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ORIGINAL RESEARCH

Severe haematologic toxicity is rare in high risk HIV-exposed infants receiving combination neonatal prophylaxis

The European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC) study group in EuroCoord*

Objectives

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. The aim of the study was to identify whether neonatal prophylaxis (NP) type is associated with the risk of severe anaemia or neutropaenia.

Methods

An individual patient data meta-analysis was conducted within six European cohorts, in infants at high risk for acquiring HIV infection. Adjusted logistic regression models were used to assess the risk of National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS) grade 3–4 anaemia/neutropaenia at ages 0–6 months. Mixture models of haemoglobin (Hb) level and $\log_{10}$-transformed neutrophil count (NC) were used to explore associations with NP type at ages 0–18 months.

Results

Of 1836 infants, 25% were preterm, 1149 (63%) had antenatal combination antiretroviral therapy (cART) exposure and 395 (22%) received NP (125 received CNP with three drugs). Overall, 117 (6.7%) infants had grade 3–4 anaemia at age 0–6 months and 140 (9.1%) had grade 3–4 neutropaenia. The presence of grade 3–4 anaemia or neutropaenia was not associated with NP type [adjusted odds ratio (aOR) 1.04 for one-drug NP and 1.60 for three-drug NP versus two-drug NP ($P = 0.879$ and $P = 0.277$, respectively) for anaemia; aOR 1.33 for one-drug NP and 1.98 for three-drug NP versus two-drug NP ($P = 0.330$ and $P = 0.113$, respectively) for neutropaenia], but was 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 level or NC were apparent by NP type.

Conclusions

A small proportion of infants experienced grade 3–4 haematological adverse events; risk of anaemia or netropenia was not associated with type of NP.

Keywords: adverse event, antiretroviral therapy, children

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Introduction

Universal antenatal HIV testing, combination 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 breast feeding have led to a dramatic decline in the number of perinatally HIV-infected children: currently, in the USA as well as in Western Europe, mother-to-child transmission (MTCT) rates are < 1% [1–3]. 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 as a consequence of 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 conducted in exclusively formula-fed infants born to women who had not received ARVs during pregnancy [7], but data

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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 [8–10].

We previously showed that the use of CNP in high-risk situations is increasing in Europe [4]. Data regarding the safety of NP, particularly CNP, are limited in both term and, concerningly, preterm infants. Haematological toxicity associated with *in utero* or early life exposure to ARVs has been well established, with some studies demonstrating that infants exposed *in utero* to cART have lower haemoglobin (Hb) levels and neutrophil counts (NCs) than those exposed to ZDV monotherapy or without ART exposure [7,11–16]. In the HPTN 040 trial, in which infants had no *in utero* ART exposure, neutropaenia (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 a European cohort collaboration, our aim was to examine haematological toxicity in infants born to women with HIV infection at high risk of MTCT, and specifically to identify whether NP type was associated with (1) the presence of severe or potentially life-threatening anaemia or neutropaenia within the first 6 months of life and (2) haemoglobin level and NC 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 subanalysis of the original data set was performed in order to investigate the occurrence and severity of haematological adverse events (specifically, anaemia and neutropaenia) related to NP. The substudy was limited to the subset of infants with at least one measurement of Hb level or NC in the first 18 months of life. The six cohorts participating in the substudy 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 related to sociodemographics, 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 (PCR) assay on at least two occasions [4]. NP was defined as any course of one or more ARVs administered with a prophylactic purpose and initiated within the first 72 h 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 neutropaenia definitions: Hb level 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 the χ^2 or Fisher's exact test and medians using Wilcoxon Mann–Whitney *U* tests. All significant tests were two-sided. Nadirs for Hb level and NC within the first 6 months of life were calculated.

