Neonatal antiretroviral prophylaxis and haematological toxicity in infants at high risk for mother-to-child transmission of HIV in Europe

European Pregnancy and Paediatric HIV Cohort Collaboration in EuroCoord

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Abstract

Background. Combination neonatal prophylaxis (CNP) is recommended in high risk situations for the prevention of mother-to-child HIV transmission, although data on its safety are limited. To identify whether neonatal prophylaxis (NP) type is associated with severe anaemia or neutropenia in the first 6 months of life, and with haemoglobin (Hb) level and neutrophil count (NC) at ages 0-18 months.

Methods. An individual patient-data meta-analysis was conducted within 6 European cohorts, on infants at high risk for acquiring HIV. Adjusted logistic regression models were conducted to assess risk of DAIDS grade 3-4 anaemia/neutropenia at ages 0-6 months. Mixture models of Hb levels and log10-transformed NC explored associations with NP type at ages 0-18 months.

Results. Of 1836 infants, 25% were preterm, 1149 (63%) had antenatal cART exposure and 395 (22%) received CNP (125 with 3 drugs). Overall, 117 (6.7%) infants had grade 3-4 anaemia at age 0-6 months and 140 (9.1%) had grade 3-4 neutropenia. Grade 3-4 anaemia or neutropenia were not associated with NP type (aOR 1.04 for 1 drug and 1.60 for 3 drugs vs. 2 drug NP; p= 0.879 and p= 0.277 for anemia and aOR 1.33 for 1 drug and 1.98 for 3 drugs vs. 2 drug NP; p= 0.330 and p= 0.113 for neutropenia), but were associated with preterm delivery. Overall 7746 Hb and NC results were available for 1836 infants up to age 18 months; no significant differences in predicted Hb levels or NC were apparent by NP type .

Conclusions. A small proportion of infants experienced grade 3-4 haematological toxicity in their first 6 months of life; risk of anaemia or netropenia was not associated with type of NP.

INTRODUCTION

Universal antenatal HIV testing, combined antiretroviral therapy (cART) during pregnancy, labour and delivery, neonatal antiretroviral prophylaxis (NP), elective caesarean section (CS) for women without optimal viral suppression near delivery and the avoidance of breastfeeding have led to a dramatic decline in the number of perinatally HIV-infected children: nowadays in the United States as well as in Western Europe, mother-to-child transmission (MTCT) rates are below 1% [1,2,3]. However, there remain missed opportunities for prevention of MTCT (PMTCT) in these settings, including late diagnosis of HIV infection in pregnant women and failure to control viral replication during pregnancy due to inadequate or lack of cART, and low adherence [2, 4, 5].

In most cases, NP consists of zidovudine (ZDV) monotherapy for 4-6 weeks [6]. International guidelines recommend the use of combination NP (CNP) with two or three antiretroviral drugs (ARVs) in specific high risk situations [6]. However, the optimal prophylactic regimen and the additional efficacy of CNP in reducing MTCT risk in such situations are not well understood. CNP was found to be superior to one drug NP in a randomized trial (NICHD HPTN 040/PACTG 1043) conducted in exclusively formula-fed infants born to women that had not received ARVs during pregnancy [7], but data in other high risk situations are limited. Furthermore, there is some controversy regarding whether high risk newborns should receive therapeutic rather than prophylactic doses of ARVs. This is based on increasing evidence of the benefits of very early cART initiation in perinatal infection with respect to restricting the viral reservoir [8, 9, 10].

We previously showed that the use of CNP in high-risk situations is increasing in Europe [4]. Data regarding safety of NP, particularly CNP, are limited both in term and, concerningly, preterm infants. Haematologic toxicity associated with *in utero* or early life exposure to ARV drugs has been well established with some studies demonstrating that infants exposed *in utero* to cART have lower

haemoglobin (Hb) levels and neutrophil counts (NC) than those exposed to ZDV monotherapy or without ART exposure [7, 11, 12, 13, 14, 15, 16]. In the HPTN 040 trial, in which infants had no *in utero* ART exposure, neutropenia (grade 2 or above) was more common in the three-drug arm than in the ZDV/nevirapine (NVP) or ZDV only arms, although there was no significant difference with respect to anaemia [7].

In an individual patient data meta-analysis of data collected from an European cohort collaboration, our aim was to examine the haematological toxicity in infants born to women with HIV at high risk of MTCT, specifically to identify whether NP type is associated with 1) severe or potentially life-threatening anaemia or neutropenia within the first six months of life and 2) haemoglobin level and neutrophil count at ages 0-18 months.

METHODS

The European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC) includes a network of cohorts of prospectively observed pregnant HIV-infected women and their infants. In an earlier pooled analysis [4], we investigated the use and effectiveness of CNP in infants born to HIV-infected mothers between 1 January 1996 and 30 June 2010 and at high risk for acquiring HIV infection, according to the US Guidelines [6] (i.e. those born to mothers who had received antenatal and intrapartum ARVs but had suboptimal viral suppression at delivery; or had received only intrapartum ARVs; or had received no antenatal or intrapartum ARVs). Additional inclusion criteria were prospective follow-up since birth and known HIV infection status. Breastfed infants were excluded.

In the present study, a sub-analysis of the original dataset was performed in order to investigate the occurrence and severity of haematological adverse events (specifically, anaemia and neutropenia) related to NP. The sub-study was limited to the subset of infants with at least one measurement of Hb levels or NC in the first 18 months of life. The six cohorts participating in the sub-study were: the Italian Register for HIV infection in children (ITLR); the Madrid Cohort of HIV-infected Children; the Catalan Cohort of HIV-infected Children (CoRISPE-Cat); the 'Victor Babes' Hospital Cohort, Bucharest, Romania; the Swiss Mother and Child HIV Cohort Study (MoCHiV) and the European Collaborative Study (ECS) (considered as Western-ECS and Ukraine-ECS).

Cohorts provided anonymized data according to a standard operating procedure, submitted using the HIV Cohorts Data Exchange Protocol as previously described [17]. Data collected included variables on socio-demographics, delivery, laboratory results and treatment/prophylaxis. Data on NP included type/number of drugs, timing and duration. Each participating cohort was responsible for ensuring that ethics approval for the analysis was in place and for compliance with local and national data protection requirements.

Definitions

HIV infection was diagnosed by the persistence of HIV antibodies after 18 months or, before 18 months by DNA or RNA polymerase chain reaction assay (PCR) on at least two occasions [4]. NP was defined as any course of one or more ARVs administered with prophylactic purpose and initiated within the first 72 hours of life, CNP being a combination of two or more ARVs. Maternal viral load and CD4 count at delivery were defined as the closest measurements prior to delivery within 8 weeks.

Anaemia and neutropenia definitions: Hb levels and NC were graded according to the revised 2004 paediatric clinical trial toxicity tables developed by the National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS) [18].

Statistical analyses

Proportions were compared using X^2 or Fisher's exact test and medians using Wilcoxon Mann–Whitney U tests. All significant tests were two-sided. Nadirs for Hb levels and NC within the first six months of life were calculated.

Univariate and multivariable logistic regression analyses

Factors potentially associated with occurrence of severe or potentially life-threatening (grade 3 or grade 4) anaemia and neutropenia versus grade 0-1-2 (considering for each study subject the nadir value in the first six months of life) were explored in univariable and multivariable logistic regression analyses. Factors examined in univariable analyses were birth period (1996-2000, 2001-2005, 2006-2009), sex, preterm delivery (≤32, 33–36, ≥37 weeks), intrapartum intravenous ZDV use, maternal viral load (<50, 50–399, ≥400 copies/mL), antenatal ART (none, 1 drug, 2 drugs, cART), maternal CD4

count (≤200 or >200 cells/µL) and NP type (none, one, two or three drugs), antenatal ART duration (≤ 4 weeks; > 4 weeks); NP duration (≤ 28 days; 29-49 days; none).

