same ultra high performance liquid chromatography technique were 0.2% to 12.7%. Concentrations less than or equal to the quantification limit were considered as equal to the LOQ. Assays were performed in the same run for each twin pair to avoid assay variability within twin pairs.

We defined twin 1 and twin 2 by their order of birth. The data collected for the pharmacological assays were date and time of ingestion by the mother for each antiretroviral medication, date and time of delivery, blood concentrations of antiretrovirals in maternal blood and umbilical cord blood for each fetus at delivery.

We considered that there was a substantial difference in concentrations within a twin pair when the ratio between twin 1 and twin 2 was below 0.67 or above 1.50. The cut-off of a 50% difference was chosen to be beyond the maximum variability of measures related to assay conditions, although assays were performed in the same run for each twin pair.

In addition to pharmacological data, we collected data concerning the type of chorionicity, HIV infection status (HIV-1 RNA viral load and CD4 count at delivery), maternal age and body mass index (BMI), pregnancy complications, delivery and neonatal outcomes (gender, birthweight, 5 minutes Apgar score). We calculated birthweight percentiles according to the French College of Obstetricians-Gynecologists curves, which are adjusted for gestational age and sex (15). We did not use data from the EPF case report forms. All data were extracted and anonymized from the patients’ obstetrical record.

Statistical analysis
For qualitative variables, data are presented as n(%) and for quantitative variables, data are presented as median [interquartile range]. To compare the two populations of twins, we used the Wilcoxon-Mann-Whitney test with R software. P value < 0.05 was considered significant.

Results

Population

We collected data for 37 pairs of twins. For 8 pairs of twins, we lacked data for one of the two twins (Figure 1). Thus, the analysis was carried out on 29 pairs of twins for which data was available on the cord blood concentration of at least one ARV for both twins at birth. There were 27 dichorionic and 2 monochorionic diamniotic twin pregnancies. Maternal drug concentrations were missing for 5 of the mothers.

The median maternal age was 32 years [interquartile range (IQR) 29; 35] and median BMI (based on reported pre-pregnancy maternal weight) was 26 kg/m$^2$ [IQR 23; 28.3] (Table 1). 10 mothers were co-infected with HBV and 1 with HCV. Chronic co-medication was reported a single patient who received L-thyroxin.

The mode of delivery was vaginal in 5 cases (3 spontaneous and 2 after induction), cesarean section in 22 cases (11 before labor and 11 during labor), and not available for 2 patients. The duration of ruptured membranes was less than 1 hour in 20 patients (19 patients who had cesarean sections and 1 with vaginal delivery). The median gestational age at delivery was 36 weeks’ gestation + 6 days [IQR 35 WG+1day; 38 WG]. There were 10 preterm deliveries, of which 4 before 34 WG. Among first twins, 1 (3%) had a birthweight <3rd percentile and 3 children (10%) had birthweights <10th percentile. Among second twins, 4 (14%) had a birthweight <3rd percentile and 8 children (28%) <10th percentile. The median difference in birthweights between twin 1 and twin 2 was 10.5% (range 8-13%).

Antiretroviral therapy was started before the beginning of pregnancy for 6 women and during the pregnancy for 23 women (information missing for 8 patients). Antiretroviral drug combinations at the time of delivery are described in Table 2. Route of administration was orally, except for intrapartum intravenous zidovudine.
HIV-1 RNA viral load at delivery was undetectable below 50 copies/mL except for 2 patients; cases # 1 and # 21 had viral loads of 239 copies/ml and 121 copies/ml respectively at delivery, suggesting compliance issues. The median CD4 count at delivery was 507 cells/mm³ [IQR 362; 578], and the percentage was 26 % [IQR 21.75; 32.25].