Univariate and multivariable logistic regression analyses

Factors potentially associated with the occurrence of severe or potentially life-threatening (grade 3 or 4) anaemia and neutropaenia versus grade 0, 1 or 2 anaemia and neutropaenia (considering for each study subject the nadir value in the first 6 months of life) were explored in univariable and multivariable logistic regression analyses. Factors examined in univariable analyses were birth

period (1996–2000, 2001–2005 or 2006–2009), sex, preterm delivery (≤ 32, 33–36 or ≥ 37 weeks), intrapartum intravenous ZDV use, maternal viral load (< 50, 50–399 or ≥ 400 HIV-1 RNA copies/mL), antenatal ART (none, one drug, two drugs or cART), maternal CD4 count (≤ 200 or > 200 cells/μL), NP type (none, or one, two or three drugs), antenatal ART duration (≤ 4 weeks or > 4 weeks) and NP duration (≤ 28 days, 29–49 days, or 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 [protease inhibitor (PI)-based; nonnucleoside reverse transcriptase inhibitor (NNRTI)-based; PI + NNRTI-based; or only nucleoside reverse transcriptase inhibitor (NRTI) or classes other 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 data set. 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 intravenous ZDV prophylaxis, NP 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 five imputations 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' (Stata Corporation, College Station, TX). For all analyses using imputed data, estimates were combined across the imputed data sets based on Rubin's rules. Finally, the multivariable analyses were repeated on the 500 imputation generated data sets.

Longitudinal analysis

Two mixed models were used including all Hb levels or NCs for each patient within his/her first 18 months of life to explore associations between these haematological markers and NP type, using the 'xtreg' command in STATA. The time variable used 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, infant HIV status and 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.

Results

Population characteristics

Laboratory investigation results for Hb level and/or NC in subjects 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 of 1149) had ≤ 4 weeks of antenatal exposure and 90.2% (1036 of 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 and 125 (31.6%) three drugs, and no specific information was provided in two cases. In the two-drug group, ZDV plus single dose nevirapine (sdNVP) was administered to 124 infants (45.9%) and ZDV plus lamivudine (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 of 125; 88.1%), with 11 (8.8%) infants receiving a PI-based regimen (nelfinavir in four cases and lopinavir/ritonavir in seven) and the remaining three infants receiving other regimens. The median duration was 5.7 weeks [interquartile range (IQR) 4–6 weeks] for one-drug NP and 5.9 weeks (IQR 4–6 weeks) for CNP. The median duration of NP was available for 1228 of 1350 (90.3%) children receiving one-drug NP and 378 of 395 (95.0%) children receiving CNP.

Anaemia or neutropaenia in the first 6 months of life

Nadir Hb level and NC for up to age 6 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 6 months [4.8% (40 of 827) in term infants and 9.6% (77 of 803) in preterm infants]. A total of 140 (9.1%) infants had grade 3–4 neutropaenia [8.8% (64 of 728) in term infants and

Table 1 Characteristics of the study population (n = 1836)

	n	%
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
One drug	155	8.4
Two drugs	133	7.2
cART	1149	62.6
Missing	34	1.9
Maternal viral load at delivery		
< 50 copies/mL	409	22.3
50–399 copies/mL	444	24.2
400–999 copies/mL	105	5.7
≥ 1000 copies/mL	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 intravenous 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		
≥ 37 weeks	1336	72.8
33–36 weeks	384	20.9
≤ 32 weeks	84	4.6
Missing	32	1.7
Birth weight		
≥ 3000 g	672	36.6
2500–2999 g	664	36.2
2000–2499 g	301	16.4
1500–1999 g	108	5.9
< 1500 g	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

ART, antiretroviral therapy; cART, combination antiretroviral therapy; ECS, the European Collaborative Study; ITLR, the Italian Register for HIV Infection in Children; MoCHiV, the Swiss Mother and Child HIV Cohort Study; ZDV, zidovudine; sdNVP, single dose nevirapine.

10.3% (75 of 725) in preterm infants]. A higher, but not significantly higher, 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 versus 6.9% for anaemia and 13.5 versus 9.5% for neutropaenia, respectively; Table 2).

Observed and imputed characteristics of children included analysis for risk of grade 3–4 anaemia or neutropaenia in the first 6 months of life are reported in Appendix Table A1.

