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Paediatric HCV and HIV infection: mode of acquisition, progression and coinfection

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I, Kirsty Anne England, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

In this thesis a diverse range of topics related to paediatric HIV and HCV infection are investigated information is provided on the more specific areas of coinfection, disease monitoring methodologies and the impact of mode of acquisition of infection. Four unique epidemiological cohorts of vertically and parenterally HIV, HCV and HIV/HCV coinfected children are analysed. ALT reference ranges adjusted for age and sex in children under five years of age show that ALT levels greater than 60U/l in boys and 55U/l in girls should be regarded as elevated in the first 18 months of life while thereafter the upper limits of normal ALT levels are lower; 40U/l in boys and 35U/l in girls. There are no differences found between vertically and parenterally HCV-only infected groups in their genotype profile, proportion with consistent viraemia, consistently elevated ALT levels or evidence of two or more markers of disease progression. Parenterally HIV infected children are described for the first time and only 12% found to progress to moderate/severe clinical symptoms or immunosuppression during follow-up. The lack of treatment in this group (11% treated) suggests a more favourable disease progression in parenterally than vertically HIV infected children. The possible detrimental effects of ART on ALT levels in HIV/HCV coinfected children are demonstrated along with the possible need for differential management of children infected via different routes given the faster progression to immunosuppression in parenterally coinfected children. The survey of current practices and policies for care of HIV/HCV coinfected children reveals that in general the management practices vary widely in terms of testing high risk groups for coinfection, which laboratory tests to carry out in comparison to those performed on HIV-only and HCV-only infected children and the opinions on optimal treatment for this group emphasise the importance of research in this area to inform clinical guidelines.

PAEDIATRIC HCV AND HIV INFECTION: MODE OF ACQUISITION, PROGRESSION AND COINFECTION

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ABBREVIATIONS

HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
MTCT	Mother-to-child transmission
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
ALT	Alanine aminotransferase
EPHN	European Paediatric HCV Network
ECS	European Collaborative Study (of children born to HIV infected women)
IFN	Interferon
HAART	Highly active antiretroviral therapy
ART	Antiretroviral therapy
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
CDC	Centres for Disease Control
PENTA	Paediatric European Network for the treatment of AIDS
WHO	World Health Organisation
BHIVA	British HIV Association
CHIVA	Children's HIV Association
SD	Standard deviation
OR	Odds ratio

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1. PAEDIATRIC HCV AND HIV INFECTION: MODE OF ACQUISITION, PROGRESSION AND COINFECTION

1.1 Global epidemiology and biology of Hepatitis C virus (HCV)

HCV is a noncytopathic virus of the Flaviviridae family which infects the liver, replicates at a high rate and in 10-30% of patients causes acute, limited infection with normally asymptomatic inflammatory liver disease reconciled by the cellular immune response (Chisari 2005, Hoofnagle 2002). However, HCV can cause chronic hepatitis in 70-90% of infected people, with elevated alanine aminotransferase (ALT) levels and progressive hepatic fibrosis, cirrhosis, liver failure and hepatocellular carcinoma (Chisari 2005, Hoofnagle 2002). Six genotypes and over 90 subtypes of HCV have been isolated since its discovery in 1988 (Choo *et al* 1989). Repeated percutaneous exposure to contaminated blood is the most efficient mode of HCV transmission. HCV transmission via accidental needlestick injuries, sex with an infected partner or mother-to-child transmission is less efficient (Alter 2007). Over the last 20 years, HCV has emerged as a global public health concern with an estimated 180 million people currently infected worldwide (WHO 2007). This equates to a global prevalence of approximately 3%, but prevalence rates vary greatly by geographical region and also between specific sub-groups within countries or regions. Specifically, injecting drug users are the highest risk group with recent global prevalence estimates of at least 50% (Aceijas and Rhodes 2007). In Northern and Central Europe HCV prevalence ranges from 0.1% to 1.2% on a population level, compared to higher prevalence in Southern Europe of 2.5% to 3.5%, although there are isolated areas of Italy and Greece where HCV prevalence is as high as 20% due to historical widespread unsafe medical procedures (Esteban *et al* 2007). In Eastern Europe the extensive injecting drug use

epidemic and the substantial use of unsafe medical procedures has resulted in higher prevalence rates up to 10% in blood donors and health workers and as high as 92% in high risk groups such as haemophiliacs (Esteban *et al* 2007, Aceijas *et al* 2004).

1.2 Acquisition of paediatric HCV infection

1.2.1 Vertical transmission

HCV infection in children is predominantly acquired via mother-to-child transmission (MTCT) and occurs in approximately 5% of children born to HCV infected mothers, although transmission rates can be as high as 15% depending on maternal HIV coinfection status and maternal HCV RNA viral load (Newell and Pembrey 2002, Tovo *et al* 1997, Zanetti *et al* 1998, EPHN 2001, Gibb *et al* 2000, Granovsky *et al* 1998, Resti *et al* 1998, Thomas 1998, Paccagnini *et al* 1995, Ruiz-Extremera *et al* 2000). Prevention of MTCT relies on the diagnosis and treatment of women infected with HCV before they become pregnant as there is currently no treatment suitable for use in pregnancy and no post-exposure prophylaxis available to the newborn, as there is for HIV infection. Studies have investigated the risk of MTCT during delivery with the hypothesis that, like HIV, vertical transmission of HCV may be reduced by elective caesarean section but to date there is strong evidence that elective caesarean section does not reduces the risk of HCV transmission despite some findings of a protective effect in smaller studies (EPHN 2001, Gibb *et al* 2000a, Tovo *et al* 1997). Similarly, HCV has been found in breast milk but there is strong evidence to suggest that MTCT does not occur through breastfeeding and breastfeeding is not contraindicated among HCV infected mothers (Kumar and Shahul 1998, Lin *et al* 1995, Mast *et al* 2005, EPHN 2001).

1.2.2 Parenteral transmission

HCV infection via contact with contaminated blood or blood products is now rare after the implementation of donor screening in many countries in the early 1990s, however, blood safety is not a priority in some countries and others lack the resources to implement donor screening (Alter 2007, Hladik *et al* 2006). The most important factor in the spread of HCV infection is unsafe medical injections with approximately 2.3 to 4.7 million people worldwide infected in this way, mostly in resource limited settings (Kane *et al* 1999) accounting for approximately 40% of HCV infections worldwide (Hauri *et al* 2004). In resource-limited countries, sterile syringes are often unavailable, injections are often administered by non-health professionals and the use of oral medication is not prioritised (Alter 2007). An example of the potential impact of these unsafe procedures was demonstrated by the reuse of glass syringes during the treatment of schistosomiases in Egypt until the early 1980s which resulted in the largest outbreak of iatrogenic transmission of a bloodbourne pathogen ever recorded and the effects of which are still seen today in the form of high population prevalence rates stemming from this initial reservoir of infection (Alter 2007, Frank *et al* 2000). In addition, HCV has also been shown present in the environment on needles, syringes and infusion bags and the infectivity of the virus after drying and storage has been confirmed (Kamili *et al* 2007). The transmission of HCV via contact with infected individuals or family members has been investigated and some limited evidence suggests that HCV prevalence is higher in children with infected parents, independent of possible MTCT or other routes of transmission (El-Raziky *et al* 2004). Despite the predominance of vertically transmitted HCV infection in children there remain children infected parenterally as a result of isolated, usually hospital-based outbreaks and there is compelling evidence to suggest that procedures causing these outbreaks continue

globally (El-Raziky *et al* 2004, Gibb *et al* 2000, O'Riordan *et al* 1998, Prati 2006, Patel *et al* 2006, Davaalkham *et al* 2006).

1.3 Monitoring HCV-related infection/disease progression

1.3.1 Liver biopsy

In the absence of HCV-related clinical symptoms, liver biopsy is sometimes used to assess the extent of liver fibrosis and the likely risk of progression to liver disease, but this is an invasive technique for what is largely an asymptomatic disease and the benefits of biopsy can not always be justified in many cases, especially in children. New, non-invasive techniques such as the Fibro Scan are being developed (Kettaneh *et al* 2007, Stauber and Lackner 2007) but clinicians rely largely on biochemical markers of infection such as ALT levels to indicate liver-related abnormalities which may require further investigation. The combination of, for example, elevated ALT levels with persistence of HCV RNA viraemia can also indicate individuals at higher risk of HCV-related disease progression (EPHN 2005a). There is, however, a general dissatisfaction with the sensitivity of serum ALT as a marker of liver disease, especially in the case of chronic HCV infection where despite normal ALT levels, some patients have been found to have some evidence of liver abnormalities (Prati *et al* 2002).

1.3.2 ALT levels

ALT levels provide a measure of liver function with high levels often indicating some degree of impairment. However, the definitions of abnormal ALT levels vary and levels are also affected by age, sex and race, making their interpretation in terms of liver function

difficult (Jamal *et al* 2003). Definitions of normal or abnormal ALT and the resulting clinical decisions have been largely based on reference ranges of normal ALT levels provided by laboratories carrying out the test. The populations from which these reference ranges have been extrapolated are undefined and are likely to vary widely. Consequently, a number of recent studies have called into question the use of these reference ranges (Prati 2002, Mohamadnejad *et al* 2003, Brinkmann *et al* 2003, Piton *et al* 1998, Kariv *et al* 2006) and identified new adult reference ranges calculated from defined "healthy" populations which in general have shown the revised upper limit of normal to be lower than those currently used. For example, a significantly lower upper limit of normal for ALT levels in 17,496 healthy subjects aged 15-90 years, 37.5U/l compared to 52U/l recommended by laboratory reference ranges, was found (Kariv *et al* 2006). These studies also indicate the importance of accounting for sex and body mass index (BMI) when calculating the appropriate ALT ranges for individuals. Piton *et al* identified four appropriate upper limits of ALT levels: 31U/l for females with BMI≤23, 42 U/l for males with BMI≤23, 44 U/l for females with BMI>23 and 66 U/l for males with BMI>23 after comparing different methods for identifying the "true" upper limit of normal, which in some cases varied by 20%– 45% (Piton *et al* 1998).

Such evidence from adult studies suggest that the true upper limit of normal ALT levels is lower than previously identified and therefore it is possible that those patients with "normal" ALT levels who go on to develop liver disease in fact had abnormal ALT levels if one applies upper limits suggested by these studies. This may help explain why the sensitivity and specificity of ALT levels in identifying patients with liver abnormalities is

not always high and may be important in terms of increasing the accuracy of this commonly used biological marker.

ALT levels are also used to indicate liver function in paediatric populations, often to assess the extent of liver damage due to hepatitis B, chronic hepatitis C, autoimmune hepatitis or non-alcoholic fatty liver disease (Zacharakis *et al* 2007, Kerkar 2005, EPHN 2005a, Davison *et al* 2006, Quirós-Tejeira *et al* 2007). As with adults, many paediatric studies refer to normal or elevated ALT levels but few provide details of the specific values or the populations and methods by which these were defined. Commonly studies define elevated ALT levels as twice the upper limit of normal but without indicating what the upper limit of normal is in terms of ALT levels. Previous studies attempting to quantify reference ranges for ALT levels have been based on cross-sectional data collated from several different populations. In a clinical setting, ALT levels are assumed to be normal or abnormal based on reference ranges from laboratories, as with adults, and a commonly used upper limit of this normal range is approximately 80U/l in children younger than 12 months and 40U/l in children older than 12 months (Nelson Paediatrics 17th Edition, EPHN 2005a). However, to date few studies have investigated the accuracy of these ranges and therefore guidelines on the appropriate reference ranges for ALT levels in children and their variation with sex or BMI, shown to be important in adults studies, remain unqualified.

1.3.3 Genotype

Five major HCV genotypes have been identified with varying geographical distributions. Genotype 1a is prevalent in northern Europe and the USA, genotypes 1b and 2 in southern Europe, 3 in the Indian subcontinent and among drug users in Europe and 4 in Africa and

the Middle East (Dusheiko *et al* 1994). Adult studies have linked HCV genotype with differential disease progression and response to interferon (IFN) therapy (Le Guillou-Guillemette *et al* 2007, Torres-Puente *et al* 2008, Powis *et al* 2008) but similar studies in children are limited. Recently, Bortolotti *et al* demonstrated increasing prevalence of genotypes 3 and 4 in vertically infected children in Italy between 1990 and 2002 and higher rates of spontaneous viral clearance early in life in children with genotype 3. They also found a high proportion of children with genotype 3 responding to IFN therapy and speculated that the increase in prevalence of genotypes 3 and 4 in recent years could significantly impact on the epidemiology of paediatric HCV infection in years to come (Bortolotti *et al* 2005).

1.3.4 HCV RNA viral load

The extent to which HCV RNA viral load predicts disease progression in HCV infected children remains unclear, predominantly due to a lack of prospective data for large numbers of infected children over a long enough period of time. As a result, its usefulness as a tool for monitoring HCV progression may be limited but associations have been made between active HCV infection and a high proportion of positive HCV RNA test results in the context of a large European prospective study. In addition, low HCV viral activity was associated with viral clearance in the same study (EPHN 2005a). In adult studies the majority of evidence points towards a lack of association between RNA viral load and severity of liver disease (Heller and Seef 2005, McCormick et al 1996, Puoti et al 1999, Gervais et al 2001, Poynard et al 2001, DeMoliner et al 1998, Freeman et al 2003). However, a few studies have found increased liver severity among HCV infected adults with high viral loads (Hisada et al 2005, Kato et al 1993, Mita et al 1994, Gretch et al

1994), although this may be due to the specialised groups studied in these cases, e.g. injecting drug users (Heller and Seef 2005). Both in adult and paediatric studies, no cut-offs for HCV RNA viral load which either predict worse or better HCV-related disease progression have been identified and therefore the predominant use of viral load information is to monitor the presence of infection, often in response to treatment. There has been some research on the prognostic value of HCV RNA viral load in predicting HIV disease progression within a UK cohort of HIV/HCV coinfected males with inherited bleeding disorders. In this group, an HCV RNA viral load measurement of greater than 5.9 $\log^{10}$cp/ml predicted progression to AIDS and all-cause mortality (Herrero-Martinez et al 2002).

1.3.5 Treatment

Currently available treatment is not suitable for children less than 3 years of age (Jacobson *et al* 2002), and although promising results have been obtained in older children treated with a combination of peg-IFN and ribavirin this combination therapy has not been rigorously evaluated in younger children (Wirth *et al* 2005). Additionally, in children there are issues relating to the side effects of IFN treatment and specifically its negative effect on growth. However, despite this, there is a low rate of treatment discontinuation in paediatric treatment trials (Fischler 2007).

1.4 Natural history of paediatric HCV infection

Most available information about paediatric HCV infection is derived from a limited number of cohorts of vertically infected children, who were followed up after their mothers

were identified as infected during screening in pregnancy (Newell and Pembrey 2002, Seef 1997, EPHN 2000, Ferrero *et al* 2003, Resti *et al* 2003, EPHN 2005a). The natural history of HCV acquired parenterally during childhood remains unqualified due to the small numbers of children infected in this way and the limited longitudinal studies available. Additionally, the asymptomatic nature of HCV infection in childhood means that a great many parenteral HCV infections likely remain undiagnosed until much later in adolescence or adulthood.

At present, the duration of follow-up of vertically HCV-only infected children is too short to determine long-term disease progression, but three modalities in the natural history of vertically acquired HCV infection have been identified; an estimated 20% of children may clear infection, 50% will develop chronic asymptomatic infection (intermittent viraemia, usually normal ALT levels and hepatomegaly rarely) and 30% will develop chronic active infection (persistent viraemia, frequently abnormal ALT levels and in some cases hepatomegaly) (EPHN 2005a). Evidence suggests that chronic HCV acquired parenterally in childhood is mild with a low rate of progression even after 20 years of infection and that eradication of HCV without treatment is more common than in patients acquiring infection in adulthood (Vogt *et al* 1999). Overall, HCV is considered to be a slow-moving fibrotic disease (Guido *et al* 2003).

The literature relating to the natural history of paediatric HCV infection focuses on patterns and clearance of viraemia, ALT levels and progression to liver fibrosis. Patterns of viraemia vary over time and so assessing difference in positivity can be problematic; however, in a recent study 44% of 105 vertically HCV infected children were found to have

persistently positive HCV RNA PCR test results (EPHN 2005a). A comparative figure for parenterally infected children is not available.

Clearance of viraemia has been reported in paediatric studies, however, the range of estimates for viral clearance in both vertically and parenterally infected children is wide, ranging from 0-93% and 13-55% respectively, though two thirds of these studies include fewer than 20 children (EPHN 2000, Vogt *et al* 1999, Ni *et al* 1994, Bortolotti *et al* 1997, Palomba *et al* 1996, O'Riordan *et al* 1998, Ceci *et al* 2001, Matsuoka *et al* 1994, Rerksuppaphol *et al* 2004, Sasaki *et al* 1997, Giacchino *et al* 1998) (Table 1.1). In a recent European study, 21% of 155 vertically infected children were found to have cleared viraemia, at a median age of 14.9 months (EPHN 2005a). In another study, 67 HCV-infected children who had acquired infection during preoperative blood transfusions were followed up and viral clearance occurred in 45% (Vogt *et al* 1999). In a direct comparison of clinical outcomes in vertically and transfusion infected children, viral clearance was 13% and 27% respectively but this difference was not statistically significant (Rerksuppaphol *et al* 2004). Additionally, children vertically coinfected with both HIV and HCV appear to be considerably less likely to clear their HCV infection than those infected with HCV only (EPHN 2005a).

Table 1.1 Clearance of HCV viraemia in published paediatric studies

Study details	No. HCV infected children	Mode of acquisition of HCV infection	Proportion clearing HCV viraemia (95% CI)
European Paediatric HCV Network 2005	155	vertical	21% (15.1 – 28.6%)
Rerksuppaphol *et al* 2004	16	vertical	13% (1.6 – 38.3%)
Resti *et al* 2003	62	vertical	19% (10.4-31.4%)
Ceci *et al* 2001	30	vertical	93% (77.9 – 99.2%)
Tovo *et al* 2000	104	vertical	17% (10.6 – 26.0%)
Giacchino *et al* 1998	9	vertical	33% (7.5 – 70.1%)
Bortolotti *et al* 1997	14	vertical	14% (1.8 – 42.8%)
Sasaki *et al* 1997	11	vertical	45% (16.7 – 76.6%)
Palomba *et al* 1996	7	vertical	0
Vogt *et al* 1999	67	parenteral	45% (32.6 – 57.4%)
Rerksuppaphol *et al* 2004	15	parenteral	18% (4.3 – 48.1%)
O'Riordan *et al* 1998	12	parenteral	42% (15.2 – 72.3%)
Sasaki *et al* 1997	17	parenteral	18% (3.8 – 43.4%)
Ni *et al* 1994	8	parenteral	13% (0.3 – 52.7%)
Matsuoka *et al* 1994	22	parenteral	55% (32.2 – 75.6%)

ALT levels have been shown to peak (up to 5 times the level of normal) early in life in vertically infected children; in 70 vertically HCV infected children, ALT levels peaked during the first 6 months in 37% and between 6 and 12 months of age in 63% (Resti *et al* 2003). This was also seen in another recent European study where ALT levels in 187 HCV-only infected children were shown to peak in the first 2 years of life (EPHN 2005a).

Additionally, this study found at least one abnormal ALT level (> 40UI/L) in 44% of children and an abnormal ALT level more than 75% of the time in 33% of children (EPHN 2005a). Rerksuppaphol *et al* found persistently abnormal ALT levels (>55IU/l) in 7% of 15 parenterally infected children and in 25% of 16 vertically infected children (Rerksuppaphol *et al* 2004). This suggests that elevated ALT levels may be more frequent in vertically infected children but it is important to highlight the different definitions of 'abnormal' used to assess ALT levels and the difficulties in subsequent comparisons between studies.

In a study of 112 children living in Italy and Spain with chronic HCV infection acquired before 10 years of age from any source, 78% had fibrosis during a median of seven years of infection (Guido *et al* 2003) and similarly, 83% of 224 HCV infected children from Spain, Italy and Belgium with vertical or parenteral HCV exposure, showed progression to chronic infection during a mean of six years of follow-up (Jara *et al* 2003). In the former study, fibrosis stage was positively associated with age at biopsy and duration of infection with 83% of patients with fibrosis infected for longer than ten years (Guido *et al* 2003). The relationships between ALT level, gender, HCV genotype, route of infection and development of fibrosis are unclear but in a study of 112 parenterally and vertically infected European children, these factors did not determine fibrosis stage (Guido *et al* 2003).

Once chronic infection has developed, the source and time of infection is reported as not associated with serological or histological features (Jara *et al* 2003). Two percent of children with chronic HCV infection acquired vertically and parenterally were found to develop severe hepatitis and cirrhosis over a mean period of 6.2 years of follow-up in a

study of 224 children (Jara *et al* 2003); persistent liver damage was found in most of these children which suggests that progression to liver disease should be expected in chronically infected children who thus require close monitoring. This is highlighted by the higher rate of fibrosis seen in adolescents in comparison to younger children (Jara *et al* 2003). In contrast to previously mentioned studies on clearance of viraemia, a study of HCV natural history in perinatally infected children suggested that clearance of the virus was unlikely and that most children would develop chronic HCV, despite intervals of remission of inflammatory activity seen in some children (Palomba *et al* 1996). This was also shown in a study of vertically infected children where 81% of 62 children were found to develop chronic HCV (Resti *et al* 2003).

1.5 Global epidemiology and biology of Human Immunodeficiency Virus (HIV) infection

The human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS) which is characterised by opportunistic infection and cancers associated with the immune system (Barre-Sinoussi *et al* 1983, Gottlieb *et al* 1981). HIV is a ribonucleic acid (RNA) virus which primarily targets the CD4 cells of the immune system and primary infection is associated with a reduction in CD4 cells and an increase in plasma HIV RNA viral load without clinical manifestations (Ho *et al* 1995). In general there are three stages of HIV infection; rapid viral replication lasting several weeks after which an immune response, which may be accompanied by symptoms, occurs and HIV antibodies are produced (seroconversion), followed by a clinically asymptomatic period during which viral replication continues and finally clinical symptoms develop. This period from primary infection lasts approximately ten years in untreated persons, following which the untreated

HIV infected person develops AIDS and usually dies within two years (Munoz *et al* 1989, Goedert *et al* 1989, Mocroft *et al* 1997, Rothenberg *et al* 1987, Zaba *et al* 2004).

In 2007 the estimated number of people living with HIV worldwide was 33.2 million with 2.5 million new infections and 2.1 million AIDS-related deaths in 2007 alone. The majority of people living with HIV (67% of 33.2 million) and the largest group of those newly infected with HIV (38% of 2.5 million) live in sub-Saharan Africa and 61% of those living with HIV in this region are women (UNAIDS 2007. In Europe the highest rates of newly diagnosed HIV infection are in the East (211 per million people) in comparison with the lower rates in Western Europe (83 per million people) and the lowest rates of all in Central Europe (9 per million people). In Eastern Europe the predominant mode of transmission continues to be injecting drug use, although heterosexual transmission is on the increase, while in Central and Western Europe heterosexual transmission remains the principal route of infection (EuroHIV 2007).

1.6 Acquisition of paediatric HIV infection

Paediatric HIV infection occurs predominantly via mother-to-child transmission which may occur before (across the placenta), during (via microtransfusions during labour), and after delivery (through breastfeeding) (Nduati *et al* 2000, Newell 1998). The most important factors associated with an increased risk of MTCT of HIV are maternal plasma HIV RNA viral load, vaginal delivery, and mode and duration of breastfeeding (Thorne and Newell 2000, Wilfert and Stringer 2004, Scarlatti 2004, Mofenson 2003). Elective caesarean section delivery before onset of labour and rupture of membranes, prophylactic antiretroviral therapy during pregnancy, intrapartum and/or neonatally, and breastfeeding

avoidance, independently, substantially reduce mother-to-child transmission rates, (Thorne and Newell 2000) which range from 15% to 40% without intervention. In settings where all interventions can be safely implemented, the advent of highly active antiretroviral therapy (HAART) has reduced the HIV vertical transmission rates to below 2% (ECS 2006, Warszawski *et al* 2008).

Less is known about paediatric HIV infection acquired parenterally via receipt of infected blood or blood products or contact with non-sterile hospital instruments despite an estimated 80,000 to 160,000 individuals (children and adults) infected in this way annually (Kane *et al* 1999). In the early years of the HIV epidemic parenteral acquisition of infection was most common among specific subgroups like haemophiliacs who received large amounts of transfused blood or associated products (Jones in Mok and Newell 1995). However, this group of parenterally infected children is now relatively small due to the implementation of donor screening in most developed countries in the early 1990s but there remain groups of children infected through unsafe medical injections and new infections are occurring globally with often large numbers of children infected from a single source (Bagchi 2007, Morris 2006, Visco-Comandini *et al* 2002, Yerly *et al* 2001, Popova *et al* 1999, Hersh *et al* 1993, Pokrovski 1992, Hersh *et al* 1991). Parenterally HIV infected children are unique both in terms of the way in which they were infected and the fact that they often have significant comorbidities given the circumstances under which infection occurred (Popova *et al* 1999) (Table 1.2). The published literature on parenterally HIV infected children worldwide is sparse as the majority of outbreaks are in less developed countries where surveillance and reporting of infected cases is poor. However, increases in surveillance techniques demonstrate the extent of the parenteral epidemic. For example, in

Romania national AIDS case surveillance definitions were revised in 1989 and reported AIDS cases rose from 13 in 1989 to 1168 by the end of 1990. 93.7% of these 1168 AIDS cases were children of whom 1086 (99.3%) were under 4 years of age and 683 (62.4%) were abandoned children living in public institutions (Hersch *et al* 1991). Four hundred and twenty three of the paediatric cases were the result of blood transfusions and infection via improper use of needles and syringes is strongly suspected to be the cause of most of the other cases (Hersh *et al* 1991). Other large outbreaks are being discovered and recent screening in Kazakhstan has identified 72 HIV infected children and although information is scarce, it is reported that six of these children have died to date. Recent estimates from Kazakhstan suggest that a minimum total of 2700 children may have been infected with HIV by contaminated blood transfusions or nosocomial transmission (Morris 2006).

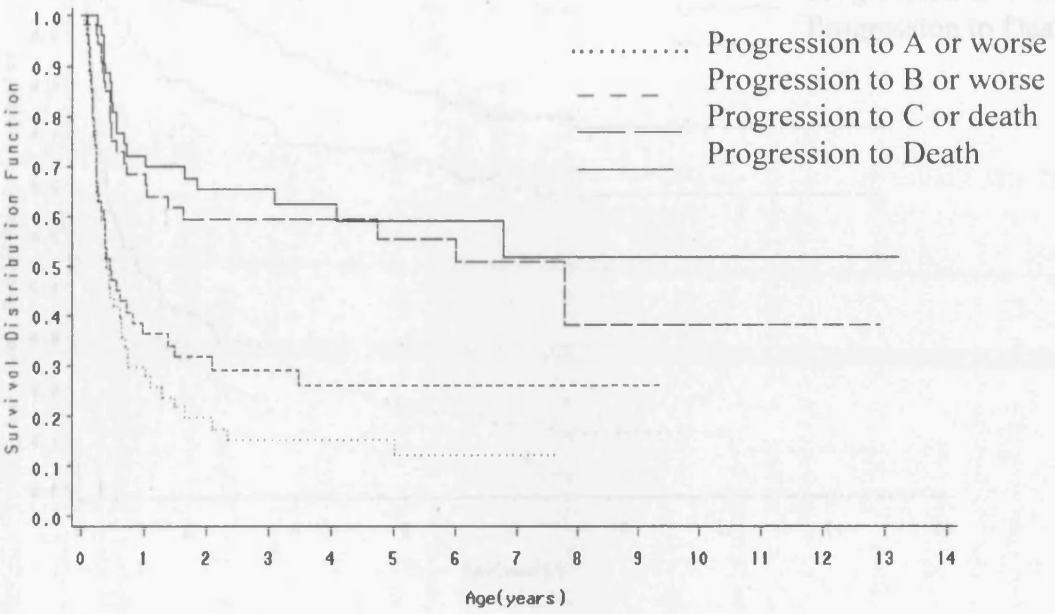
Table 1.2 Details of published studies on parenterally HIV infected children

Study author and publication year	Setting	Details
Morris 2006	Kazakhstan children	Retrospective screening has identified 72 children with HIV, 6 of whom have died. Screening continues and an estimated minimum total of 2700 children could be HIV infected.
Yerly *et al* 2001	Libyan children – a sub-sample of a larger group of approximately 400 children infected with HIV from a hospital source.	148 children screened for HIV, HCV and HBV infection. 147 HIV infected, 47% and 33% of whom were also HCV and HBV infected respectively.
Popova *et al* 1999	Russian children	124 nosocomial infections identified during hospital treatment. Approx 9 years after infection, 26% of 124 children had died.
Hersch *et al* 1993	Abandoned Romanian children living in public institutions.	101 children screened for HIV infection, 20% infected, all less than 4 years of age.
Hersch *et al* 1991	Romanian surveillance	Revised surveillance techniques resulted in 1168 cases of AIDS reported by 1990. 1094 in children under 13 years of age. 1086 younger than 4 years of age and 683 abandoned and living in public institutions.

1.7 Natural and treated history of paediatric HIV infection

The characteristics of paediatric HIV in vertically infected children has been well documented both in treated and untreated individuals (Blanche *et al* 1997, ECS 2004, ECS Pediatrics 2001, Thorne *et al* 2002, Pliner *et al* 1998, Palumbo *et al* 1998, Dunn *et al* 2003, Prendergast *et al* 2007, Little *et al* 2007). In the absence of effective antiretroviral therapy (ART) or other supportive prophylactic management, a quarter of vertically HIV-only infected children will have progressed to serious disease or death by the age of 1 year, with a 40% cumulative incidence of HIV-related disease or death by 6 years of age (The French Pediatric HIV Infection Study Group and European Collaborative Study 1997, ECS 2001) (Figure 1.1). HIV-related disease progression is measured by levels of immunological and virological manifestations, specifically CD4 cell count and HIV RNA viral load, and the presence of non-specific clinical symptoms such as hepatomegaly, splenomegaly, lymphadenopathy and HIV-related manifestations or opportunistic infection. HIV infection in children is categorised according to clinical and immunological categories defined by the Centres for Disease Control (CDC), last updated in 1994. These guidelines categorise HIV infected children in terms of whether they are asymptomatic, mildly symptomatic, moderately symptomatic or severely symptomatic (Table 1.3) and whether they have no immunosuppression, moderate immunosuppression or severe immunosuppression (Table 1.4). The World Health Organisation also provides guidelines on clinical and immunological categories for the staging of HIV infection in children which are similar to those produced by the CDC (WHO 2005).

Figure 1.1 Progression to CDC clinical categories in untreated vertically HIV infected children (ECS 2001)



Since recommendations for the use of more active combination antiretroviral ARTs were introduced in 1997-98 and paediatric antiretroviral therapy became more widely available for children at an earlier stage in their disease progression before serious clinical or immunological deterioration, children have been shown to be substantially less likely to progress to serious HIV-related diseases or death and most HIV-infected children now treated with combination ART remain symptom free for at least ten years (ECS 2001) (Figure 1.2).

Figure 1.2 Progression to CDC clinical categories in treated vertically HIV infected children (ECS 2001)

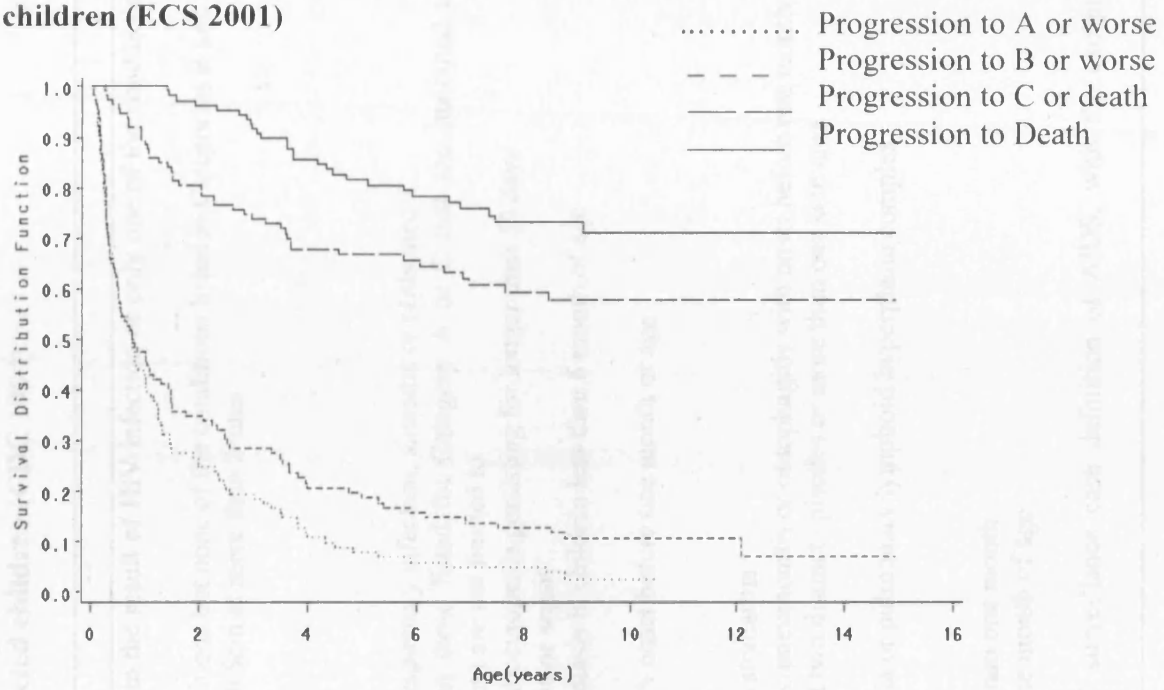


Table 1.3 Centres for Disease Control Clinical categories for HIV infected children (CDC 1994)

CDC Clinical Category	Definition
Category N (asymptomatic)	No signs or symptoms considered to the result of HIV infection or only one of the conditions listed in Category A
Category A (mildly symptomatic)	Two or more of the following conditions but none of the conditions listed in Categories B and C - Lymphadenopathy larger than 0.5cm at more than 2 sites - Hepatomegaly - Splenomegaly - Dermatitis - Parotitis - Recurrent or persistent upper respiratory infection, sinusitis or otitis media
Category B (moderately symptomatic)	Symptomatic conditions other than those listed for Category A or C that are attributed to HIV infection. Some examples include but are not limited to - Anaemia, neutropenia or thrombocytopenia persisting for longer than 30 days - Bacterial meningitis, pneumonia or sepsis - Persistent oropharyngeal candidiasis in children less than 6 month of age - Cardiomyopathy - Cytomegalovirus infection, with onset before one month of age - Diarrhoea, recurrent or chornic - Hepatitis - Recurrent herpes simplex virus stomatitis - Herpes simples virus bronchitis, pneumonitis or oesophagitis with onset before one month of age - Herpes zoster involving at least two distinct episodes or more than one skin area - Leiomyosarcoma - Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex - Nephropathy - Nocardiosis - Persistent fever lasting longer than one month - Toxoplasmosis, onset before one month of age - Disseminated varicella
Category C (severely symptomatic)	Any condition listed in the 1987 surveillance case definition of AIDS, with the exception of lymphoid interstitial pneumonia

Table 1.4 Centres for Disease Control Immunological categories for HIV infected children (CDC 1994)

CDC immunological category	Definition Children younger than 12 months of age	Children aged 1 to 5 years	Children aged 6 to 12 years
Category 1 **(no immunosuppression)**	CD4 cell count greater than 1499 cells/mm^3	CD4 cell count greater than 999 cells/mm^3	CD4 cell count greater than 500 cells/mm^3
Category 2 **(moderate immunosuppression)**	CD4 cell count between 740 and 1499 cells/mm^3	CD4 cell count between 500 and 999 cells/mm^3	CD4 cell count between 200 and 499 cells/mm^3
Category 3 **(severe immunosuppression)**	CD4 cell count below 750 cells/mm^3	CD4 cell count below 55 cells/mm^3	CD4 cell count below 200 cells/mm^3

Few studies have reported information on parenterally HIV infected children and therefore the natural history of children infected in this way and the impact of underlying morbidity and age at infection remains unqualified. Popova *et al* presented information on 124 nosocomially infected children from Russia of whom 33 (26%) had died 9 years after infection. They suggested that in the first year of life, HIV infection in these children could rapidly progress to AIDS due to their background of often severe disease prior to contracting HIV (Popova *et al* 1999).

Associations between mode of acquisition and/or age at acquisition of infection and HIV disease progression are difficult to interpret because of confounding factors including comorbidities, and delayed diagnosis after parenteral infection. A study of UK haemophiliacs infected with HIV showed a higher survival rate ten years after seroconversion, a longer time from infection to AIDS diagnosis and longer survival after AIDS diagnosis in patients seroconverting before 15 years of age compared to those seroconverting at older age groups up to 55 years of age (Darby *et al* 1996). More recently, data from over 13000 parenterally HIV infected individuals were analysed and the estimated survival of children infected before 14 years of age was higher than in other age categories and similar in those infected before five years of age and those infected between five and 14 years of age (Collaborative Group on AIDS incubation and HIV survival 2000).

It may therefore be hypothesised that, as is found in adults, the earlier a child is infected with HIV the slower the progression to severe disease. However, it is biologically plausible that those vertically infected or infected early in infancy may elicit a faster and more severe

disease progression as a result of an under-developed immune system at the time of infection. Several studies have investigated this to a certain extent by looking at differences in the disease progression of vertically infected children acquiring infection in utero and those acquiring infection post-partum or via breastfeeding. One study demonstrated that children who were infected in utero had a significantly lower mean initial HIV RNA viral load after infection and a more gentle decrease in viral load than those who were infected through breastfeeding. However in multivariable Cox Proportional Hazards analysis, the timing of infection was not associated with the risk of progression to AIDS or death in infected children (Rouet *et al* 2003). Similarly, Shearer *et al* demonstrated differences in initial peak HIV RNA viral load between those vertically infected early or and those infected late but this difference disappeared shortly after birth and both groups subsequently responded to the virus in a similar way (Shearer *et al* 1997). However, mortality was significantly lower for those with late versus early vertical infection in a meta-analysis study of mortality in infants born to HIV infected mothers in Africa (Newell *et al* 2004).

1.8 Treatment of paediatric HIV infection

There are three classes of antiretroviral drugs currently available for the treatment of HIV in children; Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs) (Table 1.5).

Table 1.5 Principle antiretroviral drugs available for use in children

NRTIs	NNRTIs	PIs
Abacavir	Efavirenz	Lopinavir-Ritonavir
Lamivudine	Nevirapine	Nelfinavir
Didanosine		Amprenavir
Stavudine		Ritonavir
Tenofovir		Fosamprenavir
Zalcitabine		Saquinavir
Zidovudine		
Emtricatabine		

There are several guidelines for the treatment of HIV infected children from organisations including CDC, Paediatric European Network for the treatment of AIDS (PENTA) and WHO in addition to individual country national guidelines, for example CHIVA in the UK, but all are similar in that they recommend different times for initiation of treatment depending on the age of the child and their clinical and immunological status (Table 1.6) and different therapy regimes depending on the age of the child (Table 1.7). The debate as to when to initiate treatment stems from the need to balance the prevention of disease progression against the issues of adherence, life-long treatment dependence, resistance, the limited available paediatric drugs and treatment side effects. Recent findings from the large South African Children with HIV Early Antiretroviral Therapy Study showed that early initiation of therapy resulted in 96% survival in comparison to 84% survival in those randomised to deferred initiation of therapy (Violari *et al* 2007). Additionally, a recent European multi-centre study of vertically HIV infected children demonstrated a beneficial effect of HAART initiation before five months of age on CD4 cell count recovery (ECS 2006).

Table 10: Summary of HAART recommendations in which to initiate therapy

category A or above (Sharland *et al* 2004)

Age	Clinical indication for treatment	Immunological/virological indication for treatment
Infants younger than 1 year	CDC stage B or C	• All children with CD4%<25-35% • Strongly consider children with viral load>1 million cp/ml • Many experts treat all asymptomatic infants
Children 1 to 3 years of age	CDC stage C	• All children with CD4%<20% • Strongly consider children with viral load>250 00 cp/ml
Children 4-8 years of age	CDC stage C	• All children with CD4%<15% • Strongly consider children with viral load>250 000 cp/ml
Children 9-12 years of age	CDC stage C	• All children with CD4%<15% but with less urgency than in younger children • Strongly consider in children with viral load>250 000cp/ml
Children 13-17 years of age	CDC stage C	• All adolescents with CD4 cell count between 200 and 350 cell/mm^3

Table 1.7 Summary of PENTA recommendations on which ART to start in children with CDC clinical stage B or greater (Sharland *et al* 2004)

Age	Recommended treatment regime
Infants	2 NNRT[1] + PI (Lopinavir/r or Nelfinavir) or 2NRTI[1] + NNRTI (Nevirapine)
Children	2NRTI[1] + PI (Lopinavir/r or Nelfinavir) or 2NRTI[1] + NNRTI (Efavirenz or Nevirapine[2])

[1]Dual NRTI combination recommended: zidovudine plus lamivudine or didanosine; didanosine plus lamivudine; abacavir and lamivudine. D4T is not recommended as first-line therapy.
[2]Nevirapine would be the preferred for children under 3 years of age

1.9 HIV coinfections

In addition to HCV, there are a number of other viral infections which, given shared transmission routes, are common among adults and children infected with HIV. The interactions between HIV and other infections are complex but it is possible that information may be gained about HIV/HCV coinfection by looking at the way these other coinfections present. Recent estimates suggest that approximately 10% of HIV infected adults are coinfected with Hepatitis B virus (HBV) (Soriano et al 2006). In many resource rich countries, including the majority of Europe and the United States, infants are immunised against HBV infection resulting in a limited population of HIV/HBV coinfected children from which information can be gathered. Information available on HIV/HBV coinfection is thus predominantly based on adult populations, which could be extrapolated to inform paediatric research. However, differing disease outcomes have been reported from vertically infected children in comparison to older children, possibly indicating differences between HIV/HBV coinfected adults and children (HPPMCS 2006). Evidence from adult studies suggests that the effect of HBV coinfection on HIV disease progression is minimal with no impact on progression to AIDS or virological and immunological

responses to HAART. (Lessell and Leen 2004, Puoti et al 2002, Cooley and Sasadeusz 2003, Konopnicki et al 2005, Nunez and Soriano 2005). Despite this, studies have shown an increase in liver-related mortality among HIV/HBV coinfected patients in comparison to those HIV-only infected and higher rates of chronic HBV infection and HBV replication in coinfected patients (Konopnicki et al 2005, Puoti et al 2002). Despite this seemingly detrimental effect of HIV infection on HBV progression, ALT levels have been found to remain lower than in HBV only infected individuals (Puoti et al 2002). It is biologically plausible that the weakened immune system in HIV infected individuals may limit their ability to mount an immune response to HBV infection and therefore the aspects of HBV infection which normally result in destruction or inflammation of liver cells by the body's own immune system may occur to a lesser extent. Given the similarities between HBV and HCV infection, it may be possible that similar responses occur in HIV/HCV coinfected individuals.

In contrast to HIV/HBV coinfection, research focussing on HIV/Cytomegalovirus (CMV) coinfection is common among paediatric populations given the possible vertical transmission of both viruses and the increased likelihood of CMV infection in HIV infected versus uninfected infants (Kovacs et al 1999, Doyle et al 1996). In the majority of cases, CMV is transmitted through breastfeeding post-partum, although a small number of transmissions occur inutero and the clinical consequences of CMV acquired in this way are worse. As HIV is predominantly transmitted from mother to child intrapartum the majority of children with CMV/HIV coinfection will not have acquired both infections at the same time and this may impact on the disease progression of both or either infections. However, the impact of CMV on HIV disease remains controversial. Evidence suggests that children

with vertically acquired HIV who become coinfected with CMV in utero or infancy, in addition to having higher rates of central nervous system disease, also have significantly higher rates of HIV disease progression and immune deficiency than those with HIV only (Kovacs et al 1999, Doyle et al 1996). Furthermore, children with symptomatic HIV disease were found to have a higher prevalence of active CMV infection compared to those with asymptomatic disease and in those asymptomatic children, CMV infection significantly increased mortality (Frenkel et al 1990). CMV is an AIDS-defining illness and, in adults, usually occurs due to a resurgence of previously acquired latent infection. However, acquisition of CMV early in infancy in children means that the role of HIV in the clinical presentation of CMV likely differs to that in adults and it remains unclear from these studies whether immune deficiencies in HIV infected children allow the emergence of CMV disease or whether CMV infection directly causes more rapid HIV disease progression (Chandwani et al 1996). In contrast, a recent Spanish study reported no association between HIV/CMV coinfection acquired in the first year of life and HIV disease progression, immunosuppression or survival, further emphasising the complexity of HIV/CMV coinfection and HIV coinfections in general (Marin Gabriel et al 2007).

1.10 HIV/HCV coinfection

HIV and HCV share transmission routes, specifically injecting drug use and blood transfusion, making coinfection relatively common, although sexual transmission of HCV is much less frequent than for HIV (Bica *et al* 2001, Bonacini and Puoti 2000, Quirishi *et al* 2003, Rosenthal *et al* 2003). With ART for HIV infection improving and prolonging life in infected individuals (Bica *et al* 2001, Rosenthal *et al* 2003, Rockstroh and Spengler 2004),

the prevalence and effect of secondary infections and comorbidities such as HCV has increased in adult HIV infected populations (Bonacini and Puoti 2000). Symptoms related to HCV infection were not previously observed in coinfected patients because progression to liver disease or associated cancers can take as long as 20 years after HCV infection and life expectancy in HIV infected individuals was short. HCV-related mortality has become a leading cause of death in HIV-infected adults (Braitstein *et al* 2004, Sulkowski *et al* 2005). The extent to which this situation is true in coinfected children is unclear.

1.11 Acquisition of HIV/HCV coinfection in children

Both HIV and HCV can be vertically transmitted from an infected mother to her child, but in Europe the number of coinfected children is small. Vertical transmission of HIV occurs more often from women coinfected with HIV and HCV than from those infected with HIV only (Giovannini *et al* 1990, Hershow *et al* 1997, Paccagnini *et al* 1995, Papaevangelou *et al* 1998, Thomas DL *et al* 1998, Tovo *et al* 1997). For example, in a study of nearly 500 mother-child pairs, HIV transmission occurred from 16% (53/326) of mothers infected with HIV only, compared with 26% (42/161) of HIV/HCV-coinfected women, suggesting that HIV/HCV coinfection nearly doubles the HIV MTCT rate (odds ratio (OR) 1.82, p=0.001) (Hershow *et al* 1997). However, the sample sizes and transmission rates used in these studies often vary considerably; furthermore, some studies have not compared transmission from coinfected mothers with HIV-only infected mothers. Reported HIV transmission rates from HIV/HCV-coinfected mothers range from 13.3% to 30% in settings where interventions to prevent mother –to-child transmission of HIV are available and where few HIV-infected women breastfeed (Paccagnini *et al* 1994, Papaevangelou *et al* 1998, Thomas

DL *et al* 1998, Tovo *et al* 1997). The lowest HIV transmission rates reported, from the most recent studies, likely reflect the use of zidovudine in pregnancy to reduce MTCT (Connor *et al* 1994). There have been few studies to assess the effect of HIV/HCV coinfection on HIV transmission rates in the past five years. Despite one recent study finding the HIV MTCT rate to be 6.2% (10/161), lower than the 11.2% (55/491) of coinfected women from the same study investigated between 1992 and 2000 (EPHN 2001, EPHN 2005c), the impact of HAART on the prevention on HIV MTCT, which became widely used after 2000, in a maternally HIV/HCV-coinfected population remains unclear.

The effect of HIV/HCV coinfection on vertical transmission of HCV has received more recent attention than that of HIV transmission. There is substantial evidence to support an increase in HCV vertical transmission from mothers coinfected with HIV compared with mothers infected with HCV only. One study including more than 100 children born to HIV/HCV coinfected mothers found HCV transmission rates to be significantly higher with a 2.3 times increase (95% CI 1.6-3.4) (EPHN 2001). Furthermore, acquisition of HCV infection is more likely in infants who also become infected with HIV (Papaevangelou *et al* 1998, Thomas *et al* 1998), which implies that transmission of HIV in utero may facilitate transmission of HCV. However, it is also plausible that coinfected women who transmit HIV have both high HIV RNA and HCV RNA viral loads and are thus prone to transmit both viruses. Treatment during pregnancy to prevent HIV transmission by lowering HIV RNA viral load may also be vital to the prevention of MTCT of both these viruses (England *et al* 2006).

Few studies have focussed on MTCT of HIV/HCV coinfection. Reported transmission rates range from 3.6% to 9.5% (Papaevangelou *et al* 1998, Thomas DL *et al* 1998, Nigro *et al*

1997), with the lower transmission rates from the two studies including more than 150 coinfected mothers (Thomas *et al* 1998, Tovo *et al* 1997). This evidence suggests that, while transmission of HCV or HIV from coinfected mothers is more likely than from singly infected mothers, the MTCT of both viruses simultaneously occurs less frequently than single-virus transmission from a coinfected mother.

Parenteral acquisition of HIV/HCV coinfection is rarely reported yet given the ways in which children acquire HIV or HCV parenterally it is likely that parenteral acquisition of coinfection is under-reported. For example, 47% of 147 parenterally HIV infected children were found to be coinfected with HCV in a study carried out on a sub-group of Libyan children known to be nosocomially infected with HIV (Yerly *et al* 2001).

1.12 Disease progression in HIV/HCV coinfected children

In adult studies, evidence of the impact of HIV/HCV coinfection on HIV disease is limited in comparison to that on HIV only infected adults. Whereas in four studies including 116-1685 coinfected people there was no evidence of an effect of HCV coinfection on progression to HIV disease (Staples *et al* 1999, Dorrucci *et al* 1995, Sulkowski *et al* 2002, Rockstroh *et al* 2004), in two further studies with 650-1157 coinfected patients, HIV progression was substantially accelerated (Carlos *et al* 2004, Grueb *et al* 2000). Specifically, in the study by Carlos and co-workers in Spain on 902 HIV infected patients, 72% (650/902) of whom were also infected with HCV, CD4 cell count two years after the initiation of therapy was significantly lower in coinfected individuals compared with HIV-only infected individuals ($p<0.001$) (Carlos *et al* 2004). In response to treatment, the mean

increase in CD4 cell count over a two year period was significantly lower than in HCV-coinfected individuals (11% *vs* 19%, $p<0.05$) and the mean decrease in plasma HIV RNA over the same period was significantly lower in coinfected patients than in HIV-only infected individuals (5% *vs* 54%, $p<0.05$), although this HIV RNA viral load association was not significant in multivariable analysis adjusting for sex, age, CD4 cell count, use of HAART, and adherence to therapy (Carlos *et al* 2004). Differences in the effect of HIV/HCV coinfection on HIV disease progression between studies may be partly explained by suggestions that coinfected individuals with HCV genotype 1 are at an increased risk of progression to AIDS-related mortality compared with those with HCV non-genotype 1, although this relationship is not yet supported by strong evidence (Yoo *et al* 2005).

The effect of paediatric HIV/HCV coinfection on progression of HIV disease remains unclear. Only two studies (both around ten years old) have investigated this area in children to date and no differences between coinfected and HIV-only infected children were reported (Papaevangelou *et al* 1998, Nigro *et al* 1997), although plasma HIV-RNA viral load at six months of age in coinfected children was higher than in infants with HIV infection only (a non-statistically significant difference) (Papaevangelou *et al* 1998).

In HCV infected adults it is widely acknowledged that coinfection with HIV accelerates the course of HCV-associated liver disease progression, particularly in patients who are more immunodeficient. HIV/HCV coinfection is thus associated with increased liver fibrosis progression, increased rate of liver decompensation, cirrhosis, hepatocellular carcinoma, and liver-related mortality, particularly due to the hepatotoxicity of many drugs used to treat HIV infection (Bonacini and Puoti 2000, Rosenthal *et al* 2003. Rockstroh and

Spengler 2004, Mohsen *et al* 2002, Nelson and Thomas 2001, Tedaldi *et al* 2003, Fuster *et al* 2004, Mathews and Bhagani 2003). However, in HCV only infected children it is generally assumed that progression of HCV-related disease is slower than in HCV only infected adults (although there are few studies where timing of infection in adults is known with any precision); the same may be true of HIV/HCV coinfected children (Guido *et al* 1998, Garcia-Monzon *et al* 1998).

Only four studies have reported results on progression of HCV in coinfected infants, with between two and 26 coinfected children included. Three studies report chronically evolving hepatitis in at least 50% of coinfected children (Papaevangelou *et al* 1998, Thomas *et al* 1998, Nigro *et al* 1997), which is similar to that found in HCV-only infected children (EPHN 2005a). One study reported the proportion of coinfected children with persistently abnormal ALT levels (>40 UI/l) to be similar to that in HCV-only infected children, but the proportion of coinfected children with a high proportion of positive HCV RNA PCR results was greater than in HCV-only infected children, albeit not significantly so (EPHN 2005a). Overall, insufficient details and a lack of formal comparisons limit conclusions about HCV disease progression in the context of paediatric HIV/HCV coinfection (England *et al* 2006).

1.13 Treatment of HIV/HCV coinfected children

The current guidelines for HIV/HCV coinfection treatment in adults vary depending on the stage at which HIV and/or HCV is diagnosed. If patients are identified before HIV therapy is indicated then HCV treatment should be considered in order that a better response to HIV therapy might be elicited once necessary (as HCV treatment is a one-off course it is

anticipated that the infection could be eliminated before HIV progression indicated treatment). If coinfection is diagnosed at a point where the patient requires, or is currently receiving, HIV therapy then this treatment should be optimised before HCV treatment is considered (England *et al* 2006).

To date, there are no studies focussing specifically on the treatment of either HCV or HIV in children coinfected with both viruses. Decisions regarding the treatment of coinfection in these children are based on adult data or extrapolate recommendations for the treatment of paediatric HIV or HCV alone. The concomitant use of ART and anti-HCV therapy is complicated by the interactions of many drugs as most HAART agents have the potential to cause hepatotoxicity (Nelson *et al* 2005). Treatment with IFN alfa-2b plus ribavirin is most commonly recommended for effective HCV treatment in children. Although recent trials show promising results for the use of pegylated IFN (Wirth *et al* 2005), ribavirin can enhance the phosphorylation of didanosine, thus increasing the risk of associated toxicity and therefore the use of both these agents simultaneously should be avoided (England *et al* 2006). Similarly, ribavirin and zidovudine are both associated with anaemia and, where possible, should not be administered together (England *et al* 2006). Didanosine and zidovudine are both major components of many antiretroviral regimens, especially those available to children, where the hepatotoxic nature of many HIV drugs is more problematic given the limited number of paediatric treatments available. It is therefore harder to find paediatric anti-HIV therapy that does not exhibit hepatotoxic properties. All NNRTIs are hepatotoxic, especially nevirapine, which can cause or intensify liver disease in HCV-infected children (Alberti *et al* 2005). PI-based combination antiretroviral therapy regimens including Lopinavir, Ritonavir, Nelfinavir, and Indinavir have inconsistently been reported

as associated with exacerbation of chronic liver disease, albeit in rare cases (Alberti *et al* 2005). Treatment combinations including NRTIs are more appropriate, although some have been shown to cause severe hepatotoxicity in rare cases (Working group of ART and medical management of HIV infected children 2006). At present there are no guidelines on how to resolve these issues in children and so the management of paediatric coinfection remains difficult and continues to be based on guidelines for the treatment of HIV and HCV separately (England *et al* 2006).

IFN-based HCV treatment is not recommended in children before 3 years of age but HIV treatment may be started immediately in infants with clinical symptoms and severe immunological and virological impairment and increasingly in those without symptoms (Working group of ART and medical management of HIV infected children 2006, Violari *et al* 2007). This means that in many cases HIV treatment will commence long before HCV therapy can be considered (Alberti *et al* 2005). As HCV infected children exhibit few clinical symptoms in the first ten to 15 years and are therefore unlikely to require HCV treatment, HIV treatment may continue for many years without the need for concomitant HCV therapy. However, as it remains unclear whether HIV coinfection increases HCV disease progression in children, the possibility that HCV treatment in this group may be necessary earlier than in HCV-only infected children must be considered. As with adult populations, HIV treatment in HIV/HCV coinfected children should be initiated as per guidelines for HIV-only infected children. However, close monitoring is essential to ensure that HIV treatment is not exacerbating HCV progression and the use of nevirapine should be avoided where possible. If and when HCV therapy becomes necessary, care should be taken to ensure than CD4 cell counts are above the recommended levels for the initiation of

HIV therapy before initiation of interferon, which is known to lower the CD4 cell count (Chung *et al* 2004). Additionally, it may be necessary to modify the HIV treatment to ensure drug interactions, specifically between ribavirin and zidovudine or didanosine, do not result in increased liver toxicity and fibrosis (England *et al* 2006).

To date no guidelines exist as to the most appropriate non-therapeutic clinical management of children coinfected with HIV and HCV. The current guidelines from BHIVA on HIV/HCV coinfection in adults suggest that, given the shared transmission route of both infections and the implications of coinfection on treatment in comparison to HIV infection alone, it is necessary to screen all HIV infected individuals for HCV infection (Nelson *et al* 2005). Once coinfection has been established, the guidelines recommend carrying out a variety of liver function tests in addition to HCV genotyping and HCV RNA viral load testing.

2. AIMS AND METHODS

2.1 Rationale for this work

HCV and HIV infection in children has been well documented in terms of MTCT and progression of vertically acquired infection (See sections 1.2, 1.4, 1.6 and 1.7). However, research in the context of parenteral infection is limited and it remains unknown whether disease progression differs in children infected in utero or intra-partum compared with those acquiring infection later in infancy or childhood. Limited evidence does however suggest that there are some differences in the initial disease progression of children infected with HIV early or later in infancy, possibly due to infection during early immune maturation (Rouet *et al* 2003, Shearer *et al* 1997, Newell *et al* 2004). In light of these potential differences it is possible that parenterally infected children, who are receiving care based on evidence from studies of vertically infected children, are not receiving optimal clinical management. As the number of children with parenteral infection is likely to continue to increase worldwide, improved knowledge of paediatric HCV and HIV acquired by different routes and at different ages will allow treatment and management to be tailored to a more individual level. The issues of increasing prevalence/diagnosis of parenterally acquired HIV and HCV infection and the lack of guidelines informing their management are exacerbated by the lack of data available on parenterally HIV or HCV infected children. This is largely due to the difficulties in screening or diagnosing children who may have been parenterally infected years previously, meaning that biological and clinical follow-up information is minimal, and also due to the inadequate surveillance or data collection systems in resource limited countries where the majority of parenteral HIV and HCV infections now occur.

In addition to HIV and HCV infection acquired singly, evidence on all aspects of paediatric HIV/HCV coinfection is limited but specifically regarding HIV and HCV disease progression in the context of coinfection. As the effects of coinfection become more apparent in adult populations (Bonacini and Puoti 2000, Braitstein *et al* 2004, Sulkowski *et al* 2005), it is important to understand the impact of HIV/HCV coinfection in a paediatric setting in order to inform clinical management and treatment. Additionally, investigating paediatric HIV/HCV coinfection according to mode of acquisition of infection (vertical or parenteral) will advance current knowledge in this area and add to the understanding of the effect of differentially acquired HIV and HCV on disease progression.

As a result of the limited knowledge on paediatric HIV/HCV coinfection, there are currently no guidelines for the clinical management of this group of children. The only guidelines available to clinicians are on the management and treatment of HIV or HCV singly infected children (Sharland *et al* 2005, EPHN 2005b) or on adult HIV/HCV coinfection (Nelson *et al* 2005) and for this reason coinfected children may not be receiving the optimal clinical care. Surveying current policies and practices of European clinicians with regards the management and treatment of HIV/HCV coinfected children allows an assessment of how these difficulties are overcome and helps emphasise the need for specific guidelines for the clinical management of this group.

2.2 Scientific hypothesis

a) There are differences in the biological and clinical profile of HIV and/or HCV infected children according to whether they became infected vertically, via mother-to-child transmission, or parenterally via contact with infected blood or blood products. Given the timing of vertical infection during early immune maturation, it is plausible that vertically infected children may respond less well to the infection and their subsequent disease progression my be worse.

b) The biological and clinical profile of HIV/HCV coinfected children differs from that of singly HIV or HCV infected children. An accelerated HIV disease progression has been observed in some adult studies comparing HIV/HCV coinfected individuals to those with HIV only. The extent to which differences exist in paediatric populations remains unknown.

2.3 Aims and Objectives

Aim

To investigate the effect of mode of acquisition of infection (vertical vs parenteral) on markers of infection and disease progression in HCV infected, HIV infected and HIV/HCV coinfected children.

Objectives

1. To establish age and sex specific references ranges for ALT levels in children to better identify children with elevated ALT levels in clinical practice.
2. To compare biological markers of HCV infection in parenterally and vertically infected children to clarify whether routinely collected clinical data differ by mode of acquisition of infection.
3. To describe the natural history, allowing for treatment, and disease progression in parenterally HIV infected children and assess the pattern in light of published material relating to vertically HIV infected children.
4. To determine immunological, virological and clinical profiles of HIV/HCV coinfected children, to identify and describe predictors of disease progression and quantify differences by mode of acquisition of infection.
5. To investigate current European policies and practices for the clinical management and treatment of children coinfected with HIV and HCV to assess the need for clinical consensus and development of treatment and management guidelines specific to this group.

2.4 Data sources

2.4.1 European Paediatric HCV Network (EPHN)

The European Paediatric HCV Network was established in 1999 at a time when little was known about paediatric HCV. The Network specifically aimed to estimate HCV MTCT rates and timings, identify factors associated with increased risk and thus inform possible interventions to reduce MTCT and to elucidate the natural history of paediatric HCV infection. This European cohort study was therefore established to prospectively collect data from clinicians in 30 centres in ten European countries (UK, Italy, Spain, The Netherlands, Ireland, Sweden, Greece, Norway, Germany and Belgium). Enrolment of mother-child pairs was based on the identification of women diagnosed as HCV infected before or during pregnancy and the follow-up of their children, or of children HCV seropositive within one month of birth for whom maternal infection could be confirmed. Clinicians completed paper study forms (Appendix 5) after routine clinical visits and sent these to the coordinating centre at the UCL Institute of Child Health, London, UK. Data from these forms were entered into a Microsoft Access database and checks were made for completeness, logicality and quality of data. All mother-child pairs were given a unique numeric identifier and all data were anonymously linked with no names appearing on any study forms or the study database. Data collection ceased in 2006 at which point 2272 mother-child pairs had been enrolled and 247 HCV infected children followed-up (EPHN 2005a, EPHN 2005c).

The information collected at different stages in the follow-up process are described in Table 2.1. Maternal information was collected at enrolment, usually during pregnancy, on mode

of acquisition of HCV infection, history of injecting drug use and HIV infection status. Maternal laboratory tests carried out during pregnancy and at delivery for anti-HCV antibody, qualitative and quantitative HCV RNA PCR and ALT levels were routinely collected and additionally information on HCV genotype and HCV and/or HIV treatment were recorded. After delivery, information was collected on mode of delivery, duration of labour, any obstetric interventions, and birth weight, gestational age, gender and neonatal ART HIV therapy if applicable. Paediatric follow-up was recommended at birth, 6 weeks, 3, 6, 9, 12, 18 and 24 months and then every 6 months if the child was HCV infected and annually if they were uninfected. At each follow-up visit clinical examination data were collected on the child's height, weight and HIV infection status as well as clinical symptoms in nine categories (growth failure, hepatomegaly, splenomegaly, nephropathy, cardio-pulmonary, skin disorders, haematological disorders, auto-immune diseases and others). Data were also collected on Hepatitis A and B immunisations, ultrasound examinations, liver biopsy results, genotype and HCV treatment. In addition to clinical examination, local laboratory testing of blood samples for anti-HCV antibody, qualitative and quantitative HCV RNA PCR, ALT, haemoglobin, white blood cells, lymphocytes, neutrophils and immunoglobulin was carried out. Liver biopsies were performed according to local practise and interpreted by the local pathologist (Table 2.1).

The data from children enrolled in the EPHN are utilised in Chapters three, four and five (Figure 2.1).

Figure 2.1 Data sources and their use in this thesis

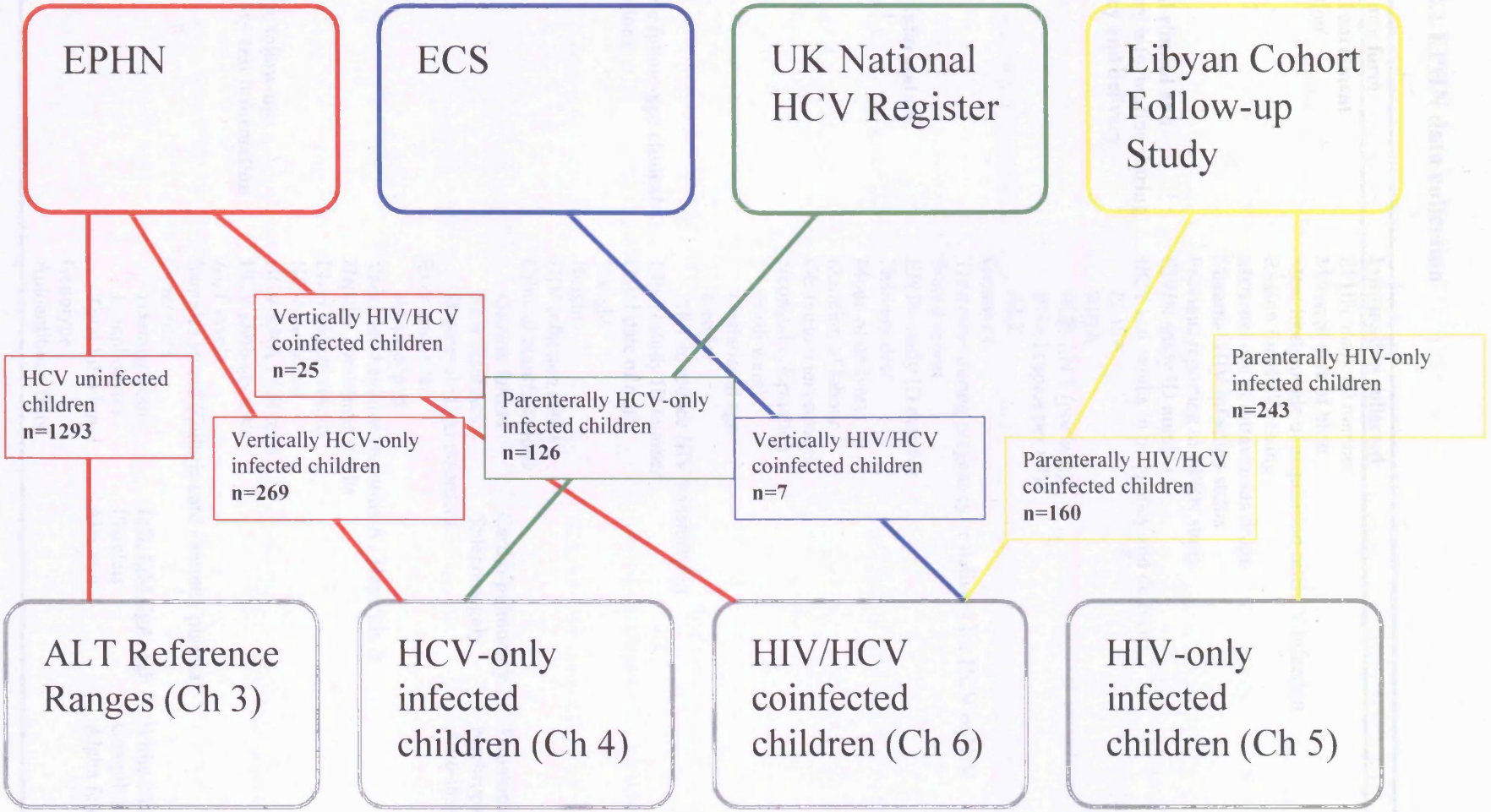


Table 2.1 EPHN data collection

EPHN study form	Information collected		
Maternal enrolment information	EPHN study ID number		
	Maternal date of birth		
	Most likely mode of acquisition of HCV infection		
	Reason for HCV testing		
	Maternal use of intravenous drugs		
	Maternal HIV infection status		
	Previous reporting to EPHN study		
Maternal clinical and laboratory information during pregnancy and delivery	EPHN study ID number		
	HCV test results in pregnancy and delivery		
	ELISA		
	RIBA		
	PCR-RNA (pos/neg)		
	RNA (copies per ml)		
	ALT		
	Genotype		
	Treatment during pregnancy or delivery for HCV or HIV		
	Stored serum		
Delivery information	EPHN study ID number		
	Delivery date		
	Mode of delivery		
	Duration of labour		
	Obstetric interventions		
	Neonatal information		
	Birth weight		
	Gestational age		
	Gender		
	ART to reduce HIV transmission		
Paediatric follow-up: clinical examinations	EPHN study ID number		
	Child date of birth		
	Weight		
	Height		
	HIV infection status		
	Clinical manifestations		
	Growth failure	Cardio-pulmonary	Hepatomegaly
	Skin disorders	Splenomegaly	Nephropathy
	Haematological disorders		Auto-immune disease
	Ever breast-fed		
	Age stopped		
	Vaccinated against Hepatitis A / Hepatitis B		
	Hepatic ultrasound results		
	Liver biopsy results		
	HCV treatment		
Paediatric follow-up: Laboratory test information	HCV RNA PCR tests		
	HCV antibody tests		
	ALT levels		
	Samples stored (Guthrie card / serum / plasma)		
	Other tests		
	Haemoglobin	IgG, IgM, IgA, IgE	White blood cells
	Lymphocytes	Platelets	Coagulation screen
	Total bilirubin	Albumin	Alpha fetoprotein
	Genotype		
	Autoantibody tests		

2.4.2 European Collaborative Study (ECS)

The European Collaborative Study (ECS) was set up in 1986 at a time when very little was known about the rate of HIV MTCT, risk factors for transmission or the natural history of vertically-acquired HIV infection. With funding from the UK Medical Research Council and the European Commission, a multi-centre cohort study was established with sites across Western Europe, to address these research questions. In 1998 the cohort extended into Central Europe, with two centres in Poland, and in 2000, to Ukraine. The ECS now covers ten European countries, with continuing enrolment and follow-up in 25 clinical centres from Spain, Italy, the United Kingdom, Germany, Belgium, Sweden, Netherlands, Denmark, Poland and Ukraine. More than 8000 mother-child pairs had been enrolled in the study by January 2008.

Routinely collected information is recorded at enrolment and follow-up visits by clinicians and documented on paper forms (Appendix 6) which are sent to the UCL Institute of Child Health, London, UK for data entry and analysis. All data is anonymously linked and each mother-child pair in the study is given a unique numeric identifier at enrolment and this is used in all data collection and analysis, no names are written on study forms or recorded in the ECS database. (ECS 2001).

Children born to women identified as HIV infected before or during pregnancy or at delivery are followed up according to a standard protocol collecting detailed clinical and laboratory information. The follow-up schedule includes clinical and laboratory data collection at birth, 3 and 6 weeks, 3, 4.5 and 6 months then every 3 months until 2 years of age. Subsequently HIV infected children are examined at least twice at year.

At enrolment maternal information on country of birth, ethnic group, obstetric history, transmission risk group, current clinical status and HIV treatment during pregnancy is recorded. Additionally, data from maternal laboratory investigation during pregnancy and at delivery are recorded and include HIV DNA PCR, HIV RNA PCR, anti-HIV antibody and CD4 cell count tests. Perinatal information on antiretroviral therapy administered during labour or at delivery, mode of delivery, duration of labour, perinatal problems and infant feeding method is collected at, or shortly after, delivery. Detailed paediatric data on clinical abnormalities along with developmental assessments, antiretroviral therapy administered, immunisations given and maternal health are then collected at each follow-up visit where blood samples are also tested for anti-HIV antibody, HIV RNA PCR, immunoglobulin, CD4 and CD8 cell count, lymphocyte and neutrophils. Testing is carried out locally and the assays used are recorded. Data collection at each point during enrolment and follow-up can be seen in Table 2.2.

The data from children enrolled in the ECS are utilised in Chapter six (Figure 2.1).

Table 2.2 ECS data collection

ECS study forms	Information collected			
Perinatal information	ECS ID number	Child's data of birth	Sex	
	Gestational age	Birth weight	Mode of feeding	
	Obstetrician details	Antiretroviral therapy during labour/delivery		
	Mode of delivery	Duration of labour	OFC	
	Time from rupture of membranes to deliver	Perinatal problems	Living with parents or adopted	
Maternal information at delivery	ECS ID number	Mother's date of birth	Country of birth	
	Marital status	Ethnic group	Obstetric history	
	Likely mode of acquisition of HIV infection	Date of first positive HIV test	Current clinical status	
	Details of treatment during pregnancy			
Maternal laboratory investigations during pregnancy and at delivery	ECS ID number	HIV RNA PCR (collected since 1994)	HIV DNA PCR	
	Total lymphocytes	CD4 cell count (collected since 1992)	CD8 cell count	
	IgG	IgA	IgM	
	P24 Ag	HIV Elisa		
Paediatric medical examinations: 3 and 6 weeks, 3, 4.5 and 6 months	ECS ID number	Weight	Height	
	OFC	Recurrent fever	Bacterial infection	
	Communicable disease	Skin infection	Non-infectious skin eruption	
	Palpable lymph nodes	Chronic paratoid swelling	Oral candida	
	Upper respiratory tract infection	Lower respiratory tract infection	Opportunistic infection	
	Hepatomegaly splenomegaly	Neurological abnormality	Developmental assessment	
	Loss of developmental milestones	Neonatal ART details	HIV treatment details	
	Hospital admissions	Immunisations	Child care	
	Breastfeeding	Health of mother		
Paediatric medial examinations: 9, 12, 18 and 24 months and older	ECS ID number	Weight	Height	
	Head circumference	Paediatrician details	HIV infection status	
	Child care	Preschool/Schooling	Treatment	
	Communicable diseases	Oral candida	Hospital admissions	
	Renal abnormalities	Gastro-intestinal abnormalities	Hepatic abnormalities	
	Cardio-vascular abnormalities	Central nervous system abnormalities	Respiratory abnormalities	
	Malignancy abnormalities			
Paediatric laboratory test results	ECS ID number			
	HIV ELISA	HIV DNA PCR	HIV RNA PCR (collected since 1994)	
	T8	T4 (collected since 1992)	IgG	
	IgA	IgM	Absolute lymphocyte	
	Neutrophil	Platelet	Haemoglobin	
	Toxo IgG Latex	Tetanus IgG	CMV IgG	

2.4.3 UK National HCV Register

The UK National HCV Register collects information on children known to be infected with HCV in the UK (Harris *et al* 2000). The majority of these children have been identified as part of several surveillance and research initiatives, including the UK Department of Health "Lookback" study (Department of Health 1995) in 1995 identifying individuals, including children, who received blood products from HCV infected donors and the British Paediatric Surveillance Unit study to assess the prevalence of HCV in UK children during 1997 and 1998 (Gibb *et al* 2000). To date there are approximately 246 individuals enrolled in the Register who were children at infection, most (76%) of whom acquired infection parenterally although as follow-up continues the balance shifts and the majority of new paediatric enrolments are vertically infected children (personal communication Dr Helen Harris, 2007).

The UK National HCV Register is based on active surveillance methods whereby clinicians following-up HCV infected children record routinely collected data on study forms on an annual basis and send the forms (Appendix 7) to the Register coordinating centre at the Health Protection Agency, London, UK. Data are recorded on clinical signs of liver disease, any other medical conditions, HCV RNA PCR and anti-HCV antibody test results, HCV genotype, liver function results such as ALT levels, liver biopsy results, hospital treatment and admissions (Table 2.3). Data from the UK National HCV Register is linked to national UK cancer and death registers to accurately monitor any major clinical disease progressions. All data within the Register is anonymously linked using unique identifiers for each individual and no names are recorded on the study forms or database. For the

purposes of this thesis, a database of HCV infected children which was a subset of the larger Register database was provided by the Health Protection Agency. Liver biopsies were carried out locally and additionally biopsy samples were sent to the Register coordinating centre at the Health Protection Agency in London for independent scoring (Harris *et al* 2000).

The data from children enrolled in the UK National HCV Register are utilised in Chapter four (Figure 2.1).

	Date of birth
	Ethnic group
	Country of birth
	GP details
	Risk factors for HCV acquisition
	Other chronic viral infection
	Other significant medical conditions
Current clinical status	Signs and symptoms of liver disease
Test results	Hepatitis B test results
	Date of first positive HCV PCR test
	HCV PCR test results
	HCV genotype
	Liver function test results
	Livery biopsy results
Antiviral drug treatment	HCV treatment details
	Response to treatment
	Antiviral drug trial participation
Current management	Hospital admissions or outpatient care details
	Alcohol intake at first diagnosis
	Current alcohol intake
Follow-up form	
Patient details	Register ID number
Current clinical status	Is patient alive
	Signs or symptoms of liver disease
	Other significant medical conditions
Test results	HCV PCR test results
	HCV antibody test results
	HCV genotype
	Liver function test results
	Haematology test results
	Liver biopsy results
Antiviral drug treatment	HCV treatment details
	Response to treatment
	Antiviral drug trial participation
Current management	Hospital admissions or outpatient care details
	Current alcohol intake

2.4.4 Libyan Cohort Follow-up Study

The Libyan Cohort Follow-up Study was established in 1999 in a collaborative between the Benghazi Infectious Disease and Immunity Center and two Italia centres (Bambino Gesù Children's Hospital, Rome and L.Sacco Hospital, Mi

agreement was in response to a nosocomial outbreak of HIV infection at Al-Fateh Children's Hospital, Benghazi, Libya whereby approximately 400 children became infected with HIV and many became coinfected with HCV. Follow-up examinations take place at clinical centres in Milan and Rome and began soon after children were exposed in1998/1999 and continues to date although enrolment remains static (Yerly *et al* 2001, Visco-Comandini 2002). Paediatric HIV infection was confirmed by HIV serological and virological analysis and vertical transmission was excluded by carrying out HIV serology and virology on parents of HIV infected children (Yerly *et al* 2001).

Data are collected at biannual routine follow-up examinations and entered into a Microsoft Access database. Data collected include quantitative HIV RNA PCR and quantitative and qualitative HCV RNA PCR test results, ALT levels and CD4 cell counts among other detailed data on clinical symptoms of disease and HIV and HCV treatment administered. More detail on the data collected at enrolment and follow-up visits can be found in Table 2.4.

The data from children enrolled in the Libyan Cohort Follow-up Study are utilised in Chapters five and six (Figure 2.1).

Table 2.4 Libyan Cohort Follow-up Study data collection

Libyan Cohort Follow-up Study forms	Information collected		
Enrolment	Study ID number		
	Sex		
	Date of birth		
	Place of residence		
	Date of first positive HIV test		
	Date of first visit to Benghazi hospital		
	Number of visits to Benghazi hospital during 1997 and 1998		
Diagnosis	Other medical conditions and diagnosis dates		
Aids defining illnesses	AIDS defining illnesses and diagnosis dates		
Antiretroviral therapy	Antiretroviral therapy drugs, start and stop dates		
Follow-up	Study ID number		
	Hospital admissions		
	CDC stage		
	Weight		
	Height		
	BMI		
	Evidence of fever		
	Evidence of diarrhoea		
	Evidence of candida		
	Evidence of failure to thrive		
	HIV RNA viral load	HCV infection status	HCV RNA viral load
	AST	ALT	Creatinine
	Cholesterol	Triglycerides	Bilirubin total
	White blood cell count	PTL	Hb
	Lymphocyte count	Neutrophils	CD4 %
	CD4 copies/ml	CD8 %	CD8 copies/ml

2.5 Biases

By using HIV and HCV data from four different cohorts there are possible biases which could occur from the differing design and data collection methods and also from the children that they collect data on, e.g. children who are vertically versus parenterally infected. All four cohorts analysed as part of this thesis prospectively collected data which was reported to study coordinating centres by clinicians following routine examinations at participating clinical centres according to a set protocol. All cohorts collected similar data from these routine examinations on virological, immunological, clinical and treatment profiles of HIV and/or HCV coinfected children. The similar design and data collection

methods of the cohorts means that biases resulting from combining data from different sources are minimised before any data analysis takes place (Appendices 5, 6 and 7) .

The children enrolled in the four cohorts differ predominantly by their mode of acquisition of infection; vertically versus parenterally. Vertically infected children are from birth cohorts, with data collected from birth and regularly thereafter in children who were only later confirmed as infected and therefore there was no selection bias based on disease progression or maternal factors or treatment and all vertically infected children were included (EPHN 2005a, ECS 2002). Parenterally infected children are from two different sorts of cohorts. Those children from the UK National HCV Register were identified largely based on exposure to a known contaminated source and therefore selection and enrolment into the cohort was not related to disease progression as may have been the case had they been identified after referral to specialist services for abnormal liver function tests. Children from the Libyan Cohort Follow-up Study were identified in relation to exposure to a known contaminated source and therefore similarly, their enrolment was not biased by selection of particular groups or children with more advanced disease progression.

In the UK National HCV Register analysed in this thesis, enrolment in the study and consequently the start of follow-up information was often a number of years after infection occurred. There is therefore also the possibility that some children exposed to contaminated blood or blood products and consequently infected with HCV, who would otherwise have been enrolled in the Register, cleared the virus prior to testing and therefore were never diagnosed as infected. The result of this would be to underestimate the level of HCV virus

clearance in this cohort and possibly to overestimate disease progression if those who did not clear the virus were those most likely to progress to HCV-related disease. Children enrolled in the Libyan Cohort Follow-up Study were diagnosed much sooner after HIV or HCV infection than those in the UK Register as the source of infection was identified within two years of the first possible infection. As a result, parenterally infected children from this cohort have information available much sooner after infection reducing any bias related to late diagnosis. As a result, this group of parenterally infected children are a very unique group to study.

Another possible source of bias may be the timing of measurements from these children which are available from the time of infection (birth) for vertically infected children from the ECS and EPHN cohorts, whereas parenterally infected children are not followed up from the point of infection and therefore there are fewer and sometimes no measurements during early infection.

The biases anticipated by the use of these different cohorts and the differences between the information available for children enrolled in them will be dealt with in either the analysis or the interpretation of the results. Some specific biases such as the age at which infection occurred or the age at which measurements were taken, relative to the age at infection, can be adjusted for using specific statistical techniques which are discussed in individual chapters throughout this thesis. Other biases, in terms of the context in which the data was collected and any possible biases in terms of selection or late diagnosis will be considered at the interpretation stage of the data in the Discussion Chapter of this thesis.

2.6 Definitions

2.6.1 HCV infection

For the purposes of inclusion in analyses for this thesis, vertically HCV infected children were defined as those with a positive HCV antibody result at or after 18 months of age and/or two consecutive positive HCV RNA PCR test results at any age who were born to mothers with HCV infection confirmed before or during pregnancy (EPHN 2005a). Parenterally HCV infected children were those infected before 18 years of age who tested positive to HCV antibodies with a known parenteral risk factor for infection, exclusion of vertical acquisition of infection and for whom follow-up information was available before 18 years of age.

2.6.2 HIV infection

Vertical HIV infection was established by the detection of HIV in at least two blood samples taken on separate occasions, and/or the persistence of HIV antibody beyond 18 months of age in children whose mothers had documented HIV infection in pregnancy or at delivery (ECS 2002). Parenteral HIV infection was defined by serological and virological confirmation of HIV and exclusion of mother-to-child transmission by laboratory exclusion of HIV in mothers of infected children. The date of HIV infection in parenterally infected children from the Libyan Cohort Follow-up Study was determined using the midpoint between 1st January 1998 (the first possible date of infection) and the date of their first positive HIV test or, if this was unavailable, the midpoint (1st January 1999) of the period that infection was known to have occurred – January 1998 to December 1999. As

nosocomial infection was isolated to a specific period of time the timing of possible infection can be estimated with sufficient accuracy.

2.7 Data analysis

The data management of all four cohort studies was carried out using Microsoft Access databases and statistical analyses were performed using STATA software version 9.0 (Stata Corporation, Texas, USA) and SAS software (v9.01, SAS Institute, Cary, North Carolina, USA). Data cleaning was carried out before the use of data from all databases and checks on data quality, logistic quality and completeness of data were performed in Microsoft Access prior to statistical analysis. The specific statistical analysis methods for each piece of work can be found at the beginning of each individual Chapter.

Information specifically for analysis in this thesis were provided by the cohort coordinators in the form of abbreviated datasets and permission was granted for their use in all the analyses presented. Additionally, advice on the use of the data was provided by the cohort coordinators.

2.8 Role of the researcher

The data analysed in the following chapters were provided by a variety of study teams and therefore I will clarify my role in the research carried out for this thesis.

Data on vertically HCV infected children from the EPHN presented in Chapters 3, 4 and 6 were provided by the EPHN coordinating team at the UCL Institute of Child Health, London, UK in the form of a Microsoft Access database. I had personally carried out some data entry on this project in order to carry out some previous analyses not forming part of this thesis (EPHN 2005d, EPHN 2005e) and had access to the original study forms to check any queries which arose as part of the data cleaning process. Data on parenterally HCV infected children for the analyses presented in Chapter 4 were provided by the Health Protection Agency, London, UK in the form of a Microsoft Access dataset. The interpretation of this dataset and queries arising from the analysis were carried out with the assistance of the UK National HCV Register study coordinator at the HPA, Dr Helen Harris. The aims and objectives of the analyses were agreed with my supervisors Professor Marie-Louise Newell and Dr Claire Thorne and additionally Dr Lucy Pembrey contributed to the planning of these analyses in her role as EPHN coordinator. The methodological and technical development of the analyses along with the interpretation of results for this thesis were my own responsibility.

The data on vertically HIV infected children from the European Collaborative Study analysed as part of Chapters 5 and 6 were provided by the ECS coordinating team at the UCL Institute of Child Health, London, UK in the form of a Microsoft Access database. I had carried out some data entry for the ECS and had access to the original study forms and clinician contacts for any queries which arose from the data cleaning process. The aims and objectives of the analyses were developed with my supervisors Professor Marie-Louise Newell and Dr Claire Thorne who were also the lead members of the ECS team. The

methodological and technical development of the analyses along with the interpretation of results for this thesis were my own responsibility.

Data on children from the Libyan Cohort Follow-up Study analysed as part of Chapters 5 and 6 were provided by Dr Guido Castelli-Gattinara, Bambino Gesù Children's Hospital, Rome, Italy and Dr Alessandra Vigano, L. Sacco Hospital, Milan, Italy in the form of two Microsoft Access databases. I carried out data cleaning and interpretation of the databases in consultation with Dr Castelli-Gattinara and Dr Vigano and then the aims and objectives of the analyses were developed with my supervisors Professor Marie-Louise Newell and Dr Claire Thorne. The methodological and technical development of the analyses along with the interpretation of results for this thesis were my own responsibility.

3. AGE AND SEX RELATED REFERENCE RANGES OF ALANINE AMINOTRANSFERASE (ALT) LEVELS IN CHILDREN

3.1 Introduction

Definitions of normal or abnormal alanine aminotransferase (ALT) levels have been largely based on reference ranges provided by laboratories carrying out the ALT tests (Piton *et al* 1998). The populations from which these reference ranges have been extrapolated are undefined and are likely to vary widely. There is some doubt about the validity of ALT as a marker of liver disease, especially in the case of chronic HCV infection where patients with normal ALT levels have been found to have minimal to mild liver abnormalities (Prati *et al* 2002). Many paediatric studies refer to normal or elevated ALT levels, often as twice the upper limit of normal, but few provide details of the specific cut-off values or the populations and methods by which these were defined. To date no studies have investigated the accuracy of these ranges in children which consequently remain unqualified.

3.2 Methods specific to this chapter

The reference group for the calculation of ALT reference ranges was children born to HCV infected mothers enrolled in the EPHN but who were confirmed as HCV uninfected themselves on the basis of a negative HCV antibody test after 18 months of age and/or at least two negative HCV-RNA PCR tests and fewer than two positive PCR tests before 18 months. Additionally, children with no positive PCR test results who were antibody negative between 9 and 18 months were considered to be HCV uninfected. ALT testing

was performed locally from birth (EPHN 2005a). All HCV uninfected children with at least one ALT measurement recorded during the first five years of follow-up after birth were included in this analysis.

3.2.1 Statistical analysis

Univariable and multivariable linear regression analyses were used to identify factors associated with variations in ALT levels while accounting for within-child repeated measures and between child differences that could not be measured, e.g. the use of data from different clinical centres, using a maximum likelihood random effects estimator on the intercept. Linear regression adjusted for age at ALT measurement (categorised into five groups: 6-monthly groups until 2 years of age and those over 2 years of age to account for the larger number of measurements and wider variation in ALT levels in the first two years of life), gender and weight (expressed as a standard deviation z-score representing the deviation from the median of the reference group previously used to calculate the UK 1990 Growth Standards) (Cole *et al* 1998, EPHN 2005d). Adjusting for weight in linear regression allowed adjustment for appropriate size-for-age without using BMI which is a less appropriate measure in younger children (Pietrobelli *et al* 1998).

Centiles for ALT levels over age were calculated using maximum penalized likelihood methods and LMS software (LMS 1.22, Institute of Child Health, London) (Cole and Green 1992). Models accurately representing the ALT centiles were developed separately for males and females, using appropriate equivalent degrees of freedom for each of the three curves making up the model – median, coefficient of variation and skewness. For the

purposes of this analysis, age at ALT measurement was transformed to better reflect the non-monotonic distribution of ALT over age by stretching the age scale at younger ages where there were many measurements and shrinking it at older ages where there were fewer measurements. This was done using splines fitted on a power-transformed scale which were then redrawn on the original scale (LMS Handbook, LMS 1.22, Institute of Child Health, London).

3.3 Results

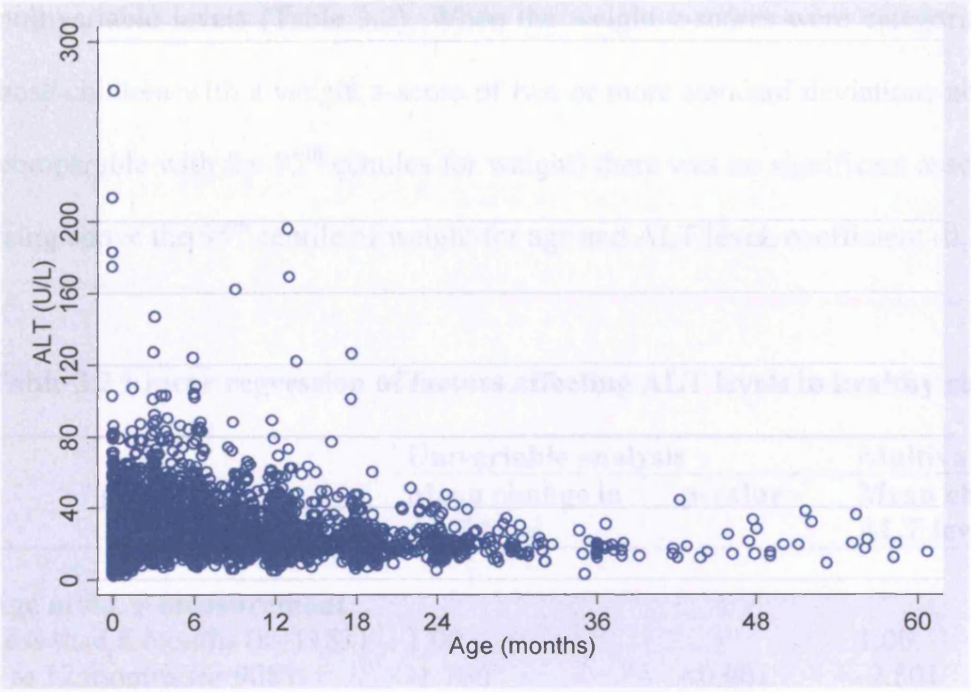
A total of 1293 HCV uninfected children, 709 (55%) male and 579 (45%) female (5 had no sex recorded), had at least one ALT measurement during 5 years of follow-up from birth with 5011 ALT measurements available overall (median 3 observations per child, range 1 to 10). Median ALT level per child was 24 U/l (range 6-117 U/l). The median age at ALT measurement was 6 months (range 0 – 4.9 years) with 3811 observations (76.1%) in the first 12 months of life from 1255 children, 1020 (20.4%) between 12 and 24 months from 721 children and 180 (3.6%) after 24 months of age from 133 children (Table 3.1). The distribution of ALT measurement over age can be seen in Figure 3.1. Baseline characteristics were similar regardless of the combination of tests used to exclude HCV infection. The rare high ALT values in uninfected children seen in Figure 3.1 were not indicative of continuously high values for specific children but were one-off events predominantly in the first six months of life when ALT levels are known to peak (EPHN 2005c). There is no evidence that they were indicative of any liver assault in these children based on concurrent clinical examinations and reporting of any clinical abnormalities of any kind. A recent survey with a 96% response rate confirmed that only 4% of children

enrolled in the EPHN were non-white but individual level data on ethnicity were not available (personal communication Dr Lucy Pembrey, November 2007).

Table 3.1 Key characteristics of 1293 children used to calculate ALT reference ranges

	Children used to calculate ALT reference ranges (n=1293)
Sex	
Male (%)	709 (55%)
Female (%)	579 (48%)
Missing	5
Median number of ALT measurements during follow-up (range)	3 (1-10)
Number of ALT measurements at specific ages	
Before 12 months of age (no. of children)	3811 (1255)
12 to 24 months of age (no. of children)	1020 (721)
After 24 months of age (no. of children)	180 (133)
Median age at ALT measurement (range)	6 months (1 month – 4.9 years)
Year of birth	
Before 1999	88 children
1999 to 2002	842 children
After 2002	363 children
Median ALT measurement per child (range)	24 U/l (6-117 U/l)

Figure 3.1 Distribution of ALT measurements in HCV uninfected, healthy children under five years of age.



3.3.1 Factors associated with changes in ALT levels

Linear regression analysis assessed the association between ALT levels and age at ALT measurement, sex and age-adjusted weight z-score (Table 3.2). ALT level decreased significantly with increasing age (non-parametric test for trend, $z=-15.1$, $p<0.001$) and ALT levels in all age categories were significantly lower in comparison to measurements taken in the first six months in both univariable and multivariable linear regression. For example, in multivariable analysis, there was a mean decrease in ALT levels of 11.6 U/l in those measurements taken after 24 months of age compared to those taken before 6 months of age (Table 3.2). Female children had significantly lower ALT levels during follow-up than male children and this association remained significant in multivariable analysis with a

mean decrease in ALT levels of 1.5 U/l in female compared to male children (Table 3.2). ALT levels significantly increased with increasing weight z-scores at univariable and multivariable levels (Table 3.2). When the weight z-scores were categorised to represent those children with a weight z-score of two or more standard deviations above the median (comparable with the 95th centiles for weight) there was no significant association between being above the 95th centile of weight for age and ALT level, coefficient -0.308 p=0.863.

Table 3.2 Linear regression of factors affecting ALT levels in healthy children

	Univariable analysis		**Multivariable analysis ***	
	Mean change in ALT level	**p-value**	**Mean change in ALT level**	**p-value**
Age at ALT measurement				
Less than 6 months (n=1183)	1.00		1.00	
6 to 12 months (n=908)	-1.766	<0.001	-2.501	<0.001
12 to 18 months (n=627)	-4.339	<0.001	-5.280	<0.001
18 to 24 months (n=279)	-7.654	<0.001	-8.842	<0.001
Greater than 24 months (n=130)	-10.241	<0.001	-11.554	<0.001
Sex				
Male (n=709)	1.00		1.00	
Female (n=579)	-1.569	0.005	-1.499	0.036
Age-adjusted weight z-score (per standard deviation) (n=1188)	1.107	0.001	1.065	0.001

* Adjusting for all variables shown

3.3.2 Age and sex specific centiles for ALT levels

Centiles of ALT levels over age showed the maximum 95th centile over five years was at three months of age in males (ALT 58.9U/l) and one month in females (ALT 55.7U/l). This

upper value of the reference range decreased with increasing age and was lowest at two years in males (ALT 34.8 UI/L) and five years in females (ALT 28.4 UI/L). Up to five years of age, the 95th centile for ALT levels was consistently higher in males than in females (Table 3.3). The 50th centile for ALT levels over age in males and females shows a peak in ALT levels during the first six months of life and a gradual decrease in ALT thereafter (Figure 3.2). This pattern is consistent with those found in HCV infected children where ALT levels have been shown to peak in the first 2 years of life and decrease thereafter (EPHN 2005a, Resti *et al* 2003).

3.3.3 Interpretation of ALT level centiles

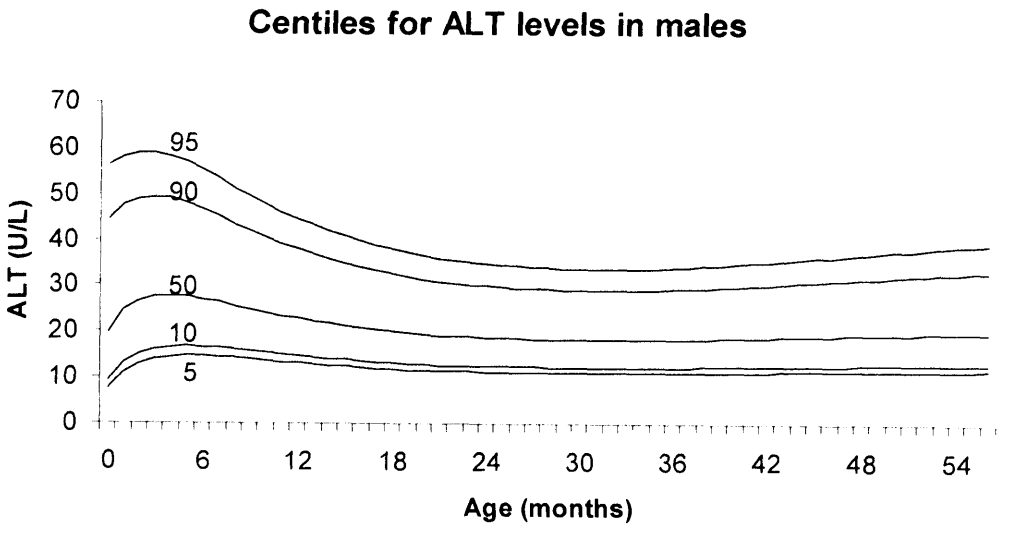
The ALT level centiles represent the distribution of ALT levels in the reference population, where the 50th centile is the median ALT level at each age. The 95th centile for each age is the point at which only 5% of the population will have larger ALT levels and therefore this is the centile commonly chosen to represent the upper limit of "normal".

Table 3.3 Reference values for Alanine aminotransferase levels for males and females at selected ages

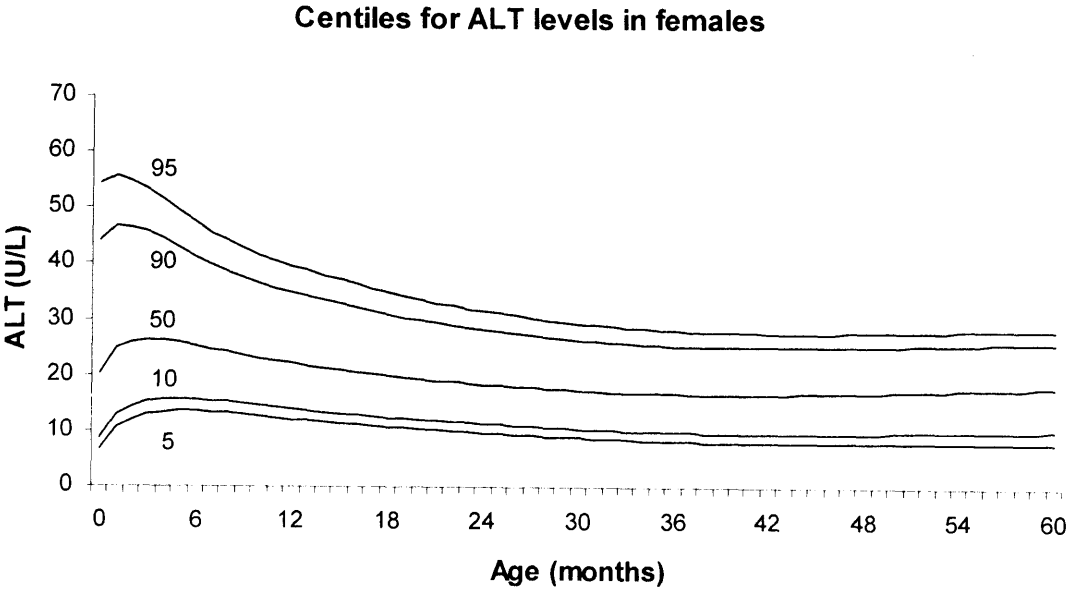
		ALT levels (UI/l)				
		Centiles				
Age	Sex	5	10	50	90	95
Birth	Male	7.45	9.19	19.78	44.47	56.43
	Female	6.78	8.75	20.36	44.09	54.26
1 month	Male	11.27	13.33	24.69	47.73	58.03
	Female	10.56	12.79	24.73	46.71	55.71
3 months	Male	13.97	16.11	27.47	49.32	58.87
	Female	12.96	15.16	26.36	45.73	53.44
6 months	Male	14.66	16.67	27.10	46.77	55.32
	Female	13.52	15.52	25.28	41.24	47.40
12 months	Male	13.11	14.71	22.89	38.04	44.59
	Female	12.10	13.83	22.07	34.87	39.63
18 months	Male	11.80	13.16	20.00	32.55	37.97
	Female	10.77	12.39	19.87	30.89	34.83
2 years	Male	11.29	12.53	18.73	30.00	34.84
	Female	9.73	11.61	18.37	28.18	31.53
5 years	Male	11.67	13.01	19.91	33.18	39.16
	Female	8.02	10.13	17.88	26.00	28.35

Figure 3.2 Centiles for ALT levels in a) males and b) females over age.

a)



b)



3.4 Key points

- ALT levels in a population of HCV uninfected, healthy children significantly decreased with increasing age after a peak in the first six months of life.

- ALT levels were significantly and consistently higher in males than females up to five years of age and also peaked earlier in girls.

- ALT levels in children increased significantly with increasing weight-for-age but no statistically significant increase in ALT levels in children with weight-for-age above the 95th centile compared with those below the 95th centile was found.

- From the pattern of ALT levels over age, it would be more appropriate to use above and below 18 months of age as the cut-off for differing reference ranges rather than above and below 12 months as has been used previously.

- Calculation of ALT centiles showed that an ALT level of greater than 60U/l in boys and 55U/l in girls should be regarded as elevated in the first 18 months of life while in children older than 18 months the upper limits of normal ALT levels are lower; 40U/l in boys and 35U/l in girls.

- Reference ranges in children younger than five years of age calculated from a population of healthy HCV uninfected children with low likelihood of liver-related

abnormalities appear to be lower than previously reported.

4. THE IMPACT OF MODE OF ACQUISITION ON BIOLOGICAL MARKERS OF HEPATITIS C VIRUS INFECTION

4.1 Introduction

Information on the impact of mode of acquisition of paediatric HCV infection on biological markers of infection, including HCV viraemia and ALT levels, used to assess disease progression in the absence of liver biopsy, is limited. In a recent European study, 44% of 240 vertically HCV infected children followed-up for a median for 4.2 years, were found to have consistently positive HCV RNA PCR test results, while 7% were found to have consistently negative HCV RNA PCR results despite positive antibody (EPHN 2005a). A comparative figure for parenterally infected children is not available, but in a small study comparing clinical outcomes in 16 vertically and 15 post-transfusion infected children, viral clearance, although undefined, was 13% in vertically infected and 27% in parenterally infected children but the difference was not statistically significant likely due to the small numbers of children in each group (Rerksuppaphol *et al* 2004).

It is plausible that given the infection of vertically infected children during early immune maturation, or the possible adaptation of the immune system of the vertically infected children following early exposure in utero, the natural history and disease progression in parenterally infected children acquiring HCV later in childhood might differ from that of their vertically infected counterparts. To inform the management of parenterally HCV infected children worldwide, and in the light of continued outbreaks and diagnoses of new cases, it is vital to understand any differences between this group and vertically infected children, upon whom the majority of paediatric HCV guidelines are based.

4.2 Methods specific to this chapter

For the purposes of this chapter, a dataset of vertically HCV infected children from the European Paediatric HCV Network and a dataset of parenterally HCV infected children from the UK National HCV Register were analysed (See section 2.3.1 and section 2.3.3).

4.2.1. Definitions

A recent study from 54 children enrolled in the EPHN suggested that between one third and a half of vertically HCV infected children acquired infection in utero and half acquired infection late intrauterine or peripartum. Although in a few children postnatal acquisition could not be ruled out (Mok *et al* 2005). However, it was not feasible to accurately estimate the timing of infection before delivery for the purposes of the analyses in this chapter and so here infection was assumed to have occurred at birth in vertically HCV infected children. The estimated date of HCV infection in the parenterally infected study population was the date of transfusion or receipt of blood products for the 76 (60%) children where this was known. Fifty (40%) children were transfused outside the UK or received multiple transfusions during childhood, and thus the date of infection could not be accurately estimated. These 50 children were consequently excluded from specific analyses which involved knowing the exact age at infection, for example, comparing factors in those infected before and after 12 months of age. Clearance of HCV viraemia was defined as having occurred in children with confirmed HCV infection whose last two consecutive HCV RNA PCR test results were negative, irrespective of antibody status. A sustained virological response (SVR) to HCV treatment was defined by a negative HCV RNA PCR test six months or more after the cessation of treatment (Poynard 2003). ALT levels greater

than 60U/l in boys and 55U/l in girls are regarded as elevated in the first 18 months of life while in children older than 18 months the upper limits of normal ALT levels are 40U/l in boys and 35U/l in girls (See Chapter 3).

4.2.2 Statistical analysis

Comparisons between parenterally and vertically infected children were carried out using Chi-squared or z-tests when comparing proportions or Mann Whitney ranksum tests when comparing medians (Kirkwood and Sterne 2003). Logistic regression identified factors associated with ALT, PCR and hepatomegaly summary variables. Children with two or more RNA PCR test results, for whom 75% or more of these PCR results were positive, were defined as being consistently HCV viraemic and similarly, children with 75% or more ALT z-scores greater than 2 standard deviations (SD) (equivalent to the 95th centile on the reference range), were defined as having consistently elevated ALT z-scores, assuming that they had at least two ALT levels recorded during follow-up.

4.3 Results

Data on 395 HCV infected children were available from the datasets provided by the EPHN and UK National HCV Register. This included 269 vertically infected and 126 parenterally infected children. Seventy-six (60%) parenterally infected children had a known date of infection and the median age at infection for this group was 19 months of age. Follow-up in vertically infected children was more intensive; and the larger number of median follow-up visits in the vertically infected group is likely a reflection of the different study protocols

with the EPHN recommending follow-up approximately every six months and the UK National HCV Register carrying out annual follow-up (EPHN 2005a, Harris *et al* 2000).

The male to female sex ratio in the vertically infected children was approximately 1:2 reflecting the increased likelihood of vertical HCV infection in girls compared to boys (EPHN 2005c). Conversely, the male to female sex ration in the parenterally infected children was approximately 2:1, $p<0.001$ (Table 4.1). HCV genotype was recorded for 165 (42%) children, 115/269 (43%) vertically and 50/126 (40%) parenterally infected. No significant differences were found in HCV genotype profiles by mode of acquisition when looking at each genotype individually ($\chi^2=4.85$, $p=0.301$) or genotype 1 in comparison to any other genotype ($\chi^2=1.99$, $p=0.158$) (Table 4.1).

Ten vertically infected children and 39 parenterally infected children received anti-HCV therapy during follow-up. Nine (90%) vertically infected children received IFN-alpha with the remaining vertically infected child receiving IFN-alpha in combination with Ribavirin. Eighteen (46%) parenterally infected children received IFN-alpha and 12 (30%) received IFN-alpha with Ribavirin. The remaining nine (23%) parenterally infected children received Pegylated IFN alpha with Ribavirin. Parenterally infected children were significantly more likely to have received HCV treatment than vertically infected children (Table 4.1) and the median age at the start of treatment was 5.7 years (range 2.3-11.7 years) for the vertically infected and 11 years (range 1.9-17.3 years) for the parenterally infected. There were no significant differences in the proportion of vertically and parenterally infected children achieving a SVR following treatment (Table 4.1). Seventeen (44%)

parenterally infected children who received HCV treatment had genotype information available (ten children had genotype 1, one had genotype 2 and six had genotype 3). Only two vertically infected children who received HCV treatment had genotype information available (one had genotype 1 and the other had genotype 2). A higher proportion of children with genotype 3 achieved a SVR (66.7% (4/6)) than children with genotype 1 (18.2% (2/11), Fisher's exact p-value=0.073.

Parenterally HCV infected children with a known date of infection began follow-up at an earlier age than those with no known infection date and had a longer duration of follow-up but the two parenterally infected groups did not differ in terms of their gender, HCV genotype or treatment profile (Table 4.2).

Table 4.1 Key characteristics and follow-up profiles of vertically and parenterally HCV infected children

	Mode of acquisition		
	Vertically infected n=269 (%)	**Parenterally infected** n=126 (%)	**Comparison** (χ^2/z-test or Mann Whitney)
Sex			
Male	96 (38.9)	69 (63.9)	χ^2=18.91, p<0.001
Female	151 (61.1)	39 (36.1)	
Missing	22		
Median age at infection	Birth	19.13 months (n=76) (range 2.1days-13.5 yrs)	N/A
Known date of infection			
Yes	269*	76 (60.3)	N/A
No	0	50 (39.7)	
Median age at start of follow-up	Birth	12.2 yrs (range 12.0months-17.9yrs)	N/A
Median number of follow-up test dates	4 (range 1-25)	2 (range 1-10)	Mann Whitney=11.74 p<0.001
median duration of follow-up	2.8 yrs (range 1month-17.9yrs)	2.5 yrs (range 1month-10.0yrs)	Mann Whitney=2.59, p=0.010
HCV Genotype			
1	55 (47.8)	29 (58)	χ^2=4.845, p=0.304
2	18 (15.7)	5 (10)	
3	31 (27.0)	13 (26)	
4	11 (9.6)	2 (4)	
5	0	1 (2)	
Missing	154	76	
Treated			
No	259 (96.3)	87 (69.0)	χ^2=58.57, p<0.001
Yes	10 (3.7)	39 (31.0)	
Response to treatment			
No response	5 (50.0)	15 (38.5)	χ^2=1.037, p=0.595
Sustained virological response	3 (30.0)	15 (38.5)	
Unknown	5 (20.0)	9 (23.1)	

* All vertically HCV infected children were assumed to have been infected at birth

Table 4.2 Difference between parenterally HCV infected children with and without known dates of infection

	Parenterally infected children		
	Known infection date n=76 (%)	**Unknown infection date** n=50 (%)	**Comparison** (χ^2/z-test or Mann Whitney)
Sex			
Male	48 (63.2)	34 (68.0)	χ^2=0.31, p=0.577
Female	28 (36.8)	16 (32.0)	
Median age at infection (range)	19.13 months (2.1days-13.5 yrs)		N/A
Median age at start of follow-up (range)	10.5 yrs (12 months – 17.9 yrs)	14.0 yrs (4.6 yrs – 17.9 yrs)	Mann Whitney=4.42, p<0.001
Median number of follow-up test dates (range)	3 (1-10)	2 (1-7)	Mann Whitney=-5.04, p<0.001
median duration of follow-up (range)	4.3 yrs (1 month-10.1 yrs)	6.1 months (1 month-6.4 yrs)	Mann Whitney=-4.89, p<0.001
HCV Genotype			
1	26 (57.8)	3 (60.0)	χ^2=1.31, p=0.859
2	5 (11.1)	0	
3	11 (24.4)	2 (40.0)	
4	2 (4.4)	0	
5	1 (2.2)	0	
Missing	31	45	
Treated			
No	53 (69.7)	34 (68.0)	χ^2=0.04, 0.837
Yes	23 (30.3)	16 (32.0)	
Response to treatment			
No response	5 (21.7)	10 (62.5)	χ^2=7.04, p=0.0.030
Sustained virological response	12 (52.2)	3 (18.3)	
Unknown	6 (26.1)	3 (18.3)	

4.3.1 Clinical signs and symptoms of HCV infection

Parenterally infected children were significantly more likely to have hepatomegaly reported at least once during follow-up with hepatomegaly events occurring in 22/126 (17.5%) parenterally infected children in comparison to 27/269 (10.0%) vertically infected children ($z=-2.097$, $p=0.036$). Among the 49 children with hepatomegaly events, hepatomegaly occurred at both a longer time after infection and an older age in parenterally infected children in comparison to vertically infected children; median time since infection 10.2 years (range 8.4yrs-15.1yrs) and 4.8 years (range 3.6days-15yrs) respectively, $z=-3.814$, $p<0.001$, and median age at first hepatomegaly event 12.0 years (range 6.8yrs-17.4yrs) and 4.3 years (range 3.6days -15yrs) respectively, $z=-5.106$, $p<0.001$.

Liver biopsy was carried out on a total of 27/269 (10.0%) vertically infected children and 73/126 (57.9%) parenterally infected children. Similar proportions of vertically and parenterally infected children showed signs of chronic hepatitis, 82% and 67% respectively ($z=1.468$, $p=0.142$). Three of 27 (11%) biopsied vertically infected children and 29/73 (40%) biopsied parenterally infected children had more than one biopsy during follow-up, number of biopsies ranged from two to four. Genotype information was available for 12/27 (44%) biopsied vertically infected children and of these 12, four children had genotype 1, two had genotype 2, five had genotype 3 and one had genotype 4. Similarly, genotype information was available for 29/73 (40%) of biopsied parenterally infected children and of these 29, 16 had genotype 1, three had genotype 2, nine had genotype 3 and one had genotype 4. Children who received anti-HCV therapy comprised 22% (6/27) of vertically infected and 40% (29/73) of parenterally infected children who were biopsied.

4.3.2 HCV RNA PCR

Qualitative HCV RNA PCR was measured for 262 (of 269) vertically infected and 116 (of 126) parenterally infected children, of whom 250 and 68 respectively had at least two PCR results recorded overall. Of these 250 vertically infected and 68 parenterally infected children, 21 (9%) and 37 (32%) respectively had two PCR test results recorded, 30 (12%) and 21 (8%) respectively had three PCR tests recorded, 43 (17%) and 10 (9%) respectively had four PCR tests recorded and 156 (62%) vertically infected children and no parenterally infected children had five or more PCR test results recorded during follow-up. Among the children with two or more PCR tests, a higher proportion of parenterally infected children had PCR results which were all positive than vertically infected children, 34/68 (50.0%) and 101/250 (40.4%) respectively, this difference was of borderline statistical significance, z=-1.420, p=0.079. Approximately 60% of both vertically and parenterally infected children were consistently viraemic (75% or more HCV RNA PCR test results positive); 145/250 (58.0%) and 37/68 (54.4%) respectively, z=0.532, p=0.595.

Logistic regression was used to identify any factors associated with being consistently HCV viraemic in 300 children (232 vertically infected and 68 parenterally infected) who had information available on all the variables in Table 4.3. In univariable and multivariable regression, the odds of being consistently HCV viraemic was significantly associated with having consistently elevated ALT z-scores and children who were consistently viraemic were over 10 times as likely to have consistently elevated ALT z-scores than those who were not consistently viraemic (Table 4.3). Overall, the adjusted odds ratios were similar to the unadjusted odds ratios for all the variables included in the logistic regression, this is not

surprising given the lack of associations between consistent viraemia and most of the variables under consideration in univariable analysis. Although not significantly so, being female, parenterally infected or receiving anti-HCV therapy reduced the odds of being consistently HCV viraemic. In contrast, ever having evidence of hepatomegaly increased the odds of being consistently HCV viraemic but again, not significantly so.

Due to the small number of children with genotype information, this variable was not part of the logistic regression model in Table 4.3 to maximise the number of children included. Univariable logistic regression was carried out on the subset of 125 children with genotype information available separately; the odds of being consistently viraemic was higher in children with HCV genotype 1 versus any other genotype but this association did not reach statistical significance (unadjusted OR=1.94 (95% CI 0.92-4.08, p=0.082 (n=125)).

Clearance of HCV viraemia was seen in similar proportions of vertically and parenterally infected children, 30% (74/250) and 34% (23/68) respectively (z=-0.633, p=0.527). The median time from infection to clearance was 1.3 years (range 3 months – 10.4 years) in vertically infected children and 7.5 years (range 3.7 years – 13.5 years) in parenterally infected children.

Table 4.3 Logistic regression of factors associated with having a positive HCV RNA PCR result on 75% or more of tests during follow-up (n=300)

	Univariable logistic regression OR (95% CI)	p-value	Multivariable logistic regression* AOR (95% CI)	p-value
Sex				
Male (n=128)	1.00		1.00	
Female (n=172)	0.97 (0.61-1.55)	0.912	0.88 (0.54-1.44)	0.615
Mode of acquisition				
Vertical (n=232)	1.00		1.00	
Parenteral (n=68)	0.89 (0.52-1.53)	0.670	1.137 (0.59-2.20)	0.703
Evidence of hepatomegaly				
No (n=260)	1.00		1.00	
Yes (n=40)	1.50 (0.75-3.00)	0.255	1.20 (0.55-2.65)	0.646
Ever treated				
No (n=284)	1.00		1.00	
Yes (n=34)	0.65 (0.32-1.32)	0.232	0.67 (0.29-1.55)	0.349
Proportion of ALT z-scores above 2SD				
Less than 75% (n=263)	1.00		1.00	
75% or greater (n=37)	10.58 (3.17-35.31)	<0.001	10.28 (3.03-34.85)	<0.001

* Multivariable regression adjusting for all other factors in the table.

4.3.3 ALT levels

A total of 1404 ALT observations were recorded (range 2-1213 U/l) from 218 vertically infected and 91 parenterally infected children. Of these, 14 (6.4%) vertically infected and 52 (57.1%) parenterally infected children had one ALT level recorded, 24 (11.0%) and 21 (23.1%) respectively had two ALT levels recorded, 27 (12.4%) and 10 (11.0%) respectively had three ALT levels recorded, 24 (11.0%) and 8 (8.8%) respectively had four ALT levels

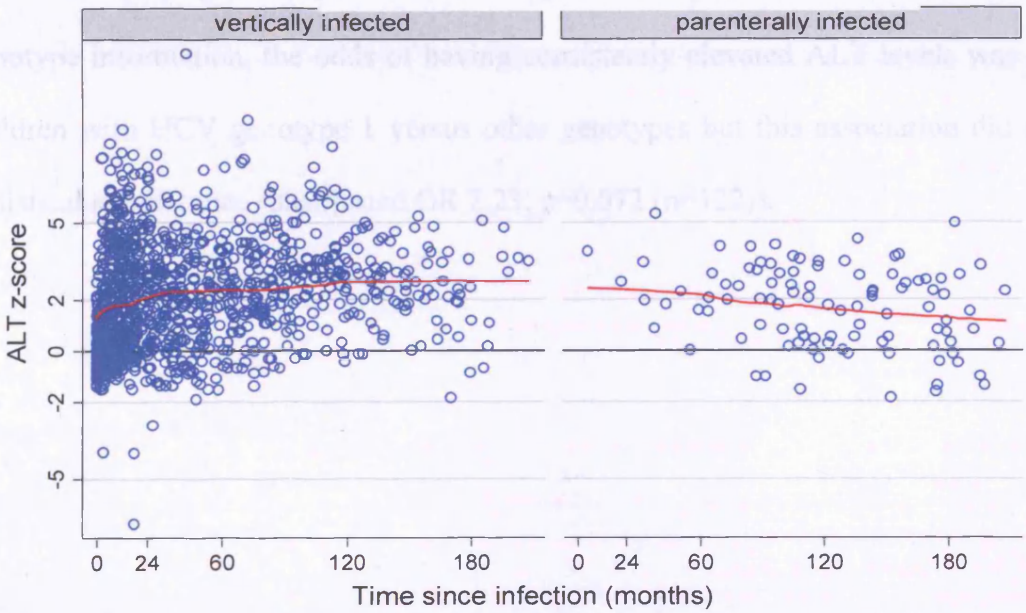
recorded and 129 (59.2%) vertically infected children and no parenterally infected children had five or more ALT levels recorded during follow-up.

At least one elevated ALT level was recorded from 137 (62.8%) vertically infected and 53 (58.2%) parenterally infected children during follow-up ($z=0.577$, $p=0.564$). Significantly fewer vertically infected than parenterally infected children had persistently elevated ALT levels, 33/218 (15.1%) and 36/91 (39.6%) respectively ($z=-4.715$, $p<0.001$). Age at infection was associated with having persistently elevated ALT levels, with 18.2% (45/247) of children infected before 12 months of age, regardless of mode of acquisition, having persistently elevated ALT results compared to 41.7% (10/24) of children infected after 12 months of age, $z=-2.733$, $p=0.006$. However, among 53 parenterally infected children, there was no difference in the proportion of children infected before and after 12 months of age with persistently elevated ALTs, 12/29 (41.4%) and 10/24 (41.7%) respectively, $z=-0.02$, $p=982$.

Age-adjusted ALT standard deviation z-scores were calculated for each ALT measurement in reference to a standard population of HCV uninfected children born to HCV infected mothers. The mean ALT z-score for vertically infected children was 2.049 (SD 2.059) and for parenterally infected children was 1.541 (SD 1.783), $t=2.949$, $p=0.03$ (Figure 4.1). The mean ALT z-score in 247 children infected before 12 months of age (2.033, SD 2.033) was also significantly higher than the mean ALT z-score in the 24 children infected after 12 months of age (1.387 SD 1.652), $t=1.887$, $p=0.030$. Among 53 parenterally infected children, there was no difference by age at infection in terms of mean ALT z-score; mean

ALT z-score 1.64 (SD 1.37) in those infected before 12 months of age and mean ALT z-score 1.63 (SD1.66) in those infected after 12 months of age, t=0.010, p=0.992.

Figure 4.1 ALT z-score over time since infection in vertically and parenterally infected children.



____ Running mean smoother calculating smoothed values for each data point

Fifty-one children had consistently elevated ALT z-scores (defined as having 75% or more ALT z-scores 2 SD or above in those children with 2 or more ALT results recorded); 43/204 (21.1%) vertically infected children and 8/39 (20.5%) parenterally infected children. Children who had ever had evidence of hepatomegaly were over three times as likely to have consistently elevated ALT z-scores in multivariable logistic regression. Additionally,

those who were consistently HCV viraemic were over 12 times as likely to also have consistently elevated ALT z-scores in comparison to those who were not consistently viraemic. Although not significantly so, parenterally infected children were more likely to have consistently elevated ALT z-scores than vertically infected children in univariable, but not multivariable analysis (Table 4.4)

In univariable logistic regression including the 122 children with ALT z-score and genotype information, the odds of having consistently elevated ALT levels was higher in children with HCV genotype 1 versus other genotypes but this association did not reach statistical significance (unadjusted OR 2.23, p=0.072 (n=122)).

Table 4.4 Logistic regression of factors associated with having 75% or more ALT z-scores greater than 2 SD (n=205)

	Univariable logistic regression OR (95% CI)	p-value	Multivariable logistic regression* AOR (95% CI)	p-value
Sex				
Male (n=87)	1.00		1.00	
Female (n=118)	1.34 (0.68-2.64)	0.394	1.43 (0.67-3.04)	0.358
Mode of acquisition				
Vertical (n=175)	1.00		1.00	
Parenteral (n=30)	1.31 (0.54-3.18)	0.614	0.83 (0.26-2.61)	0.748
Evidence of hepatomegaly				
No (n=174)	1.00		1.00	
Yes (n=31)	4.33 (1.94-9.67)	<0.001	3.07 (1.25-7.52)	0.014
Ever treated				
No (n=187)	1.00		1.00	
Yes (n=18)	1.37 (0.46-4.07)	0.571	1.13 (0.28-4.53)	0.863
Proportion of HCV RNA PCR results positive				
Less than 75% (n=82)	1.00		1.00	
75% or greater (n=123)	14.15 (4.22-42.51)	<0.001	12.69 (3.73-43.17)	<0.001

*Multivariable logistic regression adjusting for all other variables in table.

Fifteen children were consistently HCV viraemic, had consistently elevated ALT z-scores and evidence of hepatomegaly from a total of 295 children with no missing information on any of these variables. The proportion of children with two or more of these three markers of infection was similar in parenterally (15/84, 17.9%) and vertically infected children (45/211, 21.3%), χ^2=-0.67, 0.504 and also similar in those infected before 12 months of age and after 12 months of age (52/239, 21.8% and 3/24, 14.3% respectively), χ^2=1.13, p=0.288. When only the 52 parenterally infected children with date of infection information

and no missing information on the three markers of infection were examined, similar proportions of those infected before and after 12 months of age were found to have two or more markers of infection (7/28, 25.0% and 3/24, 10.7% respectively), χ^2=1.30, p=0.254. Similar proportions of children receiving anti-HCV therapy (9/36, 25%) and those receiving none (51/259, 19.7%) had two or more markers of infection, χ^2=0.55, p=0.458 and similarly there was no statistically significant difference between the number of children with genotype 1 who had two or more markers of infection (21/73, 28.8%) and those with any other genotype (13/75 17.3%), χ^2=2.73, p=0.098 (Table 4.5).

Table 4.5 Characteristics of children with two or more markers of infection (consistently viraemic, consistently elevated ALT z-scores, evidence of hepatomegaly)

	Number of children with two or more markers of infection (%)	Comparison (χ^2, p-value)
Mode of acquisition		
Vertically infected (n=211)	45 (21.3%)	χ^2=-0.67, p=0.504
Parenterally infected (n=84)	15 (17.9%)	
Age at infection		
Younger than 12 months (n=239)	52 (21.8%)	χ^2=1.13, p=0.288
Older than 12 months (n=24)	3 (14.3%)	
Age at infection – only parenterally infected		
Younger than 12 months (n=28)	7 (25.0%)	χ^2=1.30, p=0.254
Older than 12 months (n=24)	3 (10.7%)	
Treated		
Not treated (n=259)	51 (19.7%)	χ^2=0.55, p=0.458
Treated (n=36)	9 (25.0%)	
Genotype		
Genotype 1 (n=73)	21 (28.8%)	χ^2=2.71, p=0.098
Any other genotype (n=75)	13 (17.3%)	

Table 4.6 Summary of differences between parenterally and vertically HCV infected children

Factor of interest	Difference between parenterally and vertically HCV infected children
Sex	Significantly more parenterally infected boys and vertically infected girls
Anti-HCV therapy	Significantly more parenterally infected received treatment
HCV genotype	No difference
Hepatomegaly events during follow-up	Significantly more hepatomegaly events in parenterally infected
Persistently positive HCV viraemia	More parenterally infected with persistent viraemia, borderline significance
Consistently positive HCV viraemia	No difference
Persistently elevated ALT levels	Significantly more parenterally infected with persistently elevated ALT levels
Consistently elevated ALT levels	No difference
Two or more biological markers of HCV infection	No difference

4.4 Key Points

- The analyses in this chapter represent the largest investigation of the impact of mode of acquisition of infection on biological markers of HCV infection in vertically and parenterally infected children to date.

- Vertically and parenterally HCV infected children differed in terms of some key characteristics including the male: female ratio and the proportion of children receiving anti-HCV therapy.

- Children who had consistently elevated ALT z-scores were more than 10 times as likely to also be consistently viraemic than those without consistently elevated ALT z-scores.

- Parenterally infected children had a higher odds of being consistently viraemic than vertically infected children but not significantly so.

- No differences were found in the HCV genotype profiles of vertically and parenterally infected children but a higher proportion of children with genotype 3 achieved a sustained virological response to anti-HCV therapy in comparison to children with other HCV genotypes. Additionally, in univariable logistic regression, children with genotype 1 were more likely to be consistently HCV viraemic and have consistently elevated ALT z-scores than those with any other genotype but these associations

remained of borderline significance, perhaps due to the small numbers available for analysis.

- Children who had ever had evidence of hepatomegaly were over three times as likely to have consistently elevated ALT z-scores compared to those with no evidence of hepatomegaly.

- Children who were consistently HCV viraemic were over 12 times as likely to have consistently elevated ALT z-scores as those who were not consistently viraemic.

- In multivariable logistic regression analysis, parenterally HCV infected children were less likely to have consistently elevated ALT z-scores but not significantly so.

- Similar proportions of parenterally and vertically HCV infected children had evidence of two or more markers of infection (consistent viraemia, consistently elevated ALT z-scores and evidence of hepatomegaly) and similar proportions of those infected before and after 12 months of age had two or markers of infection.

- Children who were genotype 1 were more likely to have two or more markers of infection than those with any other genotype but this association only reached borderline statistical significance, perhaps due to the small numbers available for analysis.

5. NATURAL AND TREATED HISTORY OF HIV INFECTION IN PARENTERALLY INFECTED CHILDREN

5.1 Introduction

The natural and treated history of paediatric HIV in vertically infected children has been well documented (Blanche *et al* 1997, ECS 2004, ECS 2001, Thorne *et al* 2002, Pliner *et al* 1998, Palumbo *et al* 1998, Dunn *et al* 2003, Prendergast *et al* 2007, Little *et al* 2007). However, less is known about HIV disease characteristics and progression in children who acquire HIV parenterally via receipt of infected blood or blood products or contact with contaminated hospital instruments. This group of children is relatively small due to the implementation of blood donor screening and universal sterilisation procedures in most developed countries in the early 1990s but there remain groups of children infected in this way and new infections continue to occur globally, often with large numbers of children infected from a single source (Prati 2006, Morris 2006, Visco-Comandini *et al* 2002, Yerly *et al* 2001, Popova *et al* 1999, Hersh *et al* 1993). These children are unique both in terms of their route of acquisition of infection and the fact that they often have significant comorbidities, given the circumstances under which HIV infection occurred. Popova *et al* presented information on 124 nosocomially infected children from Russia of whom 33 (26%) had died 9 years after diagnosis. They report that in the first year of life, HIV infection in these children could rapidly progress to AIDS due to their background of often severe disease prior to contracting HIV (Popova *et al* 1999). Despite continued outbreaks of parenterally acquired HIV infection globally, few studies have quantified the natural history

of children infected in this way and specifically the impact of age at infection remains unqualified.

Differences in the natural history and disease progression of HIV infected individuals by mode of and age at acquisition have been demonstrated in adults, although overall results are conflicting. Recent large studies have identified no difference in disease progression among homosexuals in comparison to heterosexuals contrary to early research suggesting faster progression in the former (Carre *et al* 1994, Prins and Veugelers 1997, Rezza 1998). It is less clear whether differences found between progression in drug users and those acquiring infection via sexual transmission are genuinely a result of differing disease risk or are confounded by the high prevalence of other-cause mortality in infecting drugs users, their poorer adherence to therapy and their worse access to HAART (Langford *et al* 2007, Babiker *et al* 2003). A study of UK haemophiliacs infected with HIV showed a higher survival rate 10 years after seroconversion, a longer time from infection to AIDS diagnosis and a longer survival after AIDS diagnosis in patients seroconverting before 15 years of age compared to those seroconverting at older age groups up to 55 years of age (Darby *et al* 1996). More recently, data from over 13000 parenterally HIV infected individuals were analysed and the estimated survival of individuals infected before 14 years of age was greater than that among individuals infected at older ages, although the high number of those infected before 14 years of age surviving to the end of the follow-up period limited the estimation of survival rates for this group (Collaborative Group on AIDS incubation and HIV survival 2000). Similarly, age at initiation of HAART was found to be inversely

associated with the magnitude and speed of CD4 cell count recovery in a recent retrospective analysis of 187 HIV infected patients (Micheloud *et al* 2008).

On the basis of adult data, it may therefore be hypothesised that the earlier a child is infected the slower the progression to severe disease. However, it is biologically plausible that those infected from MTCT or early in infancy may elicit a faster and more severe disease progression as a result of an under-developed immune system at the time of infection. Several studies have partially investigated this by comparing disease progression in vertically infected children acquiring infection in utero with those acquiring infection intrapartum and/or post-partum via breastfeeding. One study demonstrated that children who were presumed infected intrapartum, on the basis of repeated early PCR tests, had a significantly lower mean initial viral load after infection and a more gentle decrease in viral load than those who were infected through breastfeeding. However in adjusted analysis, timing of infection was not associated with risk of progression to AIDS or death (Rouet *et al* 2003). Similarly, Shearer *et al* demonstrated differences in initial peak HIV RNA viral load in those vertically infected early (n=7) or late (n=43) (initial peak HIV RNA viral load 780,000 and 243,000 respectively) but this difference disappeared shortly after birth and both groups subsequently responded to the