**Frequency of discordant cord ARV concentrations**

We analyzed 76 maternal blood drug concentrations and 214 cord blood drug concentrations. Ratios of cord blood concentrations were calculated for each drug, even if the maternal concentration was below the limit of detection, which usually reflected a long interval from last maternal dose to delivery. Considering that the difference in the concentrations for twin 1/ twin 2 was substantial if ratios were below 0.67 or above 1.50, there were discordant cord blood concentrations for one or more ARV in 9 twin pairs. Of these pairs of twins, 8 were from dichorionic pregnancies and 1 was from a monochorionic diamniotic pregnancy. Concentration ratios for the 9 twin pairs with at least one discordant concentration are reported in **Table 3**. Among those pairs, and regarding individual ARV concentrations, there were 16 ratios which differed by 50% or more. The different cord blood drug concentrations concerned 2 ratios for AZT (2/23=9%), 3 ratios for g-AZT (3/13=23%), 1 ratio for TDF (1/5=20%), 2 ratios for 3TC (2/15=13%), 1 ratio for FTC (1/3=33%), 4 ratios for LPV (4/15=27%), 1 ratio for ATV (1/3=33%), 1 ratio for RTV (1/16=6%) and 1 ratio for SQV (1/3=33%). The lowest ratio was 0.30 and the highest 2.82 except for case #34, where the ratios were 29.18 for lamivudine and 17.73 for lopinavir.

We found no evidence of differences between twin pairs which could account for differential ARV transfer (**Table 4**). Regarding birthweight, only one of the neonates (case #34 mentioned above), whose antiretroviral concentrations were near the lower limit of detection, had fetal growth restriction below the 3d percentile.

**Maternal ARV blood concentration**

Drug concentrations were available for 24 mothers. If considering the 16 different ratios (in 9 twin pairs), the ARV concentration was undetectable in none of the mothers, detectable in 5 mothers, and missing for 11 mothers. Among the 91 cord blood concentration ratios which differed by less than 50%, the maternal ARV concentrations were below the limit of detection for 12 patients (13%, concerning 4 samples for AZT, 2 for g-AZT, 1 for ABC, 1 for
LPV and 4 samples for the RTV booster. Differences in ratios were found for the nucleoside transcriptase inhibitors lamivudine, ZDV/g-ZDV, emtricitabine and tenofovir, and for the protease inhibitors atazanavir, lopinavir, ritonavir and saquinavir (Table 5).

Discussion

Main Findings

We observed different cord blood ARV concentration for at least one antiretroviral in nearly one third of twin pairs, 9 of out a total of 29 twin pairs. Differences in ratios were found for various classes, nucleoside transcriptase inhibitors (lamivudine, ZDV/g-ZDV, emtricitabine, tenofovir) and protease inhibitors (atazanavir, lopinavir, ritonavir and saquinavir).

We chose an arbitrary cut-off of more than 50%, so this is a conservative estimate of the frequency of differential placental transfer. Among the 91 cord blood concentration ratios that were not considered different, the maternal concentration was below the limit of detection for 12 patients (13%), which may underestimate the proportion of discordant cord blood samples. In view of the maximum reported differences between measured and theoretical antiretroviral concentrations for the assays, even a 10% difference between twins would be statistically significant, but this would not be expected to have a real clinical impact.

Findings in the context of what is known

To date, transplacental transfer of drugs in twin pregnancies has been neglected. Discordant vertical transmission of pathogens has been described for parvovirus (16,17), cytomegalovirus (18), zika (19) toxoplasmosis (20) and HIV (21). Differential placental transfer in twin pairs has been studied for immunoglobulins, with lower levels of antibodies in cord blood reported in case of monochorionic, compared to dichorionic twins (20).

Cord blood concentrations reflect drug transfer across the placenta and also pharmacokinetics in the fetus, both of which may differ within a twin pair. Differences in transplacental passage between twins may be due to hemodynamic, metabolic, genetic and
epigenetic influences. The issues may differ between dizygotic and monozygotic, dichorionic and monochorionic twins.

Placental transfer occurs primarily via passive diffusion depending on physicochemical properties of drugs. In our study, we observed differential transfer for each of the classes of antiretrovirals suggesting that the physicochemical properties do not explain the differential concentration levels dosed in the two twins.