In unadjusted logistic regression analysis, birth period, gestational age, and delivery viral load were significantly associated with the risk of grade 3–4 anaemia (Table 3 and Appendix Table A2 for the multiple imputation model) and of grade 3–4 neutropaenia (Table 4 and Appendix Table A3 for the multiple imputation model), while antenatal ART exposure was only significantly associated with the risk of anaemia.

In multivariate analyses, grade 3–4 anaemia was not associated with NP type (aOR 1.04 for one-drug NP and 1.60 for three-drug NP versus two-drug NP; $P = 0.879$ and $P = 0.277$, respectively), but was associated with preterm delivery [aOR 2.10 ($P < 0.0001$) for 33–36 weeks and 2.43 ($P = 0.017$) for ≤ 32 weeks versus term], maternal therapy (aOR 4.60 for two drugs versus no drug; $P < 0.0001$) and birth period (aOR 0.53 for 2006–2009 versus 1996–2000; $P < 0.047$; Table 3).

In multivariate analyses, grade 3–4 neutropaenia was not associated with NP type (aOR 1.33 for one-drug NP and 1.98 for three-drug NP versus two-drug NP; $P = 0.330$ and $P = 0.113$, respectively), but was associated with preterm delivery (aOR 2.94; $P = 0.001$ for ≤ 32 weeks versus term), maternal origin (aOR 2.60; $P = 0.010$ for not European versus European mothers) and delivery method (aOR 1.57; $P = 0.047$ for caesarean versus vaginal delivery; Table 4).

In a subanalysis including only infants exposed to cART *in utero*, no significant difference in the risk of grade 3–4 anaemia or neutropaenia was observed by NP type (Appendix Tables A4a and b).

Table 2 Anaemia and neutropaenia grades in the study population, expressed as nadir value within the first 6 months of age, by number of drugs in the neonatal prophylaxis (NP) regimen and gestational age (GA)

	One-drug NP			Two-drug NP			Three-drug NP			
	Grade 0–2	Grade 3–4	Total	Grade 0–2	Grade 3–4	Total	Grade 0–2	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 of 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
33–36 weeks of 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 of 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
Neutropaenia (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 of 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 of 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

*78 infants received no NP or this information was missing.
†67 infants received no NP or this information was missing.
‡29 infants had missing GA (16 in the one-drug group, nine in the two-drug group and four in the three-drug group).
§24 infants had missing GA (14 in the one-drug group, six in the two-drug group and four in the three-drug group).

In a subanalysis restricted to uninfected infants without *in utero* ART exposure, grade 3–4 anaemia was observed in 11 of 254 infants and grade 3–4 neutropaenia in 17 of 227 infants. In univariate analysis, preterm delivery was the only factor significantly associated with grade 3–4 anaemia [unadjusted OR 10.26; 95% confidence interval (CI) 2.62–40.22; P = 0.001]. Factors associated with increased risk of grade 3–4 neutropaenia were prematurity (unadjusted OR 7.38; 95% CI 1.63–33.42; P = 0.009) and non-European maternal origin (unadjusted OR 3.31; 95% CI 1.02–10.76; P = 0.047).

No difference was found in the rate of grade 3–4 anaemia or neutropaenia in this subanalysis for CNP versus no NP [five of 131 (3.82%) versus five of 90 (5.55%) (P = 0.551) and five of 114 (4.38%) versus nine of 81 (11.1%) (P = 0.214), respectively]. However, among infants receiving CNP, we observed that grade 3–4 neutropaenia occurred more frequently in those receiving three- versus two-drug NP [eight of 47 (17.0%) versus one of 34 (2.9%), respectively; P = 0.047], while this was not observed for grade 3–4 anaemia [four of 51 (7.8%) versus one of 39 (2.6%), respectively; P = 0.267].

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

Overall, 7746 blood test 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 NCs according to age and NP type are presented in Figs 1 and 2.