The final models included NP type, plus several variables included *a priori* (cohort [included as a random effect], birth period, antenatal ART, NP type, and infant HIV status) and factors associated with risk of the outcome in univariable analysis (if $p<0.1$).

A further analysis was conducted in the subgroup of children receiving antenatal cART, including cART type (PI based; NNRTI- based; PI+NNRTI based, only NRTI or other classes other than NNRTI or PI), adjusting for the same factors as the main model.

Missing data analysis

There was a substantial proportion of missing data in our dataset. Therefore, a multiple imputation by chained equations (MICE), was used to impute missing data (gestational age, maternal CD4 count at delivery, maternal viral load at delivery, intrapartum IV ZDV prophylaxis, children therapy exposure, in utero ART exposure, maternal region of origin, and delivery method).

We assumed that missing data were missing at random (MAR). After an initial 5 imputation to test convergence, we increased imputation to 500. We carried out imputation sensitivity analysis and checked the fit of the imputation model using the Stata command "Midiagplots". For all analyses using imputed data, estimates were combined across the imputed datasets based to Rubin's rules. Finally, the multivariable analyses were repeated on the 500 imputation generated datasets.

Longitudinal analysis

Two mixed models were performed including all Hb levels or NC for each patient within his/her first 18 months of life to explore associations between these haematological markers and NP type, using *xtreg c*ommand in STATA. The used time variable was age, expressed as 6-month periods obtained by calculating calendar differences between the birth date and the blood test date. A Log_{10} transformation for NC was performed. Associations between the potential predictors of laboratory

results over time were modelled using repeated measures generalized estimating equation models that account for correlations between measures within each subject. Correlations within subjects were modelled using the exchangeable correlation structure. The following variables were included in the models as well as NP type: gestational age, antenatal ART, maternal viral load at delivery, and infant HIV status, delivery method. The parameters of the model can be interpreted as population-averaged effects on each respective laboratory measure over time.

Statistical analyses were performed using the STATA/SE version 13.0 software package (Stata Corporation, College Station, Texas, USA).

RESULTS

Population characteristics

Laboratory investigations results for Hb level and/or NC up to 18 months of age were available for 1836 infants. Characteristics of the study subjects are summarized in Table 1.

More than 60% of infants (n=1149) had been exposed to some cART in utero (Table 1); of these, 1.31% (15/1149) had ≤4 weeks antenatal exposure and 90.2% (1036/1149) >4 weeks, with 98 (8.5%) having unknown *in utero* ARV exposure.

Of the 395 infants receiving CNP (Table 1), 270 (68.3%) received two drugs, 125 (31.6%) three drugs and no specific information was provided in 2 cases. Among the two drug group, ZDV plus sdNVP was administered to 124 infants (45.9%), ZDV plus 3TC to 123 (45.6%) and other regimens were used in the remaining 23 infants. In the three drug group, ZDV/3TC/NVP predominated (111/125, 88.1%), with 11 (8.8%) infants receiving a PI-based regimen (nelfinavir=4; lopinavir/ritonavir=7) and the remaining ~~four~~ three infants receiving other regimens. Median duration was 5.7 weeks (IQR 4–6) for one drug NP and 5.9 weeks (IQR 4–6) for CNP . Median duration of neonatal prophylaxis was available for 1228/1350 (90.3%) children receiving one drug NP and 378/395 (95.0%) children receiving CNP .

Anaemia or neutropenia in the first six months of life

Nadir Hb level and NC up to age six months were available for 1737 and 1544 infants respectively, with corresponding DAIDS grades reported in Table 2 stratified by NP type and gestational age. Overall, 117 (6.7%) infants had Grade 3-4 anaemia in their first six months (4.8% [40/827] in term and 9.6% [77/803] in preterm infants). A total of 140 (9.1%) infants had Grade 3-4 neutropenia (8.8% [64/728] in term and 10.3% [75/725] in preterm infants). A higher , but not significantly, proportion of infants receiving three drug CNP had Grade 3-4 haematological toxicity compared with those

receiving two drug CNP or one drug NP (9.1% *vs*. 6.9% for anaemia and 13.5% *vs*. 9.5% for neutropenia; Table 2). Sensitivity analysis for multiple imputation model has been reported in Appendix Table 1.

In unadjusted logistic regression analysis, birth period, gestational age, and delivery viral load were significantly associated with increased risk of Grade 3-4 anaemia (Table 3 and appendix Table 2) and of Grade 3-4 neutropenia (Table 4 and appendix Table 3), whilst antenatal ART exposure was only significantly associated with increased risk of anaemia .

In multivariate analyses, grade 3-4 anaemia was not associated with NP type (aOR 1.04 for 1 drug and 1.60 for 3 drugs *vs.* 2 drug NP; *p*= 0.879 and *p*= 0.277), but was with preterm delivery (aOR 2.10; p<0.0001 for 33-36 and 2.43; *p*=0.017 for ≤32 weeks *vs.* term) and maternal therapy (aOR 4.60 for two drugs *vs.* no drug; *p*<0.0001) and with birth period (aOR 0.53 for 2006-2009 vs. 1996-2000 *p*<0.047) (Table 3). In multivariate analyses, grade 3-4 neutropenia was not associated with NP type (aOR 1.33 for 1 drug and 1.98 for 3 drugs *vs.* 2 drug NP; *p*= 0.330 and *p*= 0.113), but was with preterm delivery (aOR: 2.94; *p*=0.001 for ≤32 weeks *vs.* term), maternal origin (aOR:2.60; p=0.010 for not European vs. European mothers) , delivery methods (aOR:1.57; p=0.047 for caesarean vs. vaginal delivery) (Table 4).

In a sub-analysis including only in infants exposed to cART in utero no significant different risk for grade 3-4 anaemia or neutropenia was observed by type on neonatal prophylaxis (Appendix. Tables 4 a) and 4 b)). In a sub-analysis restricted to uninfected infants without *in utero* ART exposure, grade 3-4 anaemia was observed in 11/254 infants and grade 3-4 neutropenia in 17/227 infants. In univariate analysis, preterm delivery was the only factor significantly associated with grade 3-4

anaemia (OR:10.26; 95%CI: 2.62-40.22; *p*=0.001). Factors associated with grade 3-4 neutropenia were gestational age (OR:7.38; 95%CI:1.63-33.42; *p*=0.009) and maternal origin (OR:3.31; 95%CI:1.02-10.76; *p*=0.047).

No difference was found considering grade 3-4 anemia or neutropenia in this sub-analysis for CNP *vs*. no NP (5/131 [3.82%] *vs*. 5/90 [5.55%]; *p* =0.551, and 5/114 [4.38%] *vs*. 9/81 [11.1%]; *p*=0.214). However, among infants receiving CNP, we observed that grade 3-4 neutropenia occurred more frequently in those receiving three *vs.* two drug NP (8/47 [17.0%] vs 1/34 [2.9%]; p=0.047); while this was not observed considering grade 3-4 anemia (4/51 [7.8%] vs. 1/39 [2.6%]; p=0.267).

Neonatal prophylaxis exposure and haematological markers in the first 18 months of life

Overall 7746 blood exams results were available for 1836 infants within the age range 0-18 months; the median number of determinations was 3 (IQR: 1-6) overall. Observed and estimated Hb levels and NC according to age and NP type are presented in Figures 1 and 2.

No significant differences in predicted Hb levels or the predicted NC in the first 18 months of life were apparent by NP type (Hb level: coefficient -0.189 [95% CI: -0.38 to 0.007], *p*= 0.102, considering CNP *vs*. one drug NP; coefficient -0.35 [95%CI: -0.75 to 0.05], *p*=0.090 considering no NP *vs* one drug NP; NC: coefficient 0.02 [95% CI: - 0.01 to 0.03], *p*= 0.178) considering CNP *vs*. one drug NP; coefficient -0.03 [95%CI: -0.10 to 0.04], *p*=0.366 considering no NP *vs* one drug NP). (Appendix Table 5)

DISCUSSION

In this large multicentre individual patient data meta-analysis, haematological toxicity of CNP in infants at high risk for perinatal HIV infection was evaluated. A minority of infants experienced grade 3-4 hematological toxicities in their first 6 months of life (6.7% for anemia and 9.1% for neutropenia). Anemia or neutropenia risks were not associated with NP type; while factors independently associated with haematological toxicity were preterm delivery, maternal viral load >400 copies/mL at delivery, and maternal ART (the latter only for anaemia). Results were similar, in the sub-analyses considering only infants born to mothers treated with combined ART with three or more drugs during pregnancy.

