Level and pattern of HIV-1-RNA viral load over age: differences between girls and boys?

European Collaborative Study*

Objective: To estimate RNA viral load patterns over age in vertically infected children that account for between- and within-individual variation, treatment and assay cut-off detection level. To investigate possible sex-based differences.

Design: A total of 118 infected children with 894 RNA viral load measurements enrolled in the European Collaborative Study were prospectively followed from birth for up to 15 years.

Methods: Fractional polynomial and mixed effects models with censored data to assess the non-linear pattern of viral load over age, allowing for repeated measures.

Results: The RNA viral load peaked at approximately 3 months of age, and gradually declined thereafter. The sex by age interaction was significant ($\chi^2 = 19.7$, $P < 0.001$); viral load peaked higher for girls than boys, but after 4 years the RNA load was consistently 0.25–0.5 $\log_{10}$ lower for girls than boys. The effects of sex and treatment on viral load vary over age ($\chi^2 = 6.31$, $P = 0.043$). Sex differences in RNA viral load relating to measurement without treatment were more pronounced than those under treatment. Disease progression was more rapid for girls than for boys up to the age of 4 years, and less rapid thereafter; the overall difference was not statistically significant.

Conclusion: Differences in RNA viral load over age between untreated boys and girls may have implications for policies for the initiation of antiretroviral therapy, but do not seem to translate into differences in progression to serious disease. The findings would suggest underlying biological explanations, which need further investigation.

AIDS 2002, **16**:97–104

Keywords: Paediatrics, sex, vertically acquired infection, viral load

Introduction

The clinical progression of disease in HIV-infected children has been well described, and without early antiretroviral treatment, approximately 20% of infected children will have been diagnosed with AIDS or will have died by 1 year of age [1]. Progression is much slower in the remaining infected children, and by 10 years of age an estimated 40% of these will have serious manifestations of HIV disease or will have died [1]. However, most infected children, irrespective of a previous diagnosis of AIDS, have no or only mild symptoms or signs at any given point in time, even when untreated [2].

The pattern of HIV-RNA peripheral viral load in vertically acquired infection has been described in several studies [3–5], mostly on a cross-sectional basis and not always taking into account repeated measurements. Although little is known about viral load patterns in vertically infected children over the first 10 years of life, the dynamics are likely to vary between

Prepared by Marie-Louise Newell, Linsay Gray, Claire Thorne, Mario Cortina-Borja and Catherine Peckham, Institute of Child Health, University College London, UK.

Correspondence to: Professor Marie-Louise Newell, Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

Tel: +44 20 7829 8699; fax: +44 20 7813 8145; e-mail: m.newell@ich.ucl.ac.uk

*for European Collaborative Study collaborators see Appendix 1.

Received: 6 July 2001; revised: 29 August 2001; accepted: 6 September 2001.

and within individual children, with levels in the first 4–6 months of life expected to reflect primary infection. In adults, viral load has been reported to differ according to sex and race in some studies, although the implications of these findings on disease progression remain unclear [6,7].

Viral load is likely to be associated with progression of disease in vertically infected children, as it is in adults, but this relationship is not well documented, especially not on a dynamic basis over a number of years. Progression to moderate or severe immune deficiency, as indicated by CD4 cell measurements, occurs rapidly in children and has been shown to be poorly associated with clinical progression [1,2].

Longitudinal information on a substantial number of infected children enrolled in the European Collaborative Study (ECS) and followed for almost 15 years provides a unique opportunity to investigate the dynamics of vertically acquired infection in an appropriately rigorous manner. It can also be used to confirm the existence, and estimate the extent, of sex differences, and to investigate the implications for the progression of disease and response to treatment. We previously described fluctuations in clinical disease over the first 10 years of life [2], and now investigate virological markers of infection up to 15 years of age.