No significant differences in predicted Hb levels or the predicted NCs 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) for CNP versus one-drug NP; coefficient −0.35 (95% CI −0.75 to 0.05; P = 0.090) for no NP versus one-drug NP; NC: coefficient 0.02 (95% CI −0.01 to 0.03; P = 0.178) for CNP versus one-drug NP; coefficient −0.03 (95% CI −0.10 to 0.04; P = 0.366) for no NP versus one-drug NP] (Appendix Table A5).

Discussion

In this large, multicentre individual patient data meta-analysis, the 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 anaemia and 9.1% for neutropaenia). Anaemia or neutropaenia risks were not associated with NP type, but were associated with several factors including preterm delivery. Results were similar in the subanalyses considering only infants born to mothers treated with cART with three or more drugs during pregnancy.

Table 3 Univariable and multivariable logistic regression analyses of factors associated with grade 3–4 anaemia in the first 6 months of life (n = 1737)

	Grade 3–4 anaemia [*n*/total (%)]	Univariate analysis (observed data)			Multivariate analysis (observed data)			Multivariate analysis (imputed data – missing data analysis)		
		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										
≥ 37 weeks	70/1262 (5.55)	1.00			1.00			1.00		
33–36 weeks	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 weeks	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 cells/μL	79/1139 (6.94)	1.00								
≤ 200 cells/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										
< 50 copies/mL	18/391 (4.60)	1.00			1.00			1.00		
50–399 copies/mL	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 copies/mL	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 intravenous 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						
Exposure of infant to therapy (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)									

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; ZDV, zidovudine.

Table 4 Univariable and multivariable logistic regression analyses of factors associated with grade 3–4 neutropaenia in the first 6 months of life (n = 1544)

	Grade 3–4 neutropaenia [*n*/total (%)]	Univariate analysis (observed data)			Multivariate analysis (observed data)			Multivariate analysis (imputed data – missing data analysis)		
		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										
≥ 37 weeks	97/1121 (8.65)	1.00			1.00			1.00		
33–36 weeks	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 weeks	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 cells/μL	104/1011 (10.29)	1.00			1.00					
≤ 200 cells/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										
< 50 copies/mL	42/358 (11.73)	1.00								
50–399 copies/mL	42/372 (11.29)	0.95	0.853	0.61–1.51						
≥ 400 copies/mL	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 intravenous 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
Exposure of infant to therapy										
≤ 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			

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; ZDV, zidovudine.

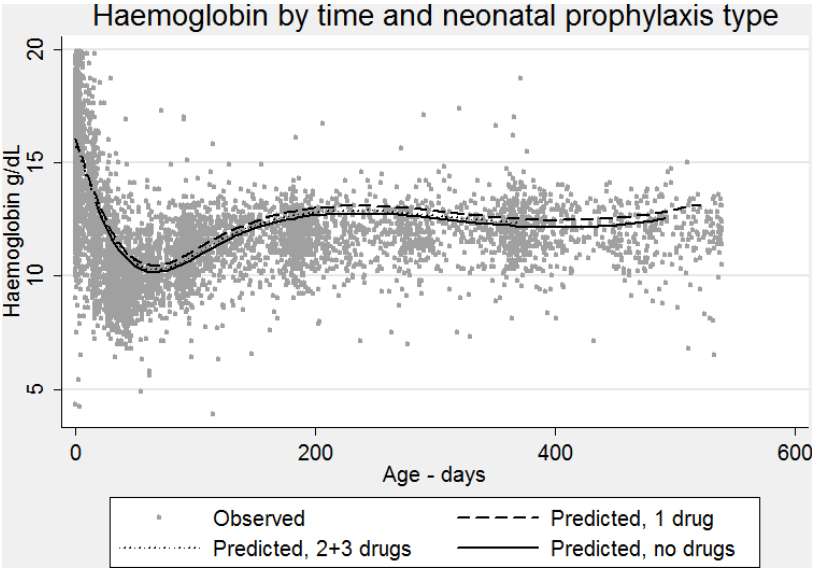

Fig. 1 Observed and estimated haemoglobin levels (g/dL) according to neonatal prophylaxis type (7746 blood test results available for 1836 infants within the age range 0–18 months).