Despite the increasing use of CNP in high risk situations, available safety data are limited. Transient hematological toxicity has been reported with the use of ZDV for MTCT prophylaxis, and some studies have reported an increased toxicity associated with the addition of a second or a third drug. In the HPTN 040/PACTG 1043 trial, 1,684 formula-fed infants born to women with a peripartum diagnosis of HIV infection, were assigned to one of three NP regimens: ZDV for 6 weeks (ZDV-alone group), ZDV for 6 weeks plus three doses of NVP during the first 8 days of life (two-drug group), or ZDV for 6 weeks plus nelfinavir and lamivudine for 2 weeks (three-drug group). Serious adverse events possibly related to study drugs were observed in 8.4% of infants, with higher rates in the three-drug group (12.2% and 4.9%) than in the ZDV-alone group (6.9% and 3.7%) or the two-drug group (6.2% and 1.8%). Neutropenia and anaemia accounted for the majority of serious adverse events; in particular Grade 2 or more neutropenia occurred in 16.4% infants receiving only ZDV, 15.0% in those receiving two drugs, reaching 27.0% in infants receiving three drugs ($p<0.0001$) [7].
In a retrospective multicenter Canadian study [19] involving 148 infants at high-risk for MTCT receiving CNP using therapeutic doses and 145 infants at low risk receiving ZDV, hematological and

growth parameters at birth, one and six months of age were evaluated. The authors concluded that CNP was generally well tolerated, but reported that 10.2% of the CNP group had potential treatment-related adverse effects (non-specific signs and symptoms, including rash, vomiting, diarrhoea, and irritability) versus none of the ZDV monotherapy recipients; furthermore, treatment was discontinued more frequently in the CNP group. In adjusted analysis, infants receiving CNP had lower Hb levels in the first six months of life compared the ZDV group (p=0.04), but there were no differences between groups for absolute NC.

In a retrospective US study [20] on 148 HIV-exposed uninfected infants, including 36 receiving CNP (most common regimen was ZDV, lamivudine, and NVP) no difference in the AE rates was observed between infants receiving three drug CNP and those receiving ZDV alone: 84% *vs.* 66% developed a grade ≥1 AE, and 11% *vs.* 17% developed a grade ≥3 AE. However, the combination of ZDV with lamivudine and NVP resulted in an increased frequency of low-grade anemia (50% *vs.* 39%). A recent small study of 33 high risk newborns prescribed NVP+ZDV+lamivudine at treatment doses within 72 hours of birth for PMTCT [21], showed that anaemia, neutropenia and hyperlactatemia were the most frequent AEs; although these were mainly mild to moderate, there were some grade 3/4 events [21].

It is difficult to compare our results with the published literature, since different toxicity grades have been used as endpoints, but our findings confirm that CNP is generally well tolerated and should be considered for newborn infants deemed at high risk of perinatally acquired HIV infection with no significant risk of serious haematologic effects. In particular, caution should be used in the case of preterm infants (who made up a quarter of our study population), particularly those born before 32

weeks gestation. This is particularly an issue when considering therapeutic doses for prophylaxis due to increased drug exposure in very preterm and / or low birth weight infants [21].

In infants not exposed to HIV in utero anemia of prematurity is common, as well as neutropenia, thus it is not surprising that prematurity remains the major risk factor for these two conditions in our study [23].

Although we did not have dosing data in our study, it is likely, given the time period during which these infants were born (all before 2011), that they would have received prophylactic and not therapeutic doses of drugs within CNP. Many questions still remain regarding the use of therapeutic doses in high risk neonates for prophylactic purposes, including pharmacokinetic and safety issues. Some of these will be addressed by the IMPAACT P1115 trial, which is investigating the effects of early intensive ART on achieving HIV remission among infants with in utero HIV infection.

We did not find a correlation between NP duration and grade 3-4 anemia or neutropenia. Conversely other authors suggested that a shorter NP may be associated with reduced incidence of haematological toxicity [23]. In a recent Spanish study, significantly higher risk for macrocytic anaemia, expressed as mean corpuscular volume (MCV), was observed among infants born to HIV infected mothers treated with cART and receiving NP with ZDV for 4 or 6 week ZDV [23]. Differences among these findings may be due to the fact that we analyse only Grade 3-4 events, and not MCV, in order to individuate severe manifestations possibly related to NP. We may speculate that shorter ZDV NP may be associated with reduced haematological toxicity, but this latter is not severe enough to be evidenced when analysing Grade 3-4 events.

In the same study, comparing two periods of exposure (2000–2001 and 2007–2013), authors reported lower incidence of anaemia and neutropenia in the second period, with higher frequency of adverse effects when using maternal regimens containing AZT [23]. Similar results were reported in a recent Brazilian study including 787 HIV exposed newborns, 25% of them presenting with anaemia at birth [24]. The risk of anaemia was associated with exposure to maternal regimens containing AZT (comparing with tenovofir) and to preterm birth [24]. Unfortunately, the small number of infants treated with CNP prevented the comparison with one-drug-NP [24]. Differently, in our analysis maternal ART type was not related to haematological toxicity, at multivariate analyses. However, it was not possible compare maternal ZDV-sparing regimens to those including ZDV due to low numbers of ZDV-sparing regimens.

Our study had several limitations. Firstly, infants received heterogeneous NP regimens and analysis by prophylaxis type was not possible. Moreover, we focused specifically on haematologic AE without considering the effect on mitochondrial, respiratory, cardiovascular, gastrointestinal systems and on infant's growth in general, since such data were not available. There are potential differences in the patient population, due to different heterogeneous scenarios in European countries which may have influenced outcomes. Given the large proportion of missing data, we performed a multiple imputation analysis, generating a large amount (500) imputation datasets.

We may not exclude channelling bias since CNP may have been preferentially prescribed to selected groups of infants (i.e. those with increased MTCT risk). In order to minimize this bias several types of adjusted analyses have been performed, whose results were very similar, corroborating our findings. However, residual confounding might persist, even after adjusting the analysis for several possibly influencing factors.

Also, we observed several differences between the original study population [4] and the present sub-study sample (i.e. a higher proportion of mothers receiving c ART, of mothers with undetectable VL, and higher proportion of caesarean deliveries). We may speculate that the subgroup of children for whom blood test results were available were born from mothers with better adherence to MTCT strategies. Unfortunately, information regarding maternal and child compliance was not available. Thus it is possible that our population is not representative of the entire population of high risk infants born to HIV-infected mothers. However, this fact should have overestimate the AE incidence, since the sub-study infants would have been more exposed to (pre and post-natal) ART than those included in the original study. Finally, type one error cannot be excluded given the high number of p-values calculated in this study.

In conclusion, in this population of infants at high risk of perinatal infection born in Europe to women with uncontrolled HIV replication, NP type was not associated with severe or potentially life-threatening haematological toxicity, and CNP appeared to be relatively safe.