Transplacental drug transfer also depends on active transport which is mediated by protein-transporters (23) expressed on both fetal and maternal side of the placental barrier (24,25). ATP-binding cassette (ABC) (26) are mostly efflux pump transporters which protect the fetus from exposure of substrates. They include P-glycoprotein (P-gp), the Breast cancer resistance protein (BCRP) and the other Multidrug resistance associated proteins (MDR) incoded by the ABCB group of genes. Gene polymorphisms may differ between dizygotic twins. Efflux transport by ABCB1 and ABCG2 has been proved for abacavir (28) and tenofovir disoproxil fumarate (27). It has been reported that etravirine, a NNRTI, may inhibit the ABCG2 and decrease transplacental transfer of TDF but not that of abacavir (29). In our study, 2 pairs of twins were exposed to abacavir and the concentration ratio did not differ for both pairs. Zidovudine transport is also mediated by the ABCB1 and ABCG2 transporters in contrast to lamivudine (30). Addionnally, P-gp is also involved in reducing the placental transport of protease inhibitors (31) and ABCB1 polymorphism alters P-gp expression levels. P-gp inhibition increases placental transfer of lopinavir (34) as well as indinavir (35) and genetic polymorphisms of transporters may play a part in differential transport in these drugs (36). Darunavir is also a substrate of efflux transporters (37) as well as the CCR5 inhibitor maraviroc (38). Thus, differential expression of transporters could result in differential drug transfer in case of dizygotic twins. Additionally, the MDR1 gene was reported to be more activated in HIV infected women than controls (39).

Differences in fetal ARV blood concentrations may also be due to interindividual variability in pharmacokinetic parameters in the fetus, as described in singletons (40). Fetal plasma protein concentrations may also affect drug concentrations and distribution volumes. In this respect, differential birthweight among twins must be considered. Although we observed no relation between birthweight or preterm delivery and differences in drug concentrations, the size of the population was small and this does not exclude a possible relation between
birth weight and transplacental drug transfer (42). Genetic polymorphism may lead to
differences in metabolism and excretion in urine, meconium or umbilical arteries of ARV
between the two twins. *In vitro* studies on human tissues reported that differences in the
expression of nuclear receptors PXR and CAR might determine the variability in fetal CYP3A7
cytochrome expression (41). The observation of differential placental transfer in one case of
monochorionic twins indicates that genetic differences do not explain all variability. Although
monochorionic twins share the same placenta, there are hemodynamic differences between
twins, even in the absence of twin-to-twin transfusion syndrome.

Dichorionic twins have separate placentas, which may influence the distribution of the
uterine blood flow. Thus the transfer of small liposoluble molecules may depend on flow
rate (23,24) and be reduced in case of elevated umbilical venous pressure (43). In case
of abnormal placental or cord attachment arterial resistance and flow rates can be modified
(46), . Also, several studies have shown differential cord blood concentrations of growth
factors in growth-restricted fetuses, compared to values in the co-twin with normal growth
(44,45). Further studies should be performed including systematic pathologic analysis of
placentas. Such investigations may offer new insights on the numerous factors involved in
placental transfer of drugs in singletons.

**Strengths and Limitations**

To our knowledge, this is the first study to describe the concentrations and transplacental
passage of xenobiotics in a case series of twin pregnancies. Since the assays were performed
in the same run for each twin pair, there was no assay variability between the results within
twin pairs. However, the study has limitations. Some data was missing. The study was not
designed to study the potential impact of differential vs concordant cord blood
concentrations on postnatal outcomes, including HIV status and potential toxicities. Cord
blood concentrations were determined at birth, which does not reflect changes in fetal
concentration earlier in pregnancy. Also, there are differences in the period between the last
maternal dose and delivery. Regarding genetic differences, although all monochorionic twins
are monozygotic, dichorionic twins of the same sex can be either dizygotic or monozygotic.
Conclusion

Cord concentrations of antiretrovirals differed in nearly one third of twins in the same pair. The molecular characteristics may explain difference in transplacental transfer between drugs but do not explain differences within twin pairs. The difference may be due to both interindividual genetic variability of placental transporters and to physiological differences between twins.
Table 1: Neonatal characteristics of 29 twin pairs