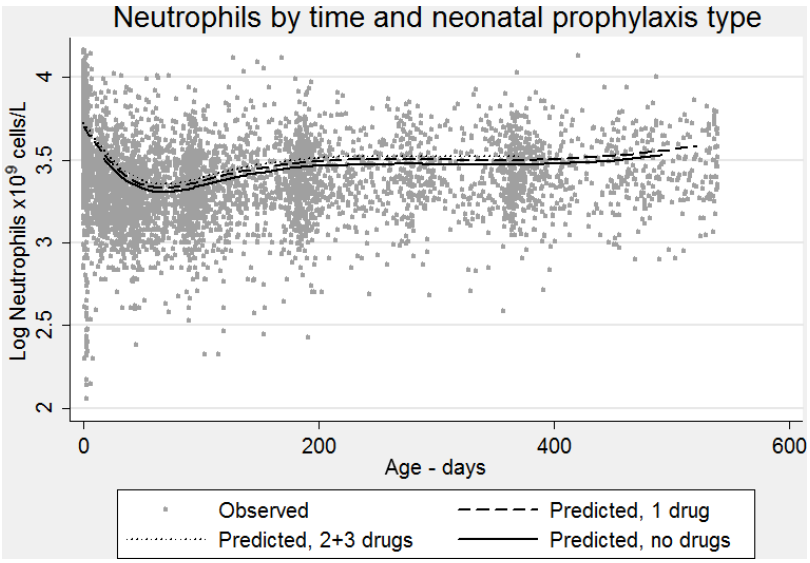

Fig. 2 Observed and estimated neutrophil levels (log cells/L × 10^9) according to neonatal prophylaxis type (7746 blood test results available for 1836 infants within the age range 0–18 months).

Despite the increasing use of CNP in high-risk situations, available safety data are limited. Transient haematological 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, 1684 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 (the ZDV-alone group), ZDV for 6 weeks plus three doses of NVP during the first 8 days of life (the two-drug group), or ZDV for 6 weeks plus nelfinavir and 3TC for 2 weeks (the 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 than in the ZDV-alone group or the two-drug group. Neutropaenia and anaemia accounted for the majority of

serious adverse events; in particular, grade 2 or greater neutropaenia occurred in 16.4% infants receiving only ZDV and in 15.0% of those receiving two drugs, reaching 27.0% in infants receiving three drugs ($P < 0.0001$) [7].

In a retrospective multicentre Canadian study [19] involving 148 infants at high risk for MTCT receiving CNP using therapeutic doses and 145 infants at low risk receiving ZDV, haematological and growth parameters at birth and at 1 and 6 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 (nonspecific 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 6 months of life compared with the ZDV group ($P = 0.04$), but there were no differences between groups for absolute NC.

In a retrospective US study [20] of 148 HIV-exposed uninfected infants, including 36 receiving CNP (the most common regimen was ZDV, 3TC and NVP), no difference in the adverse event rate was observed between infants receiving three-drug CNP and those receiving ZDV alone: 84% versus 66% developed a grade ≥ 1 adverse event, and 11% versus 17% developed a grade ≥ 3 adverse event, respectively. However, the combination of ZDV with 3TC and NVP resulted in an increased frequency of low-grade anaemia (50% versus 39%, respectively). A recent small study of 33 high-risk newborns prescribed NVP + ZDV + 3TC at treatment doses within 72 h of birth for PMTCT [21] showed that anaemia, neutropaenia and hyperlactatemia were the most frequent adverse events; 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, as 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 haematological 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 of gestation. This is particularly an issue when considering therapeutic doses for prophylaxis as a consequence of increased drug exposure in very preterm and/or low birth weight infants [21].

In infants not exposed to HIV *in utero*, anaemia of prematurity is common, as well as neutropaenia, and thus it is not surprising that prematurity remained the major risk factor for these two conditions in our study [22].