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Table 1. Characteristics of the study population

		N (n =1836)	%
Sex	Male	969	52.8
	Female	863	47.0
	Missing	4	0.2
Maternal region of origin	Europe	1051	57.2
	Sub-Saharan Africa	59	3.2
	Other	15	0.8
	Missing	711	38.8
In utero ART exposure	None	365	19.9
	1 drug	155	8.4
	2 drugs	133	7.2
	cART	1149	62.6
	Missing	34	1.9
Maternal viral load at delivery (copies/mL)	<50	409	22.3
	50-399	444	24.2
	400-999	105	5.7
	≥ 1000	341	18.6
	Missing	537	29.2
Maternal CD4 cell count at delivery	≥ 200 cells/µL	1184	64.5
	<200 cells/ µL	152	8.3
	Missing	500	27.2
Delivery method	Caesarean	1143	62.2
	Vaginal	677	36.9
	Missing	16	9.9
Intrapartum IV ZDV prophylaxis	No	385	21.0
	Yes	1071	58.3
	Missing	380	20.7
Intrapartum sdNVP prophylaxis	No	1281	69.9
	Yes	132	7.1
	Missing	423	23.0
Gestational age (weeks)	≥ 37	1336	72.8
	33-36	384	20.9
	≤ 32	84	4.6
	Missing	32	1.7
Birth Weight (g)	≥ 3,000	672	36.6
	2,500-2,999	664	36.2
	2,000-2,499	301	16.4
	1,500-1,999	108	5.9

	<1,500	43	2.3
	Missing	48	2.6
Neonatal prophylaxis	One drug	1350	73.5
	Two drugs	270	14.7
	Three drugs	125	6.8
	None	64	3.5
	Missing	24	1.3
HIV-Infected	No	1736	94.5
	Yes	100	5.4
Birth Period	1996-2000	476	25.9
	2001-2005	832	45.3
	2006-2010	528	28.8
Cohort	Catalan	575	31.3
	Ukraine-ECS	154	8.4
	ITLR	212	11.5
	Madrid	85	4.6
	Victor Babes' Hospital	46	2.5
	MoCHiV	426	23.2
	Western-ECS	338	18.4

Table 2. Anaemia and neutropenia grades in the study population, expressed as nadir value within the first 6 months of age, by number of drugs in the neonatal prophylaxis regimen and gestational age

* 78 infants received no NP or this information was missing

** 67 infants received no NP or this information was missing

[#] 29 infants missing GA (16 in one drug, 9 in two drugs and 4 in three drugs group)

[##]24 infants missing GA (14 in one drug, 6 in two drugs and 4 in three drugs group)

	One drug NP			**Two drugs NP**			**Three drugs NP**			
	Grade 0-1-2	Grade 3-4	Total	Grade 0-1-2	Grade 3-4	Total	Grade 0-1-2	Grade 3-4	Total	
Anaemia (n=1737)*; N (%)										
All[#]	1185 (93.1)	88 (6.9)	1273 (100)	247 (93.2)	18 (6.8)	265 (100)	110 (90.9)	11 (9.1)	121 (100)	χ^2=0.832 p=0.659
≤32 weeks GA	41 (87.2)	6 (12.8)	47 (100)	15 (93.8)	1 (6.3)	16 (100)	10 (83.3)	2 (16.7)	12 (100)	χ^2=0.7745 p=0.679
33-36weeks GA	536 (91.2)	52 (8.8)	588 (100)	78 (89.7)	9 (10.3)	87 (100)	46 (86.8)	7 (13.2)	53 (100)	χ^2=1.211 p=0.546
≥ 37 weeks GA	592 (95.2)	30 (4.8)	622 (100)	145 (95.8)	8 (5.2)	153 (100)	50 (96.2)	2 (3.8)	52 (100)	χ^2=0.1622 p=0.922
Neutropenia (n=1544)**; N (%)										
All[##]	1050 (90.5)	110 (9.5)	1160 (100)	197 (92.5)	16 (7.5)	213 (100)	90 (86.5)	14 (13.5)	104 (100)	χ^2=2.8832 p=0.236
≤32 weeks GA	34 (85.0)	6 (15.0)	40 (100)	13 (81.3)	3 (18.7)	16 (100)	6 (60.0)	4 (40.0)	10 (100)	χ^2=3.1731 p=0.205
33-36 weeks GA	492 (90.6)	51 (9.4)	543 (100)	69 (92.0)	6 (8.0)	75 (100)	36 (87.8)	5 (12.2)	41 (100)	χ^2=0.5483 p=0.7602
≥37 weeks GA	510 (90.6)	53 (9.4)	563 (100)	109 (94.0)	7 (6.0)	116 (100)	45 (91.8)	4 (8.2)	49 (100)	χ^2=1.3957 p=0.498

Table 3. Univariable and multivariable logistic regression analyses of factors associated with Grade 3-4 anaemia in the first six months of life

		Univariate analysis (oberserved data)			Multivariate analysis (observed data)			Multivariate analysis (imputed data – missing data analysis)		
	N=1737	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
Sex										
Male	60/918 (6.54%)	1.00								
Female	63/816 (7.72%)	1.19	0.338	0.83-1.73						
Missing	0/3 (0.00%)									
Birth period										
1996-2000	44/443 (9.93%)	1.00			1.00			1.00		
2001-2005	59/797 (7.40%)	0.72	0.123	0.48-1.09	0.83	0.428	0.52-1.32	0.84	0.454	0.53-1.33
2006-2009	20/497 (4.02%)	0.38	0.001	0.22-0.65	0.54	0.052	0.29-1.00	0.53	0.047	0.28-0.99
Gestational age (wks)										
>=37	70/1262 (5.55%)	1.00			1.00			1.00		
33-36	42/364 (11.54%)	2.22	<0.0001	1.49-3.32	2.08	0.001	1.37-3.16	2.10	<0.0001	1.39-3.18
<=32	10/80 (12.50%)	2.43	0.013	1.20-4.92	2.42	0.017	1.17-5.02	2.43	0.017	1.17-5.04
Missing	1/31 (3.23%)	0.57	0.580	0.08-4.22	0.51	0.514	0.07-3.89			
Antenatal ART (number of drugs)										
None	14/318 (4.40%)	1.00			1.00			1.00		
One	13/147 (8.84%)	2.11	0.062	0.96-4.60	2.01	0.128	0.82-4.93	2.05	0.107	0.86-4.89
Two	21/123 (17.07%)	4.47	<0.0001	2.19-9.12	4.61	<0.0001	2.01-10.60	4.60	<0.0001	2.06-10.26
cART	71/1115 (6.37%)	1.48	0.193	0.82-2.66	1.94	0.077	0.93-4.05	1.89	0.064	0.96-3.73
Missing	4/34 (11.76%)	2.90	0.076	0.89-9.35	3.76	0.039	1.07-13.24	3.38	0.055	0.98-11.71
Maternal CD4 cell count at delivery										

>200 cell /µL	79/1139 (6.94%)	1.00								
<=200 cell /µL	14/150 (9.33%)	1.38	0.288	0.76-2.51						
Missing	30/448 (6.70%)	0.96	0.865	0.62-1.49						
Maternal viral load at delivery (copies/ml)										
<50	18/391 (4.60%)	1.00			1.00			1.00		
50-399	30/435 (6.90%)	1.53	0.162	0.84-2.80	1.11	0.749	0.57-2.26	1.25	0.481	0.67-2.32
>=400	42/428 (9.81%)	2.25	0.005	1.27-3.90	1.42	0.355	0.67-3.00	1.64	0.121	0.88-3.06
Missing	33/483 (6.83%)	1.52	0.165	0.84-2.74	1.36	0.424	0.64-2.87			
Intrapartum IV ZDV prophylaxis										
No	21/341 (6.16%)	1.00								
Yes	90/1025 (8.78%)	1.47	0.127	0.89-2.40						
Missing	7/325 (2.15%)	0.34	0.014	0.14-0.80						
HIV-infected infant										
No	6/86 (6.98%)	1.00			1.00			1.00		
Yes	117/1651 (7.09%)	0.98	0.969	0.42-2.30	1.40	0.475	0.55-3.59	1.29	0.598	0.50-3.28
Neonatal prophylaxis										
Two drugs	18/265 (6.79%)	1.00			1.00			1.00		
One drug	88/1273 (6.91%)	1.02	0.944	0.60-1.72	1.10	0.744	0.62-1.97	1.04	0.879	0.60-1.81
Three drugs	11/121 (9.09%)	1.37	0.428	0.62-3.00	1.53	0.330	0.65-3.63	1.60	0.277	0.69-3.75
None	4/60 (6.67%)	0.98	0.972	0.32-3.01	1.43	0.500	0.41-4.95	1.28	0.690	0.38-4.25
Missing	2/17 (11.76 %)	1.83	0.445	0.38-8.63	1.40	0.681	0.28-7.15	1.25	0.792	0.24-6.44
In utero ART exposure										
<=4 weeks	2/47 (4.26%)	1.00								
>4 weeks	93/1218 (7.64%)	1.86	0.396	0.44-7.79						

No maternal ART	14/318 (4.40%)	1.04	0.963	0.23-4.71						
Missing	14/154 (9.09%)	2.25	0.295	0.49-10.28						
Children therapy exposure (duration of neonatal prophylaxis)										
<=28 days	40/478 (8.37%)	1.00								
29-49 days	69/1060 (6.51%)	0.76	0.190	0.51-1.14						
No therapy	4/58 (6.90%)	0.81	0.700	0.28-2.36						
Missing	10/141 (7.09%)	0.84	0.696	0.41-1.72						
Maternal origin										
Europe	80/974 (8.21%)	1.00								
Other	7/72 (9.72%)	1.20	0.655	0.53-2.71						
Missing	36/691 (5.21%)	0.61	0.019	0.41-0.92						
Delivery method										
Vaginal	42/621 (6.76%)	1.00								
Caesarean	81/1100 (7.36%)	1.10	0.643	0.74-1.61						
Missing	0/16 (0.00%)									

Table 4. Univariable and multivariable logistic regression analyses of factors associated with Grade 3-4 neutropenia in the first six months of life.

		Univariate analysis			Multivariate analysis			Imputed data Multivariate analysis		
	N=1544	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
Sex										
Male	80/815 (9.82%)	1.00								
Female	68/726 (9.37%)	0.95	0.765	0.67-1.33						
Missing	0/3 (0.00%)									
Birth period										
1996-2000	34/418 (8.13%)	1.00			1.00			1.00		
2001-2005	90/735 (12.24%)	1.57	0.031	1.04-2.38	1.44	0.125	0.90-2.30	1.43	0.135	0.90-2.27
2006-2009	24/391 (6.14%)	0.73	0.273	0.43-1.27	0.72	0.302	0.39-1.34	0.66	0.176	0.36-1.21
Gestational age (wks)										
>=37	97/1121 (8.65%)	1.00			1.00			1.00		
33-36	36/328 (10.98%)	1.30	0.201	0.87-1.95	1.21	0.369	0.80-1.85	1.22	0.357	0.80-1.86
<=32	14/69 (20.29%)	2.69	0.002	1.44-5.01	2.87	0.002	1.49-5.54	2.94	0.001	1.52-5.66
Missing	1/26 (3.85%)	0.42	0.400	0.07-0.12	0.52	0.532	0.07-4.01			
Antenatal ART (number of drugs)										
None	21/282 (7.45%)	1.00			1.00			1.00		
One	7/140 (5.00%)	0.65	0.345	0.27-1.58	0.65	0.393	0.24-1.75	0.70	0.468	0.27-1.84
Two	14/111 (12.61%)	1.79	0.109	0.88-3.67	1.78	0.187	0.76-4.19	2.09	0.075	0.93-4.70
cART	105/986 (10.65%)	1.48	0.155	0.91-2.41	1.24	0.541	0.62-2.46	1.55	0.163	0.84-2.87
Missing	1/25 (4.00%)	0.52	0.529	0.07-4.01	0.66	0.706	0.08-5.70	0.80	0.832	0.10-6.54
Maternal CD4 cell count at delivery										
>200 cell /µL	104/1011 (10.29%)	1.00			1.00					
<=200 cell /µL	20/131 (15.27%)	1.57	0.087	0.94-2.64	1.47	0.160	0.86-2.54			
Missing	24/402 (5.97%)	0.55	0.012	0.35-0.88	0.64	0.132	0.35-1.15			

Maternal viral load at delivery (copies/ml)										
<50	42/358 (11.73%)	1.00								
50-399	42/372 (11.29%)	0.95	0.853	0.61-1.51						
>=400	40/378 (10.58%)	0.89	0.620	0.56-1.41						
Missing	24/436 (5.50%)	0.44	0.002	0.26-0.74						
Intrapartum IV ZDV prophylaxis										
No	26/307 (8.47%)	1.00								
Yes	89/947 (9.40%)	1.12	0.624	0.70-1.77						
Missing	33/290 (11.38%)	1.38	0.235	0.80-2.38						
HIV-infected infant										
No	8/74 (10.81%)	1.00			1.00			1.00		
Yes	140/1470 (9.52%)	1.15	0.714	0.54-2.44	1.69	0.228	0.72-3.96	1.76	0.187	0.76-4.07
Neonatal prophylaxis										
Two drugs	16/213 (7.51%)	1.00			1.00			1.00		
One drug	110/1160 (9.48%)	1.29	0.361	0.75-2.23	1.34	0.327	0.75-2.40	1.33	0.330	0.75-2.35
Three drugs	14/104 (13.46%)	1.92	0.093	0.90-4.02	2.06	0.110	0.85-4.81	1.98	0.113	0.85-4.59
None	6/51 (11.76%)	1.64	0.328	0.61-4.43	2.81	0.077	0.89-8.78	2.70	0.075	0.90-8.07
Missing	2/15 (13.33%)	1.89	0.426	0.39-9.14	2.23	0.339	0.43-11.59	2.04	0.391	0.40-10.45
Children therapy exposure										
<=28 days	31/395 (7.85%)	1.00								
29-49 days	95/970 (9.79%)	1.27	0.261	0.83-1.95						
No therapy	6/50 (12.00%)	1.60	0.320	0.63-4.05						
Missing	16/129 (12.40%)	1.66	0.119	0.88-3.15						
In utero ART exposure										
<=4 weeks	6/45 (13.33%)	1.00								
>4 weeks	120/1192 (10.07%)	0.73	0.479	0.30-1.75						

No maternal ART	21/282 (7.45%)	0.52	0.189	0.20-1.38						
Missing	1/25 (4.00%)	0.27	0.240	0.03-2.39						
Maternal region of origin										
Europe	68/833 (8.16%)	1.00			1.00			1.00		
Other	12/70 (17.14%)	2.33	0.013	0.53-2.71	2.52	0.023	1.13-5.59	2.60	0.010	1.25-5.42
Missing	68/641 (10.61%)	1.34	0.109	0.41-0.92	1.21	0.452	0.74-1.98			
Delivery method										
Vaginal	35/532 (6.58%)	1.00			1.00			1.00		
Caesarean	111/1000 (11.10%)	1.77	0.005	1.19-2.63	1.52	0.068	0.97-2.39	1.57	0.047	1.01-2.45
Missing	2/12 (16.67%)	2.84	0.189	0.60-13.47	2.38	0.298	0.47-12.15			

Legends to Figures. Figure 1. Observed and estimated haemoglobin levels (g/dL) according to neonatal prophylaxis type (7746 blood exams results available for 1836 infants within the age range 0-18 months). Figure 2. Observed and estimated neutrophils levels levels ($Log10^9$ cells/L) according to neonatal prophylaxis type (7746 blood exams results available for 1836 infants within the age range 0-18 months)

Appendix

Supplementary table 1. Sensitivity analysis for the imputation model.

	ANEMIA			NEUTROPENIA		
	N=1737	% (observed data)	% (imputed data)	**N=1544**	% (observed data)	% (imputed data)
Sex						
Male	918	52.85		815	52.78	
Female	816	46.98		726	47.02	
Missing	3	0.17		3	0.19	
Birth period						
1996-2000	443	25.50		418	27.07	
2001-2005	797	45.88		735	47.60	
2006-2009	497	28.61		391	25.32	
Gestational age (wks)						
>=37	1262	72.65	73.99	1121	72.60	73.85
33-36	364	20.96	21.31	328	21.24	21.62
<=32	80	4.61	4.70	69	4.47	4.53
Missing	31	1.78		26	1.68	
Antenatal ART (number of drugs)						
None	318	18.31		282	18.26	
One	147	8.46		140	9.07	
Two	123	7.08		111	7.19	
cART	1115	64.19		986	63.86	
Missing	34	1.96		25	1.62	
Maternal CD4 cell count at delivery						
>200 cell /µL	1139	65.57	87.40	1011	65.48	86.61
<=200 cell /µL	150	8.64	12.60	131	8.48	13.33
Missing	448	25.79		402	26.04	
Maternal viral load at delivery (copies/ml)						
<50	391	22.51	27.16	358	23.19	28.28
50-399	435	25.04	36.83	372	24.09	36.19
>=400	428	24.64	36.00	378	24.48	35.47
Missing	483	27.81		436	28.24	
Intrapartum IV ZDV prophylaxis						
No	341	20.17	21.17	307	19.88	20.76
Yes	1025	60.62	78.83	947	61.33	79.24
Missing	325	19.22		290	18.78	
HIV-infected infant						
No	1651	95.05		1470	95.21	

Yes	86	4.95		74	4.79	
Neonatal prophylaxis						
Two drugs	265	15.26		213	13.80	
One drug	1273	73.33		1160	75.18	
Three drugs	121	6.97		104	6.74	
None	60	3.46		51	3.31	
Missing	17	0.98		15	0.97	
Children therapy exposure (maternal therapy duration)						
No therapy	58	3.34	4.50	50	3.24	4.34
<=28 days	478	27.52	29.28	395	25.58	27.36
29-49 days	1060	61.02	66.22	970	62.82	68.24
Missing	141	8.12		129	8.35	
In utero ART exposure						
No maternal ART	318	18.31	18.43	282	18.26	18.26
>4 weeks	1218	70.12	78.63	1192	77.20	78.76
<=4 weeks	47	2.71	2.94	45	2.91	2.91
Missing	154	8.87				
Maternal region of origin						
Europe	974	56.07	93.11	833	53.95	92.41
Other	72	4.15	6.89	70	4.53	7.59
Missing	691	39.78		641	41.52	
Delivery method						
Vaginal	621	35.75	36.06	532	34.46	34.73
Caesarean	1100	63.33	63.94	1000	64.77	65.27
Missing	16	0.92		12	0.78	
Cohort						
Catalan	557	32.07		503	32.58	
Ukraine-ECS	120	6.91		102	6.61	
ITLR	206	11.86		205	13.28	
Madrid	85	4.89		84	5.44	
Victor Babes' Hospital	46	2.65				
MoCHiV	411	23.66		362	23.45	
Western-ECS	312	17.96		288	18.65	

Supplementary Table 2. Univariable logistic regression analysis of factors associated with Grade 3-4 anaemia in the first six months of life obtained (imputed data – data missing analysis)

		Imputed data univariate analysis		
	N=1737	**Odds Ratio**	**P**	**95% CI**
Sex				
Male	60/918 (6.54%)	1.00		
Female	63/816 (7.72%)	1.20	0.338	0.83-1.73
Missing	0/3 (0.00%)			
Birth period				
1996-2000	44/443 (9.93%)	1.00		
2001-2005	59/797 (7.40%)	0.72	0.123	0.48-1.09
2006-2009	20/497 (4.02%)	0.38	0.001	0.22-0.66
Gestational age (wks)				
>=37	70/1262 (5.55%)	1.00		
33-36	42/364 (11.54%)	2.24	<0.0001	1.50-3.34
<=32	10/80 (12.50%)	2.44	0.013	1.20-4.93
Missing	1/31 (3.23%)			
Antenatal ART (number of drugs)				
None	14/318 (4.40%)	1.00		
One	13/147 (8.84%)	2.10	0.062	0.96-4.60
Two	21/123 (17.07%)	4.47	<0.0001	2.19-9.12
cART	71/1115 (6.37%)	1.48	0.193	0.82-2.66
Missing	4/34 (11.76%)	2.90	0.076	0.89-9.35
Maternal CD4 cell count at delivery				
>200 cell /µL	79/1139 (6.94%)	1.00		

<=200 cell /µL	14/150 (9.33%)	1.31	0.377	0.72-2.38
Missing	30/448 (6.70%)			
Maternal viral load at delivery (copies/ml)				
<50	18/391 (4.60%)	1.00		
50-399	30/435 (6.90%)	1.43	0.241	0.79-2.58
>=400	42/428 (9.81%)	2.04	0.013	1.16-3.58
Missing	33/483 (6.83%)			
Intrapartum IV ZDV prophylaxis				
No	21/341 (6.16%)	1.00		
Yes	90/1025 (8.78%)	1.21	0.453	0.74-1.97
Missing	7/325 (2.15%)			
HIV-infected infant				
No	6/86 (6.98%)	1.00		
Yes	117/1651 (7.09%)	0.98	0.969	0.42-2.30
Neonatal prophylaxis type				
Two drugs	18/265 (6.79%)	1.00		
One drug	88/1273 (6.91%)	1.02	0.944	0.60-1.72
Three drugs	11/121 (9.09%)	1.37	0.428	0.63-3.00
None	4/60 (6.67%)	0.98	0.972	0.32-3.01
Missing	2/17 (11.76%)	1.72	0.494	0.37-8.05
Children therapy exposure (duration of neonatal prophylaxis)				
<=28 days	40/478 (8.37%)	1.00		
29-49 days	69/1060 (6.51%)	0.76	0.174	0.51-1.13
No therapy	4/58 (6.90%)	0.90	0.823	0.36-2.25

Missing	10/141 (7.09%)			
In utero ART exposure				
<=4 weeks	2/47 (4.26%)	1.00		
>4 weeks	93/1218 (7.64%)	1.86	0.396	0.44-7.83
No maternal ART	14/318 (4.40%)	1.03	0.972	0.23-4.67
Missing	14/154 (9.09%)			
Maternal origin				
Europe	80/974 (8.21%)	1.00		
Other	7/72 (9.72%)	1.26	0.576	0.56-2.82
Missing	36/691 (5.21%)			
Delivery method				
Vaginal	42/621 (6.76%)	1.00		
Caesarean	81/1100 (7.36%)	1.09	0.649	0.74-1.61
Missing	0/16 (0.00%)			

Supplementary Table 3. Univariable logistic regression analysis of factors associated with Grade 3-4 neutropenia in the first six months of life obtained (imputed data – data missing analysis).

		Univariate analysis (imputed data)		
	N=1544	**Odds Ratio**	**P**	**95% CI**
Sex				
Male	80/815 (9.82%)	1.00		
Female	68/726 (9.37%)	0.95	0.765	0.67-1.33
Missing	0/3 (0.00%)			
Birth period				
1996-2000	34/418 (8.13%)	1.00		
2001-2005	90/735 (12.24%)	1.58	0.031	1.04-2.38
2006-2009	24/391 (6.14%)	0.74	0.273	0.43-1.27
Gestational age (wks)				
>=37	97/1121 (8.65%)	1.00		
33-36	36/328 (10.98%)	1.31	0.190	0.87-1.96
<=32	14/69 (20.29%)	2.69	0.002	1.44-5.01
Missing	1/26 (3.85%)			
Antenatal ART (number of drugs)				
None	21/282 (7.45%)	1.00		
One	7/140 (5.00%)	0.65	0.345	0.27-1.58
Two	14/111 (12.61%)	1.79	0.109	0.88-3.67
cART	105/986 (10.65%)	1.48	0.155	0.91-2.41
Missing	1/25 (4.00%)	0.52	0.529	0.07-4.01
Maternal CD4 cell count at delivery				
>200 cell /µL	104/1011 (10.29%)	1.00		
<=200 cell /µL	20/131 (15.27%)	1.52	0.106	0.91-2.55

Missing	24/402 (5.97%)			
Maternal viral load at delivery (copies/ml)				
<50	42/358 (11.73%)	1.00		
50-399	42/372 (11.29%)	0.89	0.595	0.56-1.39
>=400	40/378 (10.58%)	0.88	0.590	0.57-1.38
Missing	24/436 (5.50%)			
Intrapartum IV ZDV prophylaxis				
No	26/307 (8.47%)	1.00		
Yes	89/947 (9.40%)	1.17	0.493	0.75-1.83
Missing	33/290 (11.38%)			
HIV-infected infant				
No	8/74 (10.81%)	1.00		
Yes	140/1470 (9.52%)	1.15	0.714	0.54-2.44
Neonatal prophylaxis type				
Two drugs	16/213 (7.51%)	1.00		
One drug	110/1160 (9.48%)	1.29	0.361	0.75-2.23
Three drugs	14/104 (13.46%)	1.92	0.093	0.90-4.02
None	6/51 (11.76%)	1.64	0.328	0.61-4.43
Missing	2/15 (13.33%)	1.89	0.426	0.39-9.14
Children therapy exposure (duration of neonatal prophylaxis)				
<=28 days	31/395 (7.85%)	1.00		
29-49 days	95/970 (9.79%)	1.29	0.238	0.85-1.96
No therapy	6/50 (12.00%)	1.60	0.271	0.69-3.67
Missing	16/129 (12.40%)			
In utero ART exposure				
<=4 weeks	6/45 (13.33%)	1.00		

>4 weeks	120/1192 (10.07%)	0.72	0.461	0.30-1.73
No maternal ART	21/282 (7.45%)	0.52	0.189	0.20-1.38
Missing	1/25 (4.00%)			
Maternal origin				
Europe	68/833 (8.16%)	1.00		
Other	12/70 (17.14%)	2.24	0.016	1.16-4.31
Missing	68/641 (10.61%)			
Delivery method				
Vaginal	35/532 (6.58%)			
Caesarean	111/1000 (11.10%)	1.79	0.004	1.20-2.65
Missing	2/12 (16.67%)			

Supplementary Table 4 a). Sub-analysis in infants exposed to cART in utero. Univariable and multivariable logistic regression analyses of factors associated with Grade 3-4 anaemia in the first six months of life.

		Univariate analysis (observed data)			Multivariate analysis (observed data)			Univariate analysis (imputed data-missing data analysis)			Multivariate analysis (imputed data – missing data analysis)		
	N=1115	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
Sex													
Male	31/585 (5.30%)	1.00						1.00					
Female	40/529 (7.56%)	1.46	0.125	0.90-2.37				1.46	0.125	0.90-2.37			
Missing	0/1 (0.0%)												
Birth period													
1996-2000	14/203 (6.90%)	1.00						1.00					
2001-2005	40/584 (6.85%)	0.99	0.982	0.53-1.87				0.99	0.982	0.53-1.87			
2006-2009	17/328 (5.18%)	0.74	0.415	0.36-1.53				0.74	0.415	0.36-1.53			
Gestational age (wks)													
>=37	38/815 (4.66%)	1.00			1.00			1.00			1.00		
33-36	25/231 (10.82%)	2.48	0.001	1.46-4.20	2.00	0.013	1.15-3.47	2.48	0.001	1.46-4.20	1.96	0.019	1.19-3.43
<=32	8/52 (15.38%)	3.72	0.002	1.64-8.45	2.56	0.034	1.08-6.07	3.72	0.002	1.64-8.45	2.88	0.019	1.19-6.94
Missing	0/17 (0.0%)										2.00e-08	0.998	0- -
Antenatal ART (type of regimen)													

PI	30/564 (5.32%)	1.00			1.00			1.00			1.00		
Other	7/62 (11.29%)	2.27	0.065	0.95-5.40	2.17	0.098	0.87-5.43	2.27	0.065	0.95-5.40	2.07	0.131	0.81-5.34
NNRTI	25/290 (8.62%)	1.68	0.065	0.97-2.91	1.58	0.125	0.88-2.84	1.68	0.065	0.97-2.91	1.33	0.370	0.71-2.46
PI+NNRTI	9/199 (4.52%)	0.84	0.661	0.39-1.81	0.75	0.492	0.34-1.68	0.84	0.661	0.39-1.81	0.71	0.402	0.31-1.59
Maternal CD4 cell count at delivery													
>200 cell /µL	47/896 (5.25%)	1.00			1.00			1.00			1.00		
<=200 cell /µL	13/116 (11.21%)	2.28	0.013	1.19-4.36	1.83	0.089	0.91-3.69	2.10	0.024	1.10-4.02	1.71	0.135	0.86-3.48
Missing	11/103 (10.68%)	2.16	0.029	1.08-4.31	1.70	0.228	0.72-4.06						
Maternal viral load at delivery (copies/ml)													
<50	11/361 (3.05%)	1.00			1.00			1.00			1.00		
50-399	21/357 (5.88%)	1.99	0.070	0.94-4.19	1.52	0.286	0.70-3.28	1.95	0.077	0.93-4.07	1.10	0.828	0.47-2.57
>=400	28/269 (7.83%)	3.70	<0.0001	1.81-7.57	2.68	0.011	1.26-5.72	3.39	0.001	1.66-6.91	1.78	0.203	0.73-4.33
Missing	11/128 (8.59%)	2.99	0.013	1.26-7.08	1.81	0.256	0.65-5.02						
Intrapartum IV ZDV prophylaxis													
No	7/74 (9.46%)	1.00						1.00					
Yes	55/696 (7.90%)	0.82	0.640	0.36-1.88				0.66	0.316	0.29-1.49			
Missing	4/310 (1.29%)	0.13	0.001	0.04-0.44									
HIV-infected infant													

No	2/13 (15.38%)	1.00			1.00			1.00			1.00		
Yes	69/1102 (6.26%)	2.72	0.198	0.59-12.52	1.98	0.410	0.39-10.11	2.72	0.198	0.59-12.52	2.20	0.347	0.42-11.47
Neonatal prophylaxis													
Two drugs	12/173 (6.94%)	1.00			1.00			1.00			1.00		
One drug	52/873 (5.96%)	0.85	0.624	0.44-1.63	1.00	0.998	0.50-1.99	0.85	0.624	0.44-1.63	1.17	0.672	0.56-2.46
Three drugs	6/49 (12.24%)	1.87	0.236	0.66-5.28	1.23	0.709	0.41-3.73	1.87	0.236	0.66-5.28	1.18	0.768	0.38-3.65
None	0/10 (0.0%)										1.25e-08	1.000	0- -
Missing	1/10 (10.00%)	1.49	0.716	0.17-12.77	1.15	0.912	0.10-12.77	1.49	0.716	0.17-12.77	0.952	0.800	0.09-12.26
Children therapy exposure (duration of neonatl prophylaxis)													
<=28 days	28/280 (10.00%)	1.00			1.00								
29-49 days	37/718 (5.15%)	0.49	0.006	0.29-0.82	0.53	0.021	0.31-0.91						
No therapy	0/9 (0.00%)												
Missing	6/108 (5.56%)	0.53	0.171	0.21-1.32	0.54	0.241	0.20-1.50						
In utero ART exposure													
<=4 weeks	0/15 (0.00%)	1.00											
>4 weeks	64/1006 (6.36%)	397738.8	0.984	0- -									
Missing	7/94 (7.45%)	470726.5	0.983	0- -									

Maternal origin													
Europe	55/576 (9.55%)	1.00						1.00					
Other	2/26 (7.69%)	0.79	0.752	0.18-3.43				0.83	0.802	0.20-3.52			
Missing	14/513 (2.73%)	0.27	<0.0001	0.15-0.48									
Delivery method													
Vaginal	24/292 (8.22%)	1.00						1.00					
Caesarean	47/811 (5.80%)	0.69	0.150	0.41-1.15				0.68	0.146	0.41-1.14			
Missing	0/12 (0.00%)												

Supplementary Table 4 b). Subanalisys in infants exosed to cART in utero. Univariable and multivariable logistic regression analyses of factors associated with Grade 3-4 neutropenia in the first six months of life.