Materials and methods

The ECS, a prospective study, has been on-going since 1986. Children born to women known to be HIV infected at or before delivery are followed according to a standard protocol, with detailed clinical and laboratory information, including RNA viral load and CD4 cell count, in 11 paediatric centres from eight European countries [8–10]. Children are seen at birth, 3 and 6 weeks, 3, 4.5 and 6 months, and then at 3 monthly intervals until 24 months. Subsequently, infected children are examined at least twice a year, according to the same clinical and laboratory protocol; current treatment is recorded on standard forms. Parental consent is obtained before enrolment in the ECS, and the study was approved by local ethics committees. Information relating to the infected children enrolled in the ECS and entered by 1 April 2001 was included in the analysis.

The analysis presented here is based on children known to be HIV infected according to the Centers for Disease Control and Prevention definition of paediatric HIV infection [11]. A child is classified as infected after the onset of AIDS, or the detection of virus or antigen in at least two blood samples (taken on separate occasions), or the persistence of antibody beyond 18 months of age. A child is presumed uninfected if at least two blood samples are antibody negative and if no virus or antigen has ever been identified.

In the absence of standard treatment regimens for HIV-infected children, decisions about the initiation of treatment are based on individual factors, including the clinical and immunological status of the child and adherence concerns (European Collaborative Study, in preparation), and may change over time. In the early years of the ECS, antiretroviral therapy consisted of zidovudine monotherapy to children with advanced disease, but now usually consists of combination therapy at an earlier stage (European Collaborative Study, in preparation).

Laboratory tests, including HIV-RNA polymerase chain reaction and CD4 cell count measurements, were carried out locally, with the assays used recorded on forms. The HIV-RNA copy number was assessed either by nucleic acid sequence-based amplification (NASBA)/Nuclisens (Organon Teknika, Oss, the Netherlands) or Roche Amplicor Monitor, versions 1.0 and 1.5, (Roche Diagnostic Systems, Basel, Switzerland). CD4 cell counts were based on flow cytometry and expressed as the number of cells per cubic millimetre.

Statistical methods

The values of both HIV-RNA viral load and CD4 cell count were log base 10 transformed. As the assay system and type of treatment could vary over age, both assay and treatment status were introduced in a time-dependent manner. Monotherapy was categorized with no therapy because it is unlikely to be associated with a substantial reduction in viral load, an increase in the CD4 cell count, or delayed clinical progression [12]. Children were thus categorized as either treatment naive/receiving monotherapy or treated with a combination of two or more antiretroviral drugs.

The non-linear pattern of viral load over age and the repeated-measures nature of the data required modelling techniques such as fractional polynomial models [13] and mixed effects models with censored data [14]. Fractional polynomial models are flexible, are able to reveal the 'true' curve shape, and allow for modelling asymptotes. Models are compared for significant improvement in fit using likelihood ratio tests [15]. The data also call for a mixed effects model that can account for left censoring of the RNA viral load measurements. The methods described by Hughes [14] offer a modification of the usual EM estimation procedure for fitting mixed effects models with normal errors by accommodating varying censored observations arising from lower (as well as upper) detection limits. Jacqmin-

Gadda *et al*. [15] described a way of calculating the likelihood function for methods that include Hughes' as particular cases. As it was not possible to account for mixed effects models with censored data within the framework of fractional polynomial model-fitting, we used a two-step process. Initially, the data were treated as independent uncensored observations and the optimal fractional polynomial model with respect to age was ascertained. This model was then taken forward into the method allowing both repeated measures and censoring.

The model was expanded from that involving only the age terms, through the introduction of stratification by sex, treatment and CD4 cell count to assess the significance of these factors separately. The factors were assessed for significance of interaction with the age terms and, where appropriate, with one another. The significance of individual terms was assessed by comparing nested models using likelihood ratio tests [15]. There were sufficient data to expand the model as far as three-way interaction. Viral load profiles were compared for those who did and those who did not progress to serious disease or death in a descriptive manner.

Progression to serious disease in this cohort was assessed by Kaplan–Meier product limit analysis, and plots were compared by sex using the log-rank test. Regression analysis investigated the differences in $\log_{10}$ HIV-RNA viral load before and after the initiation of combination therapy, allowing for other factors.