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 International Maternal Pediatric Adolescent AIDS Clinical Trials Group (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 anaemia or neutropaenia. Conversely, other authors suggested that a shorter NP may be associated with a reduced incidence of haematological toxicity [22]. 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 weeks [23].

Differences among these findings may be attributable to the fact that we analysed 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 that the latter is not severe enough to be detected when analysing grade 3–4 events.

In the Spanish study, comparing two periods of exposure (2000–2001 and 2007–2013), the authors reported a lower incidence of anaemia and neutropaenia in the second period, with a higher frequency of adverse effects when using maternal regimens containing ZDV [23]. Similar results were reported in a recent Brazilian study including 787 HIV-exposed newborns, 25% of them presenting with anaemia at birth [24]. An increased risk of anaemia was associated with exposure to maternal regimens containing ZDV (compared with tenovofir) and with preterm birth [23]. Unfortunately, the small number of infants treated with CNP prevented the comparison with one-drug NP [23]. In contrast, maternal ART type was not related to haematological toxicity in our multivariate analyses. However, it was not possible to compare maternal ZDV-sparing regimens to those including ZDV because of 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 haematological adverse events without considering the effect on mitochondrial, respiratory, cardiovascular and gastrointestinal systems and on the infant's growth in general, as such data were not

available. There are potential differences in the patient population, as a consequence of 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 data sets with a large number (500) of imputations.

We cannot exclude the possibility of channelling bias, as 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 were performed, whose results were very similar, corroborating our findings. However, residual confounding may have persisted, even after adjusting the analysis for several factors that may have influenced the results.

Also, we observed several differences between the original study population [4] and the present substudy sample (i.e. higher proportions of mothers receiving cART, of mothers with undetectable viral load, and of caesarean deliveries). We may speculate that the subgroup of children for whom blood test results were available were born to mothers with better adherence to MTCT strategies, but this information 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, if the mothers in our subgroup had better adherence, the adverse event incidence would have been overestimated in the substudy, as the substudy infants would have been more exposed to (pre- and postnatal) ART than those included in the original study. Finally, the possibility of 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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Author contributions

EC and CT were responsible for the study concept and design. EC, CT and CL were responsible for undertaking the analyses; CL acts as guarantor for the analyses and has full access to the data set. EC, CT and CL wrote the manuscript. CT, AJ, EC, CG, LE, LG, TG, ANJ, JTRA, PR-C, CR, CT, PT and RM provided data for the study. All members of the Writing Committee participated in discussions about the design of the study, the choice of statistical analyses and interpretation of the findings, and critically reviewed the manuscript.

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Appendix

Table A1 Observed and imputed characteristics of children included in the analysis of risk of grade 3–4 anaemia (n = 1737) or neutropaenia (n = 1544) in the first 6 months of life

	Anaemia			Neutropaenia		
	n	% (observed data)	% (imputed data)	*n*	% (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						
≥ 37 weeks	1262	72.65	73.99	1121	72.60	73.85
33–36 weeks	364	20.96	21.31	328	21.24	21.62
≤ 32 weeks	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 cells/μL	1139	65.57	87.40	1011	65.48	86.61
≤ 200 cells/μL	150	8.64	12.60	131	8.48	13.33
Missing	448	25.79		402	26.04	
Maternal viral load at delivery						
< 50 copies/mL	391	22.51	27.16	358	23.19	28.28
50–399 copies/mL	435	25.04	36.83	372	24.09	36.19
≥ 400 copies/mL	428	24.64	36.00	378	24.48	35.47
Missing	483	27.81		436	28.24	
Intrapartum intravenous 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	
Exposure of infant to therapy (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	

ART, antiretroviral therapy; cART, combination antiretroviral therapy; ZDV, zidovudine.

