Maternal viral load and vertical transmission of HIV-1: an important factor but not the only one

The European Collaborative Study*

Objectives: To investigate the association between maternal RNA load, risk of vertical transmission of HIV-1, and other variables.

Methods: Plasma or serum samples from mothers of 373 children, enrolled in the prospective European Collaborative Study, were collected around time of delivery, and HIV-RNA quantified using two types of commercial assay. Women and children were followed according to a standard protocol. Adjusted odds ratios (AOR) were calculated to estimate the effect of RNA load and other maternal factors on vertical transmission.

Results: Maternal RNA levels, mode of delivery and gestational age were independently associated with transmission. Vertical transmission increased with increasing RNA levels, but there was no threshold below which transmission did not occur. The risk was more than double for women with RNA above the sample specific median [AOR 2.36 (1.23–4.52)]. Elective caesarean section was associated with a substantial and significant decrease in transmission [AOR 0.19 (0.06–0.55)], and delivery before 37 weeks gestation with an increased risk [AOR 2.67 (1.33–5.38)]. Elective caesarean section was effective in both subgroups defined by median RNA level [AORs 0.37 (0.08–1.71) and 0.15 (0.03–0.64) below and above median respectively]. The predicted rate of transmission in a woman with a low RNA load delivering by elective caesarean section or vaginally after 37 weeks is around 2%, and 11%, respectively.

Interpretation: Mother-to-child transmission of HIV-1 is multi-factorial; high RNA load is an important determinant but clearly not the only one. Interventions that target risk factors other than maternal RNA load remain important.

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Introduction

Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) has been associated with immunological and obstetrical factors [1-5], and more recently, with maternal RNA load [6-8]. Although it has been suggested that there may be a threshold level of maternal HIV-1 RNA below which transmission is unlikely [9,10], this has not been confirmed in most studies. In the American–French trial on using zidovudine to reduce vertical transmission, women with a wide range of RNA levels transmitted,

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including some with undetectable virus [11]. In this trial the reduction in viral load associated with zidovudine therapy accounted for less than 20% of the decrease in vertical transmission risk, and maternal viral load only moderately predicts vertical transmission for an individual woman [11,12]. This suggests that other factors may also play a role or that zidovudine has an effect on vertical transmission other than its effect on viral load [13]. It has recently been confirmed that elective caesarean section also substantially reduces the risk of mother-to-child transmission [14,15], although there is some debate as to its effectiveness in women who receive prophylactic antiretroviral therapy.

The aim of the present analysis was to determine the direct relationship between RNA load and mother-to-child transmission of HIV-1, through studying a large subset of women from a prospective cohort study, most of whom did not receive prophylactic antiretroviral therapy. In addition, the indirect association of RNA load with other maternal risk factors, assay type and sample material type, and these factors' independent effect on vertical transmission was investigated.

Methods

Study population and specimens

The European Collaborative Study (ECS) is a prospective study in which HIV-infected women are enrolled during pregnancy and their children followed from birth [1]. Informed consent is obtained according to local guidelines. Maternal blood samples for plasma or serum were collected according to a study protocol during a specified period from a subset of these women. Samples were frozen locally and shipped in dry ice to either of two designated central laboratories in Padua and Stockholm, where they were stored at -80^{o}C and tested for RNA in 1997/1998. In addition, maternal RNA load is now routinely measured in some ECS centres, and this more recent information was added to that from samples obtained earlier. Samples were collected between December 1987 and May 1998, with more than half being collected in 1992 and 1993.