All analyses were carried out using STATA (STATA Version 6.0; College Station, TX, USA) and S-PLUS 3.4 (Insightful, USA) in a Unix environment, utilizing a modified version of the software provided by Hughes in 1999. We used the software described by Jaqmin-Gadda *et al*. [15] to compute the multivariate normal integrals in the likelihood function.

Results

Of the 178 infected children (8.7% of the total cohort) 41 had died and 19 were no longer in follow-up in an ECS centre after the introduction of routine viral load assays. Viral load measurements were thus available on 118 infected children (Table 1). Of the 894 viral load measurements, 163 were below the assay specific cut-

Table 1. Infected children in the European Collaborative Study.

Variable	Children (n = 118)	Measurements
Number of measurements	Median (range)	Total
	7 (1–23)	894
Viral load values (copies/ml)		
Median (range)	–	14 690 (40–8 100 000)
Viral load measurement n (%)		
Actual	70 (59.3)	731 (81.8)
Below detection level	48 (40.7)	163 (18.2)
Sex n (%)		
Female	60 (50.8)	421 (47.1)
Male	58 (49.1)	473 (53.9)
Treatment status n (%)		
No or monotherapy	30 (25.4)	272 (30.4)
Combination therapy	88 (74.6)	622 (69.6)
CD4 T cell count ($\times 10^3$/l)		
Median (range)	–	0.67 (0.001–4.82)
Assay n (%)		
NASBA	–	167 (20.7)
Roche	–	639 (79.3)
Age at treatment initiation (months)		
Median (range)		31.5 (0.76–151.1)
Age at last visit (months)		
Median (range)		93.1 (0.2–179.0)
CDC immunological stage at last visit n (%)		
1 Immunologically normal		12 (10.2)
2 Moderate suppression		50 (42.4)
3 Severe suppression		50 (42.4)
Died		6 (5.1)
CDC clinical stage at last visit n (%)		
N No symptoms		15 (12.7)
A Mildly symptomatic		12 (10.2)
B Moderately symptomatic		70 (59.3)
C Severely symptomatic		15 (12.7)
Died		6 (5.1)

CDC, Centers for Disease Control and Prevention; NASBA, nucleic acid sequence-based amplification.

off value, and the median RNA viral load in the remainder was 14 690 copies per millilitre of plasma. Most children (88, 75%) had received combination antiretroviral therapy, with a median age at treatment initiation of 31.5 months. Twenty-one (11 girls and 10 boys) of the 118 children had been diagnosed with serious HIV-related disease (Centers of Disease Control and Prevention classes C or D), of whom six had died.

HIV-RNA viral load over age

The three power fractional polynomial model was not significantly better than the best two power ($\chi^2 = 3.74$, $P = 0.156$); the optimal model for log viral load and age included an inverse square root of age and a log age term. Any measurements taken on the day of birth were set to day one to resolve numerical indeterminations. The pattern of HIV-RNA viral load over age, in both a non-parametric smoother and the curve predicted from the fractional polynomial model, is seen to peak at approximately 3 months of age, with a gradual decline thereafter (Fig. 1).

A total of 805 observations with information on assay type, age, sex, CD4 cell count and treatment were used in the subsequent modelling. Nearly 80% (638/805) were assessed using a Roche assay and 20% (167/805) using a NASBA assay. The shape of the overall pattern of HIV-RNA viral load over age was similar for RNA measurements assessed by Roche or by NASBA assay.

The $\log_{10}$ RNA viral load did not differ by sex in univariate analysis ($P = 0.971$) (Table 2). A one $\log_{10}$ CD4 cell increase resulted in a significant decrease of 0.895 in $\log_{10}$ RNA viral load ($P < 0.001$). Combination therapy was associated with a 1.28 lower $\log_{10}$ viral load compared with the viral load in the no/monotherapy category ($P < 0.001$). The Roche assay was associated with a 0.404 lower $\log_{10}$ viral load than the NASBA assay ($P = 0.0128$).