		Univariate analysis			Multivariate analysis			MI Univariate analysis			MI Multivariate analysis		
	N=986	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
Sex													
Male	52/516 (10.08%)	1.00						1.00					
Female	53/469 (11.30%)	1.14	0.535	0.76-1.70				1.14	0.535	0.76-1.70			
Missing	0/1 (0.0%)												
Birth period													
1996-2000	15/185 (8.11%)	1.00						1.00					
2001-2005	67/542 (12.36%)	1.60	0.117	0.89-2.87				1.60	0.117	0.89-2.87			
2006-2009	23/259 (8.88%)	1.10	0.774	0.56-2.18				1.10	0.774	0.56-2.18			
Gestational age (wks)													
>=37	72/716 (10.06%)	1.00			1.00			1.00			1.00		
33-36	24/208 (11.54%)	1.17	0.538	0.71-1.90	1.18	0.518	0.71-1.95	1.17	0.525	0.72-1.91	1.16	0.567	0.70-1.92
<=32	9/49 (18.37%)	2.01	0.072	0.94-4.32	2.21	0.051	1.00-4.89	2.02	0.071	0.94-4.33	2.13	0.063	0.96-4.74
Missing	0/13 (0.0%)												
Antenatal ART (type of regimen)													

PI	53/471 (11.25%)	1.00			1.00			1.00			1.00		
Other	3/60 (5.00%)	0.41	0.149	0.12-1.37	0.40	0.137	0.17-1.34	0.41	0.149	0.12-1.37	0.38	0.120	0.11-1.29
NNRTI	25/276 (9.06%)	0.78	0.345	0.48-1.29	0.79	0.406	0.46-1.37	0.78	0.345	0.48-1.29	0.91	0.736	0.53-1.56
PI+NNRTI	24/179 (13.41%)	1.22	0.448	0.73-2.05	1.27	0.377	0.75-2.15	1.22	0.448	0.73-2.05	1.31	0.322	0.77-2.22
Maternal CD4 cell count at delivery													
>200 cell /µL	85/793 (10.72%)	1.00						1.00			1.00		
<=200 cell /µL	13/99 (13.13%)	1.26	0.470	0.67-2.35				0.12	<0.0001	0.68-2.36	1.31	0.415	0.68-2.54
Missing	7/94 (7.45%)	0.67	0.328	0.30-1.49									
Maternal viral load at delivery (copies/ml)													
<50	41/334 (12.28%)	1.00			1.00			1.00			1.00		
50-399	34/311 (10.93)	0.87	0.595	0.54-1.42	0.91	0.710	0.54-1.52	0.88	0.616	0.55-1.43	0.94	0.825	0.57-1.57
>=400	22/228 (9.65%)	0.76	0.334	0.44-1.32	0.76	0.399	0.41-1.43	0.78	0.380	0.46-1.35	0.77	0.378	0.43-1.38
Missing	8/113 (7.08%)	0.54	0.131	0.25-1.20	0.60	0.240	0.25-1.41						
Intrapartum IV ZDV prophylaxis													
No	5/65 (7.69%)	1.00						1.00					
Yes	68/941 (10.61%)	1.42	0.464	0.55-3.67				1.35	0.529	0.53-3.44			
Missing	32/280 (11.43%)	1.55	0.384	0.58-4.14									
HIV-infected infant													

No	3/12 (25.00%)	1.00			1.00			1.00			1.00		
Yes	102/974 (10.47%)	2.85	0.121	0.76-10.69	2.82	0.151	0.68-11.62	2.85	0.121	0.76-10.70	2.70	0.168	0.66-11.09
Neonatal prophylaxis													
Two drugs	10/129 (7.75%)	1.00			1.00			1.00			1.00		
One drug	89/804 (11.07%)	1.48	0.259	0.74-2.93	1.60	0.198	0.78-3.29	1.48	0.259	0.74-2.93	1.73	0.128	0.85-3.50
Three drugs	4/37 (10.81%)	1.44	0.557	0.42-4.89	1.24	0.737	0.35-4.46	1.44	0.557	0.42-4.89	1.29	0.699	0.36-4.64
None	0/6 (0.0%)				5.80e-08	0.998	0- -						
Missing	2/10 (20.00%)	2.97	0.203	0.55-15.94	4.09	0.121	0.69-24.16	2.97	0.203	0.55-15.94	4.98	0.073	0.86-28.78
Children therapy exposure (neonatal prophylaxis duration)													
<=28 days	27/230 (11.74%)	1.00											
29-49 days	63/651 (9.68%)	0.80	0.376	0.50-1.30									
No therapy	0/6 (0.00%)												
Missing	15/99 (15.15%)	1.34	0.396	0.68-2.65									
In utero ART exposure													
<=4 weeks	3/15 (20.00%)	1.00						1.00					
>4 weeks	102/971 (10.50%)	0.47	0.248	0.13-1.69				0.47	0.248	0.13-1.69			

Maternal of origin													
Europe	48/486 (9.88%)	1.00						1.00					
Other	4/26 (15.38%)	1.66	0.370	0.55-5.06				1.96	0.169	0.75-5.13			
Missing	53/474 (11.18%)	1.49	0.510	0.76-1.74									
Delivery method													
Vaginal	18/246 (7.32%)	1.00						1.00					
Caesarean	85/731 (11.63%)	1.67	0.059	0.98-2.83				1.67	0.057	0.98-2.84			
Missing	2/9 (22.22%)												

Supplementary table 5. Neonatal prophylaxis exposure and haematological markers in the first 18 months of life : longitudinal analysis by mixed effect model results

	Anaemia			Neutropenia		
	Coef.	**P**	**95% CI**	**Coef.**	**P**	**95% CI**
Gestational age (wks)						
>=37	1.00			1.00		
33-36	-0.36	<0.0001	-0.55--0.17	-0.01	0.533	-0.04-0.02
<=32	-0.83	0.001	-1.34--0.32	-0.02	0.673	-0.10-0.06
Missing	-0.08	0.788	-0.48--0.63	0.03	0.433	-0.05-0.11
Antenatal ART (number of drugs)						
None	1.00			1.00		
One	-0.27	0.085	-0.57-0.04	-0.03	0.188	-0.08-0.02
Two	-0.68	<0.0001	-1.01--0.34	-0.04	0.151	-0.10-0.01
cART	-0.21	0.120	-0.47-0.05	-0.03	0.162	-0.08-0.01
Missing	0.04	0.903	-0.58-0.66	0.11	0.221	-0.29-0.07
Maternal viral load at delivery (copies/mL)						
<50	1.00			1.00		
>=50	-0.32	0.001	-0.51--0.12	-0.004	0.825	-0.04-0.03
Missing	-0.10	0.368	-0.32-0.12	0.04	0.058	-0.001-0.07
HIV-infected infant						
No	1.00			1.00		
Yes	-0.82	<0.0001	-1.21--0.43	0.01	0.731	-0.06-0.08
Neonatal prophylaxis						
One drug	1.00			1.00		
Two and more drugs	-0.19	0.060	-0.39-0.01	0.02	0.218	-0.01-0.05
None	-0.32	0.117	-0.72-0.08	-0.03	0.460	-0.10-0.04
Missing	0.002	0.995	-0.73-0.74	-0.01	0.776	-0.09-0.07
Delivery method						
Vaginal	1.00			1.00		
Caesarean	-0.33	<0.0001	-0.51--0.15	-0.04	0.005	-0.07--0.01
Missing	0.74	0.074	-0.07-1.54	0.02	0.788	-0.12-0.16