<table>
<thead>
<tr>
<th></th>
<th>Twin 1 (%)</th>
<th>Twin 2 (%)</th>
<th>All children (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (WG + days), median</td>
<td>36+6</td>
<td>36+6</td>
<td>36+6</td>
</tr>
<tr>
<td>Mode of delivery, N vaginal / N cesarean</td>
<td>5 / 22</td>
<td>4 / 22</td>
<td>9 / 44</td>
</tr>
<tr>
<td>Gender, N female/ N male</td>
<td>10 / 18</td>
<td>15 / 13</td>
<td>25 / 31</td>
</tr>
<tr>
<td>Apgar score at 5 min, median [range]</td>
<td>10 [10; 10]</td>
<td>10 [9.25; 10]</td>
<td>10</td>
</tr>
<tr>
<td>Birthweight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All children, median [range] (g)</td>
<td>2620 [2270; 3005]</td>
<td>2370 [2140; 2690]</td>
<td>2615 [2181; 2830]</td>
</tr>
<tr>
<td>&lt;3rd centile, n (%)</td>
<td>1 (3%)</td>
<td>4 (14%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>3-10th centile, n (%)</td>
<td>2 (7%)</td>
<td>4 (14%)</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>10-90th centile, n (%)</td>
<td>21 (72%)</td>
<td>16 (55%)</td>
<td>35 (60%)</td>
</tr>
<tr>
<td>&gt;90th centile, n (%)</td>
<td>3 (10%)</td>
<td>1 (3%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Missing data, n (%)</td>
<td>2 (7%)</td>
<td>4 (14%)</td>
<td>7 (12%)</td>
</tr>
</tbody>
</table>
### Table 2: Antiretroviral therapy regimens in 29 mothers with twins

<table>
<thead>
<tr>
<th>Antiretrovirals</th>
<th>Dose mg/day</th>
<th>3TC</th>
<th>ABC</th>
<th>ZDV</th>
<th>ddI</th>
<th>FTC</th>
<th>TDF</th>
<th>NVP</th>
<th>ATV</th>
<th>IDV</th>
<th>LPV</th>
<th>RTV</th>
<th>SQV</th>
<th>RAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleoside/nucleoside reverse transcriptase inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3TC (n=19)</td>
<td>300</td>
<td>/</td>
<td>3 (16%)</td>
<td>17 (89%)</td>
<td>1 (5%)</td>
<td>0</td>
<td>0</td>
<td>2 (11%)</td>
<td>2 (11%)</td>
<td>3 (16%)</td>
<td>10 (53%)</td>
<td>12 (63%)</td>
<td>3 (16%)</td>
<td>0</td>
</tr>
<tr>
<td>ABC (n=3)</td>
<td>600</td>
<td>3 (100%)</td>
<td>/</td>
<td>3 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33%)</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>ZDV (n=23)</td>
<td>600</td>
<td>17 (74%)</td>
<td>/</td>
<td>0</td>
<td>3 (13%)</td>
<td>3 (13%)</td>
<td>2 (9%)</td>
<td>3 (13%)</td>
<td>10 (43%)</td>
<td>14 (61%)</td>
<td>3 (13%)</td>
<td>1 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ddi (n=1)</td>
<td>800</td>
<td>1 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>0</td>
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<tr>
<td>FTC (n=13)</td>
<td>200</td>
<td>0 (0%)</td>
<td>0</td>
<td>3 (23%)</td>
<td>0</td>
<td>/</td>
<td>3 (23%)</td>
<td>0</td>
<td>1 (8%)</td>
<td>0</td>
<td>1 (8%)</td>
<td>2 (15%)</td>
<td>0</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>TDF (n=5)</td>
<td>300</td>
<td>0 (0%)</td>
<td>0</td>
<td>5 (100%)</td>
<td>0</td>
<td>3 (67%)</td>
<td>/</td>
<td>1 (20%)</td>
<td>1 (20%)</td>
<td>0</td>
<td>2 (40%)</td>
<td>3 (67%)</td>
<td>0</td>
<td>1 (20%)</td>
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<tr>
<td><strong>Non-nucleoside reverse transcriptase inhibitors (NNRTI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NVP (n=4)</td>
<td>400</td>
<td>2 (50%)</td>
<td>0</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25%)</td>
<td>4</td>
<td>0</td>
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<td>0</td>
<td>1 (25%)</td>
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<tr>
<td><strong>Protease Inhibitors</strong></td>
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<tr>
<td>ATV (n=3)</td>
<td>200</td>
<td>2 (67%)</td>
<td>0</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>1 (33%)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IDV (n=3)</td>
<td>800</td>
<td>3 (100%)</td>
<td>1</td>
<td>3 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>/</td>
<td>1 (33%)</td>
<td>2 (67%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LPV (n=15)</td>
<td>800</td>
<td>10 (67%)</td>
<td>2 (13%)</td>
<td>10 (67%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
<td>2 (13%)</td>
<td>0</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
<td>/</td>
<td>10 (67%)</td>
<td>1 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>RTV (n=16)</td>
<td>200</td>
<td>12 (75%)</td>
<td>1 (6%)</td>
<td>14 (88%)</td>
<td>1 (6%)</td>
<td>2 (12%)</td>
<td>3 (19%)</td>
<td>0</td>
<td>2 (12%)</td>
<td>3 (19%)</td>
<td>10</td>
<td>/</td>
<td>2 (12%)</td>
<td>0</td>
</tr>
<tr>
<td>SQV (n=4)</td>
<td>750</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25%)</td>
<td>2 (50%)</td>
<td>/</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Integrase strand inhibitors (INSTI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL (n=2)</td>
<td>800</td>
<td>0 (0%)</td>
<td>0</td>
<td>1 (50%)</td>
<td>0</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>
Table 3: Concentration ratios of T1/T2 for twin pairs with at least one discordant concentration. Discordant concentrations indicated in bold type

<table>
<thead>
<tr>
<th>Case #</th>
<th>Gestational age (WG+d)</th>
<th>T1 weight g (centile)</th>
<th>T2 weight g (centile)</th>
<th>Nucleoside/nucleotide reverse transcriptase inhibitors</th>
<th>NNRTIs</th>
<th>Protease inhibitors</th>
<th>INSTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3TC</td>
<td>ABC</td>
<td>ZDV</td>
<td>g-ZDV</td>
</tr>
<tr>
<td>11 (dichorionic)</td>
<td>34+6</td>
<td>2500 (18th)</td>
<td>2180 (27th)</td>
<td>1.45</td>
<td>1.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (monochorionic)</td>
<td>37</td>
<td>2750 (50th)</td>
<td>2720 (50th)</td>
<td>1.01</td>
<td>1.28</td>
<td>2.60</td>
<td>1.00</td>
</tr>
<tr>
<td>21 (dichorionic)</td>
<td>37+6</td>
<td>2540 (33th)</td>
<td>3050 (62th)</td>
<td>1.99</td>
<td>0.83</td>
<td>1.09</td>
<td>0.77</td>
</tr>
<tr>
<td>23 (dichorionic)</td>
<td>36+6</td>
<td>3085 (92th)</td>
<td>2290 (15th)</td>
<td>0.61</td>
<td>2.60</td>
<td>0.29</td>
<td>1.37</td>
</tr>
<tr>
<td>26 (dichorionic)</td>
<td>38</td>
<td>2620 (42th)</td>
<td>MD</td>
<td>0.71</td>
<td>0.30</td>
<td>0.62</td>
<td>2.00</td>
</tr>
<tr>
<td>27 (dichorionic)</td>
<td>MD</td>
<td>MD</td>
<td>MD</td>
<td>1.03</td>
<td>1.01</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>31 (dichorionic)</td>
<td>38+1</td>
<td>3450 (97th)</td>
<td>2690 (50th)</td>
<td>0.85</td>
<td>0.83</td>
<td>0.73</td>
<td>1.09</td>
</tr>
<tr>
<td>32 (dichorionic)</td>
<td>36</td>
<td>2680 (57th)</td>
<td>2770 (67th)</td>
<td>0.90</td>
<td>1.05</td>
<td>2.82</td>
<td>0.29</td>
</tr>
<tr>
<td>34 (dichorionic)</td>
<td>30+3</td>
<td>1450 (26th)</td>
<td>1140 (1st)</td>
<td>29.18</td>
<td>2.47</td>
<td>1.69</td>
<td>17.73</td>
</tr>
<tr>
<td>All cases (mean)</td>
<td>36+1</td>
<td>2634</td>
<td>2402</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Nucleoside Reverse Transcriptase Inhibitors (NRTIs): Abacavir (ABC); Didanosine (ddI); Emtricitabine (FTC); Lamivudine (3TC); Zidovudine (ZDV); Nucleotide Inhibitors of Reverse Transcriptase (INtTI): Nevirapine (NVP); Tenofovir (TDF); Protease inhibitors (PIs): Atazanavir (ATV); Indinavir (IDV); Lopinavir (LPV); Ritonavir (RTV); Saquinavir (SQV); Integrase strand inhibitors (INST);Raltegravir (RAL); Missing data (MD)
Table 4: Maternal and neonatal characteristics in twin pairs with different or similar cord drug concentrations (medians and interquartile intervals)

<table>
<thead>
<tr>
<th></th>
<th>Discordant cord concentration (n=9)</th>
<th>Similar cord concentration (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.5 [30; 41]</td>
<td>32 [29; 35]</td>
<td>0.34</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26 [23; 27]</td>
<td>26 [24; 29]</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Neonatal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight T1 (g)</td>
<td>2650 [2430; 2833]</td>
<td>2620 [2270; 3005]</td>
<td>0.79</td>
</tr>
<tr>
<td>Birthweight T2 (g)</td>
<td>2690 [2235; 2745]</td>
<td>2295 [2065; 2620]</td>
<td>0.29</td>
</tr>
<tr>
<td>Mean birthweight of the 2 neonates (g)</td>
<td>2706 [2500; 2750]</td>
<td>2610 [2200; 2653]</td>
<td>0.33</td>
</tr>
<tr>
<td>Birthweight difference (g)</td>
<td>410 [88; 767]</td>
<td>315 [85; 503]</td>
<td>0.66</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>259 [251; 266]</td>
<td>257 [246; 267]</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 3: Twin1/Twin 2 concentration ratios of antiretrovirals in 29 twin pairs.

Twin1/Twin2 ratios were calculated even if drug concentration was below detection limit in the maternal sample. Cord/maternal ratios were not calculated for drugs which were below detection limit in the corresponding maternal samples.

<table>
<thead>
<tr>
<th>Nucleoside/nucleoside reverse transcriptase inhibitors</th>
<th>NNRTI</th>
<th>Protease Inhibitors</th>
<th>INSTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3TC</td>
<td>ABC</td>
<td>ZDV</td>
<td>g-ZDV</td>
</tr>
<tr>
<td>Number of maternal samples</td>
<td>12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Maternal concentration below detection limit n (%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>4 (24%)</td>
</tr>
<tr>
<td>Number of cord blood samples</td>
<td>30</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>Cord blood concentration below detection limit n (%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ratio of twin1/maternal</td>
<td>0.91 [0.54;0.69]</td>
<td>1.06 [0.96 ;0.99]</td>
<td>0.69 [0.54;0.69]</td>
</tr>
<tr>
<td>Ratios of concentrations, median [IQR]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>twt2/tmat</td>
<td>0.65; 1.17</td>
<td>0.91; 0.91</td>
<td>0.91; 1.56</td>
</tr>
<tr>
<td>ratio of twin2/maternal</td>
<td>0.93</td>
<td>1.09</td>
<td>1.09</td>
</tr>
<tr>
<td>ratio of twin 1/twin 2 cord</td>
<td>1.01</td>
<td>0.92</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Abbreviations:**  
interquartile range (IQR); Nucleoside Reverse Transcriptase Inhibitors (NRTI): Abacavir (ABC); Didanosine (ddI); Emtricitabine (FTC); Lamivudine (3TC); Zidovudine (ZDV); Nucleotide Inhibitors of Reverse Transcriptase (INtTI): Nevirapine (NVP); Tenofovir (TDF); Protease inhibitors (PIs): Atazanavir (ATV); Indinavir (IDV); Lopinavir (LPV); Ritonavir (RTV); Saquinavir (SQV); Integrase inhibitors (INI); Raltegravir (RAL); Not calculated (-)  
* Rounded off to the nearest hour
References


Figure 1: Flow chart

Pregnant women with twin deliveries, n = 37

No cord concentration for one of the twins, n = 3
pair n°7: acardiac twin
pair n°12: in utero fetal death
pair n°22: sample missing
Other missing data, n = 3

Cord blood concentration available for both twins, n = 29 twin pairs
dichorionic biamniotic, n = 27
monochorionic biamniotic, n = 2
monochorionic monoamniotic, n = 0