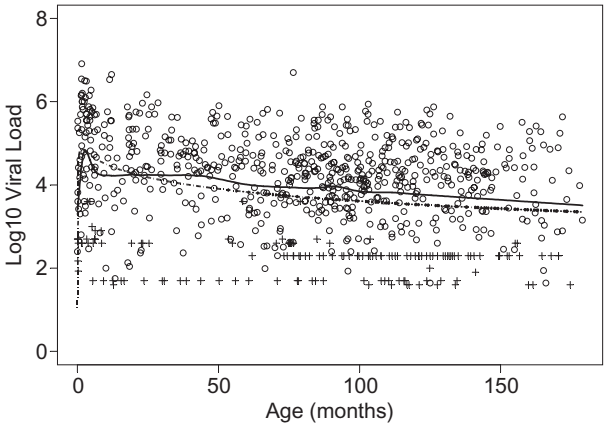

Fig. 1. Observed and predicted HIV RNA by age. —— Non-parametric smoother; – – – fractional polynomial; ○ observed; + censored.

Factors influencing HIV-RNA viral load pattern over age

Two-way interactions of each of the main effects of sex, treatment and CD4 cell count with age were assessed for significance. The sex by age interaction was significant ($\chi^2 = 19.7$, $P < 0.001$). The pattern of viral load over age in girls differed from that of boys (Fig. 2). This difference was masked in the previous model, which accounted for only the main effect of sex. Viral load in girls peaks a little earlier (1.1 months) than in boys (2.1 months), and at a higher value and declines more sharply in girls than boys, with a cross-over at approximately 4 years of age. After this age the viral load in girls is consistently lower than that in boys. At the peak the estimated HIV-RNA viral load is more than 1 $\log_{10}$ above the peak level for boys at that age, whereas after the cross-over girls have a predicted viral load 0.25–0.5 log below that for boys. Interactions of treatment ($\chi^2 = 0.599$, $P = 0.741$) and CD4 cell count ($\chi^2 = 0.235$, $P = 0.628$) with age were also investigated. Allowing for an interaction between sex and treatment, there was evidence of a difference in the effect of treatment by sex ($\chi^2 = 5.18$, $P = 0.023$), suggesting that treatment is associated with a larger decrease in the RNA viral load in boys than in girls.

A full model was fitted to incorporate the main effects of sex, treatment, assay type and CD4 cell count and two-way interactions of age by sex, age by treatment and sex by treatment, and a three-way interaction of age by sex by treatment. Because of the significant three-way interaction of inverse square root age by sex by treatment and $\log_{10}$ age by sex by treatment ($\chi^2 = 6.31$, $P = 0.043$) the viral load pattern over age is presented separately for the four strata defined by sex and treatment for Roche assay observations, at the median CD4 cell count (Fig. 3). The picture for NASBA observations is similar, but levels are a little lower than for Roche measurements. This model predicts that, for example, at age 12 months the difference in RNA viral load between untreated and treated girls is approximately 2 $\log_{10}$, and 1.5 $\log_{10}$ for treated and untreated boys. Similarly, at 15 years of age, the predicted differences would be 1 $\log_{10}$ for girls and 2 $\log_{10}$ for boys.

Progression to serious disease

In the ECS cohort the group of children with available viral load information reflects children enrolled in more recent years, when treatment was also more widely available. Therefore progression is less than would be seen in the total cohort. In the group of 118 infected children with available information about the HIV-RNA viral load, 21 children had progressed to serious HIV disease (15) or death (6). Survival estimates of the progression to CDC class C disease or death indicate that 6% (2–11%) of infected children in this group would have progressed by the age of 1 year, 17% (10–

Table 2. RNA viral load pattern over age by sex, treatment, CD4 cell count and assay in individual analyses.

Variable	Univariate coefficient	95% CI	P value
1/sqrt(Age) increase	−1.16	−1.50 to −0.822	< 0.0001
ln(Age) increase	−0.496	−0.625 to −0.366	< 0.0001
Sex			
Females	0	–	–
Males	−0.006	−0.342 to 0.355	0.971
Treatment			
No/monotherapy	0	–	–
Combination therapy	−1.28	−1.56 to −0.994	< 0.0001
1 $\log_{10}$ CD4 cell increase	−0.895	−1.14 to −0.651	< 0.0001
Assay			
NASBA	0	–	–
Roche	−0.404	−0.722 to −0.086	0.0128

CI, Confidence interval; NASBA, nucleic acid sequence-based amplification.

24%) by the age of 5 years, and 22% (13–31%) by 10 years of age. Progression to serious disease or death was similar for boys and girls (log rank test $P = 0.79$). There was no statistically significant association by treatment, and indeed children who had received combination therapy were estimated to have a non-significantly increased rate of clinical progression, reflecting late initiation of treatment.

Only seven of the 21 children were observed to have progressed to serious disease or death by 1 year of age, whereas only five of the 21 who progressed at any age had viral load measurements relating to a time before they had been diagnosed with serious disease. Given the rarity of events, analyses relating peak viral load measurements in the first months of life to the risk of progression were considered to be inappropriate.

The difference in $\log_{10}$ HIV-RNA viral load measurements before and after the initiation of combination was evaluated in a regression analysis, allowing for sex, viral load assay, initial viral load, and duration between measurements. A total of 34 children (14 girls and 20 boys) had information available, and the decrease in log viral load after the initiation of treatment was estimated to be 0.48 less in boys than in girls, but this did not reach statistical significance ($P = 0.299$).

Although progression did not differ significantly by sex, progression to serious disease or death was more rapid for girls up to approximately 4 years of age and less rapid than for boys after that age. This trend mirrors that seen for the HIV-RNA viral load pattern over age.

Discussion

Using data on the infected children enrolled in the ECS, the level and pattern of HIV-RNA viral load over nearly 15 years was described. The overall picture shows a marked peak in viral load at approximately 3

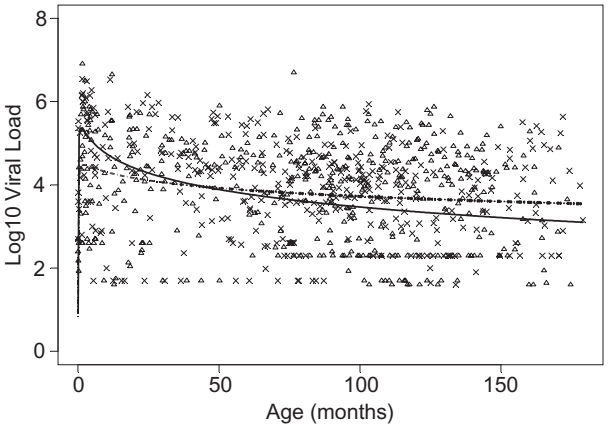

Fig. 2. Log RNA viral load by sex.
× —— Females; △ – – males.

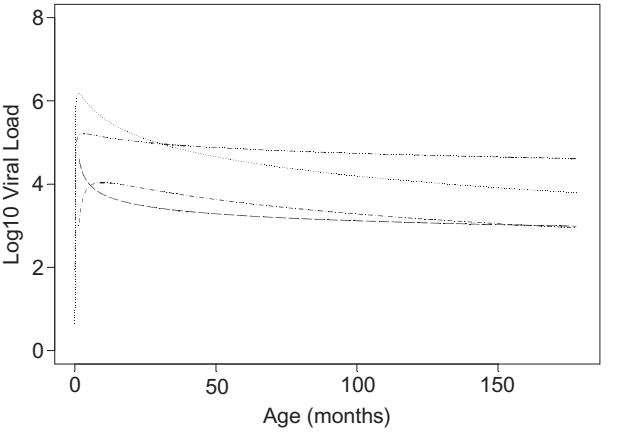

Fig. 3. Log RNA viral load (by Roche assays) by age, sex and treatment. ····· Females (no and mono); ——— females (comb); – ··· – ··· – males (no and mono); – · – · – males (comb).

months of age, probably reflecting primary infection, followed by a rapid decline in the first 5 years of life, slowing thereafter. Our analysis used fractional polynomial models that reflect the actual shape of the viral load pattern over age in vertically infected children. Although the results obtained using fractional polynomial methodology are generally sensitive to outlying values, the large number of observations available for analyses here have produced reliable models. The methodology used allows for both repeated measures within children and the censoring effect of the assay cut-off value. Generally, the assay cut-off value for detection is either set at the assay specific point, or arbitrarily half-way between zero and the cut-off. Both approaches lead to bias in the parameter estimates [13,14]. Furthermore, we were able to use longitudinal repeated measurements over 15 years rather than cross-sectional assessments at different ages.

HIV-RNA viral load has been shown in several studies to be associated with the progression of disease overall [16,17], but there is a lack of information on whether this association differs by sex. In a recent conference presentation [18], average relative and absolute CD4 cell counts were higher in girls than in boys both in infected and uninfected children. In infected children, HIV-RNA levels were lower, but not significantly so, at all time points to 18 months in untreated girls than in boys, but any relationship with the progression of disease was not investigated. Using European data, we show here that allowing for repeated measures within individuals in a model with viral load, age and sex, there was a substantial sex difference in viral load pattern over the first 15 years of life. RNA viral load levels were estimated to peak a little earlier and at substantially higher levels in girls than in boys, with a difference of more than 1 $\log_{10}$. After approximately 5 years of age the levels of RNA in girls are up to half a $\log_{10}$ below that of boys. This sex difference in the peripheral viral load pattern over age was reflected in the curve of clinical progression, although overall progression was not statistically significantly associated with sex. This may imply that for a given RNA viral load the progression of disease in boys is slower than for girls.

The data presented here were collected within a prospective cohort study, not a clinical trial and treatment decisions were thus not random. The interactions between age, sex and treatment in the assessment of patterns of viral load are complex. The association between treatment and viral load over age differed between girls and boys. For both boys and girls viral load observations under treatment were consistently and substantially lower. However, whereas the difference in RNA viral load under treatment and not under treatment increased slightly for boys, it narrowed substantially for girls. Without treatment, the measurements of RNA viral load are initially higher for girls than boys, but levels cross over at approximately 3 years of age, and thereafter the measurements relating to untreated girls are consistently lower than in boys. With treatment, viral load measurements for girls started off being higher than for boys, but after approximately 9 months of age they become lower than for boys, with very little difference at later ages. Taken together, these findings indicate possible sex-specific dynamics of viral replication.

In adults, lower HIV-RNA viral loads for women than for men have been described, tentatively suggested to be associated with hormonal levels. Hormonal influences could also play a role in children, even in the pre-pubertal stages. Our findings suggest a biological explanation in response to HIV infection. The progression of disease is generally similar in women and men, and in our study there was no difference in overall progression between boys and girls. However, few studies have successfully associated viral load patterns over a prolonged period of time with clinical progression in men and women separately, and most analyses have been cross-sectional [19]. Guidelines for the initiation of antiretroviral therapy based on viral load do not currently allow for sex, although it has been suggested that, for a similar baseline level, women may achieve viral suppression after highly active antiretroviral therapy at a faster rate than men and have a more sustained response [7].

Conclusion

The findings presented here, if confirmed, may have implications for clinical guidelines for the initiation of antiretroviral therapy in children, and suggest that cut-off levels of RNA viral load at which therapy would be recommended may need to be lower for girls than for boys, after the first year of life. For example, WHO guidelines suggest that for children over 1 year of age the initiation of treatment should be considered if the viral load is over 100 000 copies [20]. In our cohort, 22 girls and 32 boys had an HIV-RNA load above this level over the age of 1 year, with 13 girls and 18 boys being treated at or before the time of high viral load. In addition, response to treatment measured through RNA viral load may also need to be interpreted in a different way for boys and girls, with an expected lower average RNA load for boys after the initiation of treatment than for girls.

Acknowledgements

The authors would like to acknowledge support from Mrs L. Toxtle and Dr Simona Fiore (London). They

would also like to thank Prof L. Chieco-Bianchi, Prof F. Zacchello, Dr E. Ruga, Dr R. D'Elia, Dr A.M. Laverda, Dr S. Cozzani, Dr C. Cattelan, Dr A. Mazza, Prof B. Grella, Dr A.R. Del Mistro and Mrs S. Oletto (Padua); Dr C. Feiterna, and Dr R. Weigel (Berlin); Dr F. Johnstone, Dr S. Burns, Dr N. Hallam, Dr P.L. Yap, and Dr J. Whitelaw (Edinburgh); Dr B. Sancho, and Dr G. Fontan-Casanego (Madrid); Dr F. Asensi, Dr M.C. Otero, Dr A. Perez Tamarit, Dr A. Gonzalez Molina, Dr M. Gobernado, Dr J.L. Lopez, and Dr J. Cordoba (Valencia); A. van der Plas (Amsterdam); Dr B. Christensson, Dr P. Bolme, and Dr U. Ewald (Sweden); Dr G. Di Siena, and Dr E. Pontali, Prof M.F. Pantarotto, G. Mantero, and Dr P. Dignetti (Genoa); and Dr A. Hottard, Dr M. Poncin, Dr S. Sprecher, Dr B. Lejeune, Dr G. Zississ, and Prof N. Clumeck (Brussels), Dr M. Guxens, and Dr P. Martinez (Barcelona).

Appendix

ECS collaborators: Dr C. Giaquinto, Dr O. Rampon, Dr V. Giacomet and A. De Rossi (Universita degli Studi di Padova, Italy); Dr I. Grosch-Wörner (Charite Virchow-Klinikum, Berlin, Germany); Dr J. Mok (Royal Hospital for Sick Children, Edinburgh); Dr I. Bates, Dr I. de José, Dr F. Hawkins, Dr M.C. Garcia-Rodriguez, Dr C. Ladrón de Guevara, Dr J. Mª Peña, Dr J. Gonzalez Garcia and Dr J.R. Arribas Lopez (Hospital Infantil La Paz, Madrid); Prof F. Asensi-Botet, Dr M.C. Otero, Dr D. Pérez-Tamarit, Dr A. Orti, Dr M.J. San Miguel and Dr R. de la Torre (Hospital La Fe, Valencia, Spain); Dr H. Scherpbier, M. Kreyenbroek and Dr K. Boer (Academisch Medisch Centrum, Amsterdam, the Netherlands); Dr A.B. Bohlin, Dr E. Belfrage, Dr L. Navér, Dr A. Ehrnst and Prof A. Sönnerborg (Huddinge and Karolinska University Hospitals, and Karolinska Institutet, Sweden); Prof J. Levy, Dr M. Hainaut, Dr A. Peltier, Dr S. Wibaut and Dr P. Barlow, (Hospital St Pierre, Brussels, Belgium); Dr A. Ferrazin and Prof D. Bassetti, (Department of Infectious Diseases, University of Genoa, Italy); Dr A. De Maria (Department of Internal Medicine, University of Genoa, Italy); Dr C. Gotta (Department of Obstetrics and Gynecology – Neonatology Unit, University of Genoa, Italy); Dr A. Mûr, Dr A. Payà, Dr M. Viñolas, Dr M.A. López-Vilchez, Dr M. Rovira, Dr R. Carreras, Dr E. Esteban Tores, Dr S. Herrero Perez (Hospital del Mar, Universidad Autonoma, Barcelona, Spain); and Dr N.H. Valerius (Hvidovre Hospital, Denmark).

Sponsorship: The European Collaborative Study is a concerted action of the European Commission (Biomed II PL 97 2005 and QLRT-1999-30002). The Medical Research Council (UK) provided support to the coordinating centre. Collaborating centres were supported by grants from the Ministero della Sanita – Istituto Superiore di Sanita, Progetto AIDS (Padua, Genoa); the Medical Research Council (UK); the AIDS Virus Education Research Trust, the Scottish Office Home and Health Department (Edinburgh); Praeventiefonds No. 28-1704 (Amsterdam); Bundesminister fur Gesundheit (Berlin); Fonds Houtman, Office de la Naissance et de L'Enfance, Communaute Francaise de Belgique (Brussels); and the Research Foundations of the Karolinska Institutet (Stockholm).

References

1. The French Paediatric HIV Infection Study Group, European Collaborative Study. **Morbidity and mortality in European children vertically infected by HIV-1.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, **14**:442–450.
2. European Collaborative Study. **Fluctuations in symptoms in HIV-infected children: the first 10 years of life.** *Pediatrics* 2001, **108**:116–122.
3. Mofenson LM, Harris DR, Rich K, *et al*. **Serum HIV-1 p24 antibody, HIV-1 RNA copy number and CD4 lymphocyte percentage are independently associated with risk of mortality in HIV-1-infected children.** *AIDS* 1999, **13**:31–39.
4. Shearer W, Quinn TC, LaRussa P, *et al*. **Viral load and disease progression in infants infected with human immunodeficiency virus type I.** *N Engl J Med* 1997, **336**:1337–1342.
5. Paediatric European Network for Treatment of AIDS (PENTA). **HIV-1 viral load and CD4 cell count in untreated children with vertically acquired asymptomatic or mild disease.** *AIDS* 1998, **12**:F1–F8.
6. Sterling TR, Vlahov D, Astemborski J, Hoover DR, Margolick JB, Quinn TC. **Initial plasma HIV-1 RNA levels and progression to AIDS in women and men.** *N Engl J Med* 2001, **344**:720–725.
7. Moore AL, Mocroft A, Madge S, Devereux H, Wilson D, Phillips AN, Johnson MA. **Gender differences in virologic response to treatment in an HIV-positive population: a cohort study.** *J Acquir Immune Defic Syndr* 2001, **26**:159–163.
8. European Collaborative Study. **Maternal viral load and vertical transmission of HIV-1: an important factor but not the only one.** *AIDS* 1999, **13**:1377–1385.
9. European Collaborative Study. **Vertical transmission of HIV-1: maternal immune status and obstetric factors.** *AIDS* 1996, **10**:1675–1681.
10. European Collaborative Study. **Natural history of vertically acquired human immunodeficiency virus-1 infection.** *Pediatrics* 1994, **94**:815–819.
11. Centers For Disease Control. **1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age.** *MMWR* 1994, **43(RR12)**:1–10.
12. Concorde Coordinating Committee. **Concorde: MRC/ANRS randomised double-blind controlled trial of immediate and deferred zidovudine in symptom-free HIV infection.** *Lancet* 1994, **343**:871–881.
13. Royston P, Altman GD. **Regression using fractional polynomials of continuous covariates: parsimonious parameter modelling.** *Appl Stat* 1994, **43**:429–467.
14. Hughes JP. **Mixed effects models with censored data with application to HIV RNA levels.** *Biometrics* 1999, **55**:625–629.
15. Jacqmin-Gadda H, Thiebaut R, Chene G, Commenges D. **Analysis of left-censored longitudinal data with application to viral load in HIV infection.** *Biostatistics* 2000, **1**:1355–1368.
16. Abrams EJ, Weedon J, Steketee RW, *et al*. **Association of human immunodeficiency virus (HIV) load early in life with disease progression among HIV-infected infants.** *J Infect Dis* 1998, **178**:101–108.
17. Dickover RE, Dillon M, Leung KM, *et al*. **Early prognostic indicators in primary perinatal human immunodeficiency virus type 1 infection: importance of viral RNA and the timing of transmission on long-term outcome.** *J Infect Dis* 1998, **178**: 375–387.

18. Pitt J, Moye J, Matthews Y, Diaz C, Rich K, Paul M, Hoff R. **Gender differences in immunologic and virologic markers in children born to women infected by HIV.** In: *8th Conference on Retroviruses and Opportunistic Infections.* Chicago, 2001 [Abstract 513197].
19. Rezza G, Cozzi Lepri A, D'Arminio Monforte A, *et al.* **Plasma viral load concentrations in women and men from different exposure categories and with known duration of HIV infection.** *J Acquir Immune Defic Syndr* 2000, 2556–2562.
20. World Health Organisation. *HIV/AIDS Antiretroviral Newsletter* 1999; 2.