HIV-1 RNA levels were determined by either reverse transcriptase polymerase chain reaction (RT–PCR) (Amplicor Monitor, version 1.0, Roche Diagnostic Systems, Basel, Switzerland), Nucleic Acid Sequence Based Amplification (NASBA HIV-1 RNA QT) assay (Organon Teknika, Oss, Holland) or second generation NASBA (NucliSens HIV-1 RNA QT, Oss, Holland) according to the manufacturers' instructions. For the Roche assay, three different batches of kits were employed. High positive, low positive, and negative controls included in the kit were run on each plate. Mean values of the positive controls were 5.57 $\log_{10}$ HIV-1 copies/ml +/– 0.11 standard deviations for the high positive control, and 3.84 $\log_{10}$HIV-1 copies/ml +/– 0.06 standard deviations for the low positive control. The maximum inter assay difference between the positive controls was 0.27 $\log_{10}$ for the high positive controls, and 0.19 $\log_{10}$ for the low positive controls. NASBA and NucliSens use the same primer sequences; these assays achieve amplification without a thermocyclic process and have three calibrators added to each tube, generating an individual calibration curve, from which the result is deduced. Each kit of the NASBA or NucliSens was accompanied with quality assurance documents according to ISO 9001 and EN 46001. The results of the RT–PCR and NASBA assay have been shown to be highly correlated [16,17]. Laboratory personnel were unaware of the infection status of the infants. Lower levels of detection were generally at the level of 200 copies/ml for Roche, 1000 copies/ml for NASBA and 400 copies/ml for NucliSens. The test variation for the NASBA/NucliSens is approximately 0.5 $\log_{10}$, according to the manufacturer. Specimens in which HIV-1 RNA could not be detected were assigned an arbitrary value of 10 copies/ml below the relevant assay's lower limit of quantification in order to include RNA load or $\log_{10}$RNA load as a continuous variable.

The present analysis includes data from 373 pregnancies, with samples collected either during pregnancy (n = 352) or within 2 months of delivery (n = 222). Of the nine twin pairs, second-born twins were excluded to avoid duplication of information. Separate pregnancies to the same mother were counted as two events. Where repeated maternal antenatal samples were available (120 women with 321 samples), the value taken at a time that was closest to delivery was used [4,5,18-20]. Of the 373 samples, 249 (67%) were taken on the date of delivery or within 1 week either side. Maternal CD4 cell count was similarly taken as the value closest to delivery. Prematurity was defined as delivery before 37 weeks; gestational age was usually assessed by ultrasound.

Infection status was determined as previously described [1]. Briefly, definition of HIV infection in the child was defined as persistence of antibody beyond 18 months, the development of AIDS or the detection of virus by culture or DNA-PCR in at least two separate samples [21]. A child who became antibody negative and had never had a positive HIV test was presumed to be uninfected. The remaining children were of indeterminate infection status.

Statistical analysis

Univariate comparisons of maternal characteristics by transmission status and HIV-1 RNA were tested for significance using the χ^2 test for categorised variables, the non-parametric Wilcoxon's rank sum test and t-test

for continuous variables, and the Kruskal–Wallis test and ANOVA where appropriate. Spearman's rank correlation was used to assess the relationship between continuous CD4 cell count and RNA load. Logistic regression, with infant infection status as the dependent variable, was used to obtain odds ratios (OR) and 95% confidence intervals (CI), both univariate and adjusted for potential confounders. Likelihood ratio tests were used for model selection. A Pearson χ^2 test was used to assess the goodness of fit of the model to the data. Several sensitivity and subgroup analyses were performed. Analysis was performed using SAS statistical software (SAS Institute, Cary, North Carolina, USA). All *P*-values are two-sided.

Results

The analysis is based on samples from 373 pregnancies to 364 women. The characteristics of the women included (Table 1) were similar to those previously described for the overall ECS cohort enrolled around the same time [22]. Only 79 women (21%) received zidovudine, reflecting the date of enrollment. These included 21 (27%) enrolled prior to 1994 who received treatment for clinical indications, and 58 (73%) enrolled after early 1994 who received prophylactic therapy to reduce vertical transmission [23]. In contrast, 90% of women enrolled in the ECS in 1997 received prophylactic therapy [24].

Of the 373 children, 56 (15%) were infected, 292 (78%) uninfected and 25 (7%) were of indeterminate infection status (1 neonatal death, 15 lost to follow-up, nine recent births). The infection rate is similar to the 16% previously reported for the ECS for a similar time period [1]. Twenty-two children developed AIDS, nine of whom died (eight from HIV related causes and one from another cause), and deaths occurred in a further three children without AIDS.

Detection rate related to assays and sample type

RNA levels were measured in 239 (64%) plasma and 134 (36%) serum samples, with 165 (44%) of the samples analysed using NASBA [29 (18%) NASBA and 136 (82%) NucliSens] and 208 (56%) using Roche. More serum samples were analysed using the Roche assay than plasma samples (75% versus 45%, χ^2 test $P < 0.001$). RNA levels in 77 (21%) of the 373 samples tested were below the limit of detection for the assay used. Samples assayed by Roche were more likely to have detectable virus levels than those assayed by NASBA/NucliSens (85% and 72%, respectively, $P = 0.002$). There was no association between sample material type and virus detection ($P = 0.48$).

Concern has been expressed that commonly used RNA assays may be less effective in detecting the HIV subtypes that are most prevalent in sub-Saharan Africa than in detecting subtype B which predominates in Europe. When comparing the RNA load obtained by Roche RNA-PCR assay version 1.0 and NASBA tests, both were deficient in detecting subtype A [25]. Of 67 samples from 65 black women from sub-Saharan Africa, nine were analysed using the Roche assay, none were below the limit of detection and RNA values ranged from 400 to 16,000 copies/ml. Of the remaining 58 samples analysed by NASBA/NucliSens, 19 (33%) were below detection (1000 copies for NASBA and 400 for NucliSens).

Table 1. Maternal characteristics.

	Total (%)
Mothers (n = 364)	
Mode of acquisition:	
History of injecting drug use	189 (57%)
Heterosexual contact only	140 (42%)
Blood products	4 (1%)
Unknown	31
Race:	
White	279 (77%)
Black	65 (18%)
Other	19 (5%)
Unknown	1
Pregnancies (n = 373)	
Year of delivery:	
1987–1991	81 (22%)
1992	97 (26%)
1993	96 (26%)
1994–1998	99 (26%)
Zidovudine in pregnancy:	
No	294 (79%)
Yes	79 (21%)
Mode of delivery:	
Elective caesarean	93 (25%)
Emergency caesarean	30 (8%)
Vaginal	232 (62%)
Instrumental	17 (5%)
Unknown	1
Premature delivery:	
No	279 (80%)
Yes	72 (20%)
Unknown	22
Age (years) of mother at delivery:	
≤ 25	135 (37%)
26–30	139 (38%)
> 30	90 (25%)
Unknown	9
Mean (range)	27 (16–40)
Time since diagnosis of HIV:	
≤ 2 years	197 (53%)
> 2–5 years	94 (25%)
> 5 years	82 (22%)
CD4 count cells × 10^6/l:	
< 200	42 (15%)
200–499	126 (44%)
≥ 500	115 (41%)
Unknown	90
Median (range)	450 (10–1580)
Maternal AIDS by delivery:	
No	362 (97%)
Yes	11 (3%)

HIV-1 RNA load

Based on either plasma or serum samples, the overall median RNA value for all women was 5500 copies/ml (range below detection to 2,100,000); and 10,000 copies/ml among women with levels detectable by the assay used. The median RNA value for samples assayed by NASBA was 6500 copies/ml (range below detection to 1,750,000) and for samples assayed using the Roche kit 5500 copies/ml (range below detection to 2,100,000). There was no evidence of a difference in $\log_{10}$RNA values in samples assayed by either Roche or NASBA/NucliSens (nor was there if RNA viral load was categorised as ≤ 1,000, > 1,000–≤ 10,000, > 10,000–≤ 100,000 and > 100,000, or below and above 10,000). Further, in each of the subsets of plasma or serum samples, there was no evidence of an appreciable difference in viral level values according to type of assay used.

The median RNA value for plasma samples was 8500 copies/ml (range below detection to 2,100,000) and 2700 copies/ml for serum samples (range below detection to 200,000). Plasma values were significantly higher than serum values overall (Wilcoxon's test $P < 0.0001$), and in each of the subsets of NASBA or Roche assay ($P = 0.03$ and $P = 0.001$ respectively). Although this could partly reflect year of sample collection, there was no clear trend in viral levels over time (χ^2_{trend} $P = 0.06$). This difference in sample type was taken into account in subsequent analyses.

RNA load and vertical transmission

The transmission rates for women with RNA values between 1000 and 10,000 copies/ml on NASBA and Roche testing were similar (16% versus 13%). Categorising RNA relative to the median values for plasma or serum, 9% of children born to mothers with a value less than or equal to (referred to subsequently as below) the median were infected compared to 23% of children whose mothers had a RNA load greater than (referred to subsequently as above) the relevant median value (χ^2 test P <0.001). Mothers with a RNA load above the sample specific median were more than 2.5 × as likely to transmit the virus to their infant than those with a RNA load below the median value (OR 2.8, 95% CI 1.5–5.3). Similar results were obtained if a cut-off value of 10,000 copies/ml was used (OR = 2.5, 95% CI 1.4–4.5). When four categories were used (≤ 1,000, > 1,000–≤ 10,000, > 10,000–≤ 100,000 and > 100,000), the respective transmission rates were 6.0%, 14.4%, 17.5% and 41.7% (χ^2_{trend} P <0.001).

The percentage of infected children was plotted against each value of $\log_{10}$RNA (rounded to the nearest half) (Fig. 1). Samples where RNA was not measurable are shown as a separate category, although in the modelling (see below) they were included as actual values with a value just below the appropriate cut-off point for detection, as discussed in Methods. There was a clear relationship between increasing RNA load and risk of transmission, although there were also infections occurring at low RNA values or values below detection.

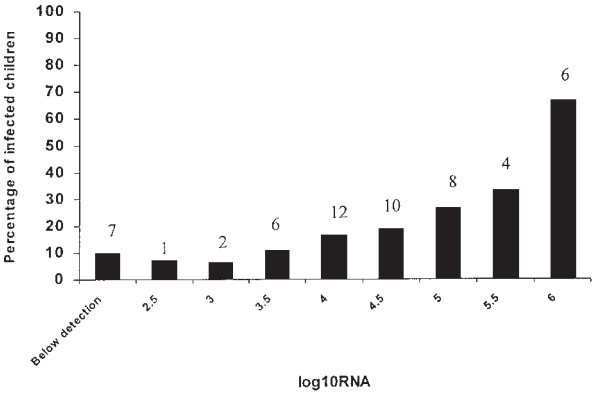

Fig. 1. Maternal peripheral RNA level and risk of transmission. Numbers above each bar represent the number of infected children.

If $\log_{10}$RNA was included in a logistic regression model the odds of infection increased by a factor of more than 2 for each unit increase in $\log_{10}$RNA (or each 10-fold increase in RNA) (OR = 2.06, $P < 0.0001$). The predicted rate of vertical transmission by $\log_{10}$RNA in this univariate model was 8.7% at 1000 copies/ml, and 16.3% at 10,000, 28.7% at 100,000 and 45.3% at 1,000,000 copies/ml.

Median RNA load was 3.86 × (0.59 $\log_{10}$) higher in transmitting women (18,000 copies/ml, range below detection to 2,100,000) than for non-transmitting women (4600 copies/ml, range below detection to 1,750,000). Thus, although values were significantly higher in mothers who transmitted ($P < 0.0001$), and there was a very clear trend of increasing RNA with increased rates of transmission, there was no threshold below which transmission did not occur. Of the 56 mothers who transmitted, seven (12%) had RNA values below detectable levels (Fig. 1). These seven samples were collected between April 1989 and January 1995, and had been stored for some time. However, the total 77 values below detection level for the assay used relate to samples taken between the end of 1987 and early 1998 and thus it is unlikely that degradation of samples during storage was a significant problem. Further, the median RNA value was lowest for samples collected most recently than for earlier samples.

Viral load and other maternal characteristics

We investigated the possible relationship of maternal factors with RNA load. These included race, zidovudine use in pregnancy, mode of delivery, maternal AIDS by delivery, premature delivery, maternal drug use in pregnancy, time since HIV diagnosis, CD4 cell

count, and duration of rupture of membranes. Lack of zidovudine in pregnancy and low CD4 cell count were most strongly associated with higher RNA load in univariate analyses. Mothers who received zidovudine during pregnancy were more likely to have an RNA load value below the median than those who did not receive antepartum therapy (70% versus 45%, $P < 0.001$). The median RNA load among treated women was 2000 copies/ml and among those not treated was 7000 copies/ml ($P < 0.001$). Of the 21 women who received zidovudine for clinical indications, 62% had a RNA load greater than the median and of the 58 receiving prophylactic zidovudine 19% had a load above the median. Mothers with CD4 cell count $< 500 \times 10^6$/l were less likely to have a RNA load below the median than those whose CD4 cell count was $\geq 500 \times 10^6$/l (37% versus 52%, $P < 0.02$). The median RNA level in the former group of women was 11,000 copies/ml and in the latter group was 4000 copies/ml ($P < 0.0001$). HIV RNA copy number was negatively correlated with CD4 cell count, with high RNA load being associated with low CD4 (correlation coefficient = 0.35, $P < 0.0001$). Moreover, viral RNA was above the relevant cut-off value in 41 of 42 (98%) women with CD4 cell counts $< 200 \times 10^6$/l, in 107 of 126 (85%) women with CD4 cell counts of 200–500 $\times 10^6$/l, and in only 91 of 115 (79%) women with counts of 500×10^6/l or greater (χ^2_{trend} $P = 0.006$).

There was some evidence that peripheral RNA load values were higher in white than in black women; 135 (47%) of 286 white women had an RNA value below the median compared to 42 (63%) of 67 samples from black women ($P = 0.023$). Among white women the median RNA value was 6000 copies/ml and among black women was 3500 copies/ml ($P = 0.05$). There was no evidence of a significant difference in CD4 cell count between white and black women, the medians being 460×10^6/l and 425×10^6/l, respectively ($P = 0.54$). In the ECS most of the black women would have acquired their infection in Africa.

Women with AIDS by the time of delivery had higher RNA loads, but because of the small number of women with AIDS (n = 11) and the association between maternal AIDS and low CD4 cell count this clinical variable was not considered further. RNA load did not vary significantly by mode of delivery, the median value being 3000 copies/ml for those undergoing elective caesarean section and 6500 copies/ml among women delivering by other modes ($P = 0.21$). RNA load in women delivering before 37 weeks did not differ substantially from that in women delivering at term; the medians were 7000 copies/ml and 5000 copies/ml, respectively ($P = 0.20$).

Vertical transmission

Vaginal and emergency caesarean section deliveries, prematurity, and low CD4 cell count were most strongly associated with infant's infection status in univariate analyses (Table 2). Children delivered vaginally or by emergency caesarean section were more likely to be infected than those delivered by elective caesarean section, with a reduction in risk of 79% associated with the latter ($P < 0.001$). Similarly, infants delivered before 37 weeks were more than twice as likely to be infected than infants who were not premature ($P = 0.01$), and infants born to mothers with a CD4 cell count greater than or equal to 500×10^6/l were nearly half as likely to be infected ($P = 0.07$). The median maternal CD4 cell count among mothers who transmitted was 380×10^6/l compared to 460×10^6/l among those who did not transmit ($P = 0.03$). Few women had a CD4 cell count less than 200×10^6/l: seven of 41 (17%) women with a count of $< 200 \times 10^6$/l transmitted compared to 39 of 227 (17%) with CD4 cell count $\geq 200 \times 10^6$/l (χ^2 test $P = 0.99$). Year of delivery (for the four categories 1987–1991, 1992,

Table 2. Maternal factors and vertical transmission.

	No. mother–child pairs	No. infected children (%)	χ^2 test *P*-value	Univariate models OR (95% CI)	RNA subgroup analyses[a]	Multivariate Model 1 OR (95% CI) n = 252	Multivariate Model 2 OR (95% CI) n = 329
Mode of delivery (n = 347)							
Vaginal and emergency CS	264	52 (20%)	$P < 0.001$	1.00	3.04 (P = 0.001)	1.00	1.00
Elective CS	83	4 (5%)		0.21 (0.07–0.59)	1.19 (P = 0.86)	0.29 (0.09–0.88)	0.19 (0.06–0.55)
Zidovudine use (n = 348)							
No	278	49 (18%)	$P = 0.121$	1.00	2.27 ($P = 0.017$)	1.00	–
Yes	70	7 (10%)		0.52 (0.22–1.20)	6.77 ($P = 0.031$)	1.43 (0.46–4.48)	–
Premature delivery (n = 330)							
No	264	35 (13%)	$P = 0.013$	1.00	1.83 ($P = 0.11$)	1.00	1.00
Yes	66	17 (26%)		2.27 (1.18–4.38)	6.22 ($P = 0.009$)	3.05 (1.38–6.72)	2.67 (1.33–5.38)
CD4 count (n = 268)							
< 500	160	33 (21%)	$P = 0.068$	1.00	2.49 ($P = 0.048$)	1.00	–
≥ 500	108	13 (12%)		0.53 (0.26–1.05)	3.86 ($P = 0.05$)	0.70 (0.33–1.49)	–
RNA viral load (n = 348)							
Below median	171	16 (9%)	$P < 0.001$	1.00	–	1.00	1.00
Above median	177	40 (23%)		2.83 (1.50–5.28)	–	2.61 (1.18–5.75)	2.36 (1.23–4.52)

[a]These are odds ratios (OR) of vertical transmission for viral load above sample type median compared to below median for each category of each variable. CI, Confidence interval; No., number; CS, caesarean section.

1993, and 1994–1998) was not associated with infection status of the child (χ^2 test $P = 0.54$).

Odds ratios for RNA load above the relevant median compared to below the relevant median on vertical transmission were calculated for different subgroups (Table 2). In each subgroup RNA load above the median was associated with a higher risk of vertical transmission than RNA load less than or equal to the median, but the difference was not always significant. As an example, in the vaginal and emergency caesarean section subgroup women in the higher RNA category were 3 × as likely to transmit whereas in the elective caesarean section subgroup their risk of transmitting did not differ significantly from that of women in the lower RNA category. However, although the odds ratios for maternal RNA load were lower in women delivering by elective caesarean section or those who delivered after 37 weeks, caution is needed in interpreting subgroup analyses because of the small numbers studied. Indeed, tests for heterogeneity of the odds ratio values were not significant, indicating that there were no significant interactions ($P = 0.38$, $P = 0.24$, $P = 0.12$, $P = 0.60$ for mode of delivery, zidovudine therapy, prematurity and CD4 cell count, respectively).

In addition, the effect of elective caesarean section on transmission risk was evaluated in women with an RNA load below and above the median. In the former the odds ratio relating mode of delivery and transmission was 0.37 (95% CI 0.08–1.71) ($P = 0.20$), and in the latter 0.15 (95% CI 0.03–0.64) ($P = 0.01$). Thus, elective caesarean section was associated with a reduced risk of transmission in both groups, although this did not reach statistical significance in the low RNA load group with only two of 16 infected children being delivered by elective caesarean section.

Multivariate logistic regression models

Mode of delivery, zidovudine use, prematurity, CD4 cell count and RNA load were included in a multivariate logistic regression model with infection status as the dependent variable (Table 2). Women delivering by elective caesarean section were less than a third as likely to transmit than women delivering vaginally or by emergency caesarean section. Women were more than 3 × as likely to transmit if they delivered prematurely, and they were more than twice as likely to transmit if their RNA viral load was above the relevant median value. Including CD4 cell count, gestational age or $\log_{10}$RNA as continuous variables made little difference to the size of the other odds ratios, or to the interpretation of the results.

The use of zidovudine was not associated with infection status; however, as previously stated, a sizeable proportion of women who received therapy were prescribed treatment for clinical indications [23]. Further, the number of treated women is small in this reduced sample. CD4 cell count was no longer associated with infection status in the presence of RNA load, a finding previously observed [19], since the sample size was substantially reduced when CD4 cell count was included, only mode of delivery, prematurity and RNA load were then included in the final model. This did not alter the magnitude of the odds ratios substantially (Table 2). As in the first multivariate model, including gestational age or $\log_{10}$ RNA as continuous variables made little difference to the results of the final model. Adding other variables into this model, such as race, centre or year of delivery also had little impact. Omitting values that were below detection level for the assay used resulted in the sample size being reduced to 263 mother–child pairs, but again made little difference to the results. This final model was also repeated based on only those deliveries occurring more than 18 months prior (thus excluding 18 cases, all uninfected); mode of delivery, premature delivery and RNA load remained significant with the odds ratios relatively unchanged. A model including $\log_{10}$RNA, mode of delivery, prematurity, and additionally terms representing assay and material type was considered; odds ratios and *P*-values for RNA load, mode of delivery and prematurity remained at similar values with material and assay used being non-significant factors.

Observed and predicted probabilities of vertical transmission were examined for each combination of RNA viral load, mode of delivery and prematurity, with predicted probabilities determined from the reduced logistic regression model described (Table 3). This indicates that the risk of transmission for a woman with a low RNA load delivering by elective caesarean section

Table 3. Observed and predicted probabilities of vertical transmission for all combinations of covariates defined by the final model.

RNA viral load	Mode of delivery	Premature	No. mother–child pairs	No. infected children	Probability of vertical transmission as observed	Probability of vertical transmission as predicted
Below	Elective CS	No	29	2	0.069	0.022
Above	Elective CS	No	30	1	0.033	0.049
Below	Elective CS	Yes	16	0	0	0.056
Above	Elective CS	Yes	8	1	0.125	0.123
Below	Other	No	103	11	0.107	0.106
Above	Other	No	101	21	0.208	0.218
Below	Other	Yes	15	3	0.200	0.239
Above	Other	Yes	27	13	0.481	0.427

after 37 weeks, for example, could be as little as 2%, and for a woman delivering vaginally in this group approximately 11%. Women with peripheral RNA levels above the median, with an infant delivered vaginally before 37 weeks had an estimated risk of transmission of 43%, but those with RNA levels below the median who delivered vaginally of a premature infant had a predicted risk of 24%. Overall model fit was very good (Pearson χ^2 4.29, 4 df, $P = 0.37$).

Discussion

In this large subset of women enrolled in the ECS, peripheral HIV RNA levels were significantly higher in women who transmitted the virus than among those who did not. When RNA copy numbers in the mothers increased 10-fold stepwise from less than 1000 to over 100,000 copies/ml, the percentage of infected children increased from 6%, 14%, 17% to 42%. These rates are similar to those reported by Burns *et al.* [26]. After adjusting for mode of delivery and prematurity we found that children born to mothers with a RNA load above the relevant plasma or serum median were more than twice as likely to be infected. When peripheral RNA load was taken into account CD4 cell count was no longer important. If a threshold could be identified below which vertical transmission is unlikely to occur then it could be argued that interventions to reduce transmission such as elective caesarean section, would not be justified. However, in agreement with other reports [6–8], we could not confirm the presence of a threshold [9,10]. Although in this analysis vertical transmission was not found to be associated with zidovudine treatment, this is likely to be due to the small number of women who received prophylactic zidovudine [13,24]. Women who received therapy to reduce transmission had a lower viral load than those who received therapy for clinical indications.

Vaginal and emergency caesarean section delivery and delivery before 37 weeks were independently and significantly associated with vertical transmission in addition to high RNA load in plasma or serum. This suggests that interventions such as elective caesarean section and avoidance of premature delivery remain important [6]. Our results add substantially to the recent findings from a randomised mode of delivery trial [14] and a large meta-analysis [15], which showed a substantial reduction in vertical transmission occurring with elective caesarean section both in women who received zidovudine and those who did not, but information on maternal RNA load was not available in these studies.

A possible limitation of our study is that both serum and plasma samples were collected and RNA load quantification performed using different kits. However, we found no evidence of a difference in RNA load values between the different assays used. This is perhaps not surprising as most samples assayed by NASBA used the second generation kit where the lower level of detection is close to the lower level of detection for the Roche test, and the different assays have been shown to be highly correlated [16,17]. We found plasma RNA levels to be higher than serum levels, which has previously been shown for the Roche and branched DNA (bDNA) assays [27,28], and marginally for NASBA [29,30], and therefore allowed for sample type in the analysis. Different handling and processing of the specimens may also have had some impact on the results. Samples were collected over a considerable length of time, with some of the samples collected several years ago, but detection was not related to year of delivery, and it is unlikely that the rate of degradation would have differed between transmitting and non-transmitting mothers. Ginocchio *et al.* [30] reported that sample collection, storage conditions and specimen processing only marginally affect RNA quantification. About a fifth of our samples had undetectable virus in the assays used, a rate similar to that reported by others [7,8]. Current HIV RNA assays have detection limits in the order of 50 copies per ml, as compared to 1000 copies per ml in the first generation NASBA assay used for some of the samples included in our study, and their ability to quantify non-B subtypes has improved.

Lack of zidovudine therapy and low CD4 cell count were both strongly associated with high peripheral RNA load, but we did not observe an association between RNA load and gestational age [19]. However, in infants born before 37 weeks high maternal RNA load was strongly associated with vertical transmission which provides further indirect evidence for the finding by Kuhn *et al.* [31], that infants born prematurely were more likely to have evidence of intrapartum transmission. We found high RNA load to be slightly less associated with transmission risk in women with a low CD4 cell count than in those with a count above 500×10^6/l, similar to other studies [7,26]. It has been suggested that until immune defences are compromised a high RNA load is required for vertical transmission, but that in the presence of severe immunosuppression virus load becomes less important. This may suggest that infected women with less advanced disease would benefit most from efforts to decrease RNA load in order to reduce vertical transmission.

Our results support the multi-factorial nature of perinatal transmission whereby high viral load in plasma or serum is an important determinant of transmission but clearly not the only one. Allowing for zidovudine use, CD4 cell count and RNA levels, mode of delivery and gestational age were also independently associated with infection in infants. This supports the need to evaluate

other approaches in addition to antiretroviral therapy to reduce mother-to-child transmission [13,32,33]. We have previously hypothesised that zidovudine treatment may decrease the risk of premature delivery, and thereby have an effect on transmission risk in addition to that due to reducing viral load [13]. Our data suggest that an elective caesarean section delivery may be beneficial in reducing the risk of vertical transmission even among women with low viral load. Indeed, the predicted probability of vertical transmission in a woman with a low viral load delivering by elective caesarean section after 37 weeks was as little as 2%, and in those delivered vaginally in this group as much as 11%.

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Appendix

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