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Maternal CD4+ microchimerism in HIV-exposed newborns after spontaneous vaginal delivery or caesarean section

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ABSTRACT

Background: Maternal CD4+ cell microchimerism may be greater after caesarean section compared to spontaneous vaginal delivery and could cause mother-to-child transmission (MTCT) in HIV-exposed newborns.
Aims: To evaluate maternal CD4+ cell microchimerism in HIV-exposed newborns after spontaneous vaginal delivery or caesarean section.
Study design and subjects: In this prospective single-centre study, neonates whose mothers were infected with HIV and had normal MTCT risk according to the German Austrian Guidelines were considered for study enrolment. Maternal CD4+ cell microchimerism in the newborns' umbilical cord blood was measured and compared by mode of delivery.
Results: Thirty-seven HIV-infected mothers and their 39 newborns were included in the study. None of the 17 (0.0%) newborns delivered vaginally had quantifiable maternal CD4+ cells (95% confidence interval (CI): 0.00–0.00) in their circulation at birth compared with four of 16 (25.0%) newborns delivered via planned caesarean section, who showed 0.01–0.66% maternal cells (95% CI: −0.06–0.16; $P = 0.02$) in their circulation. The intention to treat analysis, which included six additional newborns delivered by unplanned caesarean section, showed quantifiable maternal CD4+ cells in one (0.05%; 95% CI: −0.02–0.04) of 23 (4.3%) newborn at birth compared to four of 16 (25.0%) born via planned caesarean section (95% CI: −0.06–0.16; $P = 0.04$). There was no MTCT in any of the newborns.
Conclusion: In this small cohort, spontaneous vaginal delivery in HIV-infected women with normal MTCT risk was associated with lower maternal CD4+ cell transfer to newborns compared to planned caesarean section.
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1. Introduction

The implementation of preventive interventions for mother-to-child transmission (MTCT) of HIV, namely optimized antiviral therapy of the mother, postnatal prophylaxis of the newborn, avoidance of breastfeeding and appropriate mode of delivery, has resulted in transmission rates of <2% in developed countries in recent decades [1–3]. Regarding mode of delivery, planned caesarean section was the strategy of choice in the era prior to the use of combination antiretroviral therapy (cART) [4–6]. Now that the use of cART is established, the viral load can be suppressed below the limit of detection in the blood of pregnant women. In recent years, large studies have shown no difference in MTCT rates in the setting of successful cART after spontaneous vaginal delivery or planned caesarean section [2,7,8]. Unfortunately, even under optimal conditions, MTCT of HIV still occurs [2], and the reasons are poorly understood [9]. We aimed to study relevant factors other than viral load

Abbreviations: CD, cluster of differentiation; Ct, threshold cycle; HIV, human immunodeficiency virus; MTCT, mother-to-child transmission; min., minimum; max., maximum; cART, combination of antiretroviral therapy; DNA, deoxyribonucleic acid; a-pH, arterial cord blood pH; RNA, ribonucleic acid; PCR, polymerase chain reaction; MACS, magnetic cell sorting; EDTA, ethylenediamine tetraacetic acid; CS, caesarean section; ITT, intention-to-treat.

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that play a crucial role in MTCT. Lin et al. [10] and Kaneda et al. [11] found higher levels of maternal microtransfusions to the newborn after vaginal delivery compared to planned caesarean section in HIV-negative women. Data on the transfer of maternal CD4+ cells to newborns (maternal microchimerism) are not yet available in HIV-positive mothers. Because HIV can persist as proviral DNA in CD4+ cells [12] and reactivate HIV replication even after years of sufficient cART [13], elevated CD4+ materno-foetal cell transfer after spontaneous vaginal delivery could be associated with an elevated risk of MTCT.

Therefore, our objective was to evaluate quantitative maternal CD4+ cell microchimerism after spontaneous vaginal delivery compared to planned caesarean section and to determine whether there is an increased risk of MTCT due to proviral DNA transfer in CD4+ cells.

2. Methods

2.1. Overall study design and ethics committee approval

This prospective single-centre multidisciplinary study is registered at ClinicalTrials.gov (NCT01450059). The study was conducted in accordance with the latest version of the Declaration of Helsinki. The protocol for the study was reviewed and approved by the Ethics Committee of the Johann Wolfgang Goethe-University Clinic, Frankfurt am Main. Written informed consent was obtained from all parents or single mothers prior to study-related blood draws.

According to parental wishes and possible obstetric options, the neonates were born either by planned caesarean section, spontaneous vaginal delivery or unplanned caesarean section after intended spontaneous vaginal delivery.

2.2. Study participants

Neonates born during the study period from 31 August 2011 to 30 April 2014 whose mothers were HIV-infected and had normal MTCT risk according to the German Austrian Guidelines [14,15] were considered for enrolment. Women had to have normal MTCT risk because they are the only group of HIV-infected women eligible to give birth by spontaneous vaginal delivery. According to the 2008 guidelines, normal MTCT risk was defined as an HIV viral load 'below the limit of detection' at the end of pregnancy [14]; the 2011 guidelines specified that HIV viral load had to be below 50 copies/ml [15].

Exclusion criteria were an elevated or very high risk of MTCT, newborns from underage mothers, infants born at other hospitals, severe perinatal asphyxia, chromosomal defects, severe birth defects, and newborns who were breastfed.

All newborns received immediate postnatal support from the attending paediatrician according to the current German Austrian Guidelines [14,15] and daily ward rounds during their hospital stay. Data collection at birth included mode of delivery, maternal CD4+ and CD8+ cells in neonatal blood, weight, length, and head circumference at birth, Apgar score, arterial cord blood pH (a-pH) and sex. HIV-1 RNA was measured by PCR twice in the first year of life, with the second measurement conducted after the third month of life at the earliest. Infants' HIV infection status was classified as 'HIV uninfected' after two negative HIV-1 RNA PCR results, a negative HIV antibody test, or a negative Western blot during follow up.

Maternal information included age, placenta location at birth, antiretroviral therapy, and CD4+ and CD8+ cell counts, as well as viral load (HIV-1 RNA PCR) within four weeks prior to birth.

2.3. Blood collection

Every birth was attended by a paediatrician, who supported the newborns according to the current German Austrian Guidelines [14, 15]. The umbilical cord was clamped twice, near the newborn and the placenta, and was cut between the two clips next to the baby. The umbilical vein was punctured, and at least 0.5 ml of blood was collected in an ethylenediamine tetraacetic acid (EDTA) tube. From the mothers, 5 ml of blood was collected into an EDTA tube by venous puncture. All probes were cooled immediately to 2–8 °C and processed either in real time during regular working hours or within 72 h at the latest on weekends.

2.4. Microchimerism analysis

2.4.1. Isolation of CD4+ cells

As previously published by Willasch et al. [16], CD4+ cell isolation from umbilical cord blood was conducted using the MACS (magnetic cell sorting) technique. Here "Whole Blood MicroBeads" conjugated to a monoclonal anti-human CD4 antibody were used for magnetic cell separation conducted by the AutoMACS separator device (Miltenyi Biotec, Bergisch Gladbach, Germany). The positive fraction was used for DNA isolation as described elsewhere [17].

2.4.2. Quantification of maternal CD4+ cell microchimerism

Maternal microchimerism was quantified by real-time PCR (qPCR, TaqMan chemistry), as published by Willasch et al. [17]. Briefly, 29 sequence polymorphism markers were available for the screening of a maternal specific marker. After primer selection, amplification curves generated from artificial chimeric dilution series, consisting of maternal and foetal DNA, were used for quantification of maternal microchimerism (limit of detection of 0.01%). All probes were measured a minimum of two times. If the results of the two measurements of the same probe differed by less than one threshold cycle (Ct), they led to positive quantifiable or negative results. When the findings of a probe differed repeatedly by one Ct or more, the result was assessed as not quantifiable.

2.5. Extraction and amplification of HIV-specific nucleic acids

To determine whether HIV-specific nucleic acids were present in any of the tested compartments, whole DNA from CD4+ cells was used as a source of amplification with HIV-specific primers located in the *env* region (C2V5). The protocol described here has been used for routine genotypic tropism testing with either viral RNA or proviral DNA as the PCR template.

Whole DNA from isolated CD4+ cells was extracted using the QIAamp Viral RNA Kit (Qiagen) as described elsewhere [18].

Amplification of the C2V5 region was performed using the SuperScript™ One Step RT-PCR kit (Invitrogen) according to the manufacturer's recommendations. The reverse primers were 7791 m and 8001 m, and 6501 and 6631 were used as the forward primers. Cycling conditions were as follows: reverse transcription at 50 °C for 30 min, denaturation at 94 °C for 2 min followed by 30 cycles (94 °C 30 s, 50 °C 90 s, 72 °C 180 s), and a final step at 72 °C for 10 min. Afterwards, a nested PCR was run using the Platinum® Taq DNA Polymerase (Invitrogen) according to the manufacturer's recommendations with 7401 as the reverse primer and 7001 as the forward primer [19]. Amplification conditions were denaturation at 95 °C for 3 min, 40 cycles (95 °C 30 s, 55 °C 45 s, 72 °C 180 s), and a final step at 72 °C for 10 min.

2.6. Statistical analyses

The required sample size was calculated using preliminary data [20]. With respect to the measured maternal microchimerism of CD4+ cells in the Caesarean Section and the Spontaneous Group, a range of 0.26 was expected. From that, the calculated standard deviation was $\sigma = 0.065$ (R ~ 4 * SD). The two random check *t*-test ($\alpha = 0.05$, $\beta = 0.20$, power = 0.80) required 30 cases (n1 = n2 = 15). Micochimerism results were compared categorical with the Mantel–Haenszel test. Differences in dichotomous variable were calculated with Fisher's exact test, while ordinal and quantitative data were compared with the Wilcoxon

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