Table A2 Univariable logistic regression analysis of factors associated with grade 3–4 anaemia in the first 6 months of life (imputed data – data missing analysis) (n = 1737)

		Univariate analysis (imputed data)		
	Grade 3–4 anaemia [*n*/total (%)]	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				
≥ 37 weeks	70/1262 (5.55)	1.00		
33–36 weeks	42/364 (11.54)	2.24	< 0.0001	1.50–3.34
≤ 32 weeks	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 cells/μL	79/1139 (6.94)	1.00		
≤ 200 cells/μL	14/150 (9.33)	1.31	0.377	0.72–2.38
Missing	30/448 (6.70)			
Maternal viral load at delivery				
< 50 copies/mL	18/391 (4.60)	1.00		
50–399 copies/mL	30/435 (6.90)	1.43	0.241	0.79–2.58
≥ 400 copies/mL	42/428 (9.81)	2.04	0.013	1.16–3.58
Missing	33/483 (6.83)			
Intrapartum intravenous 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
Exposure of infant to therapy (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)			

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; ZDV, zidovudine.

Table A3 Univariable logistic regression analysis of factors associated with grade 3–4 neutropaenia in the first 6 months of life (imputed data – data missing analysis) ($n = 1544$)

		Univariate analysis (imputed data)		
	Grade 3–4 neutropaenia [*n*/total (%)]	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				
≥ 37 weeks	97/1121 (8.65)	1.00		
33–36 weeks	36/328 (10.98)	1.31	0.190	0.87–1.96
≤ 32 weeks	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 cells/μL	104/1011 (10.29)	1.00		
≤ 200 cells/μL	20/131 (15.27)	1.52	0.106	0.91–2.55
Missing	24/402 (5.97)			
Maternal viral load at delivery				
< 50 copies/mL	42/358 (11.73)	1.00		
50–399 copies/mL	42/372 (11.29)	0.89	0.595	0.56–1.39
≥ 400 copies/mL	40/378 (10.58)	0.88	0.590	0.57–1.38
Missing	24/436 (5.50)			
Intrapartum intravenous 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
Exposure of infant to therapy (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)			

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; ZDV, zidovudine.

Table A4 Univariable and multivariable logistic regression analyses of factors associated with grade 3–4 anaemia (a) and neutropaenia (b) in the first 6 months of life: subanalysis in infants exposed to combination antiretroviral therapy (cART) *in utero* [(a) $n = 1115$; (b) $n = 986$]neutropaenia

(a)

		Univariate analysis (observed data)			Multivariate analysis (observed data)			Univariate analysis (imputed data – missing data analysis)			Multivariate analysis (imputed data – missing data analysis)		
	Grade 3–4 anaemia [*n*/total (%)]	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													
≥ 37 weeks	38/815 (4.66)	1.00			1.00			1.00			1.00		
33–36 weeks	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 weeks	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)												
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 cells/μL	47/896 (5.25)	1.00			1.00			1.00			1.00		
≤ 200 cells/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													
< 50 copies/mL	11/361 (3.05)	1.00			1.00			1.00			1.00		
50–399 copies/mL	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 copies/mL	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 intravenous 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)												
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
Exposure of infant to therapy (duration of neonatal 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	64/1006 (6.36)	1.00											
≤ 4 weeks	0/10 (0.00)												
Missing	7/94 (7.45)	1.18	0.683	0.068–0.009									
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)												

Table A4 (*Continued*

(b)

	Grade 3–4 neutropaenia [*n*/total (%)]	Univariate analysis (observed data)			Multivariate analysis (observed data)			Univariate analysis (imputed data – missing data analysis)			Multivariate analysis (imputed data – missing data analysis)		
		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													
≥ 37 weeks	72/716 (10.06)	1.00			1.00			1.00			1.00		
33–36 weeks	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 weeks	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 cells/μL	85/793 (10.72)	1.00						1.00			1.00		
≤ 200 cells/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													
< 50 copies/mL	41/334 (12.28)	1.00			1.00			1.00			1.00		
50–399 copies/mL	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 copies/mL	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 intravenous 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)												
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
Exposure of infant to therapy (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 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)												

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; ZDV, zidovudine.

Table A5 Neonatal prophylaxis exposure and haematological markers in the first 18 months of life: longitudinal analysis by mixed effect model

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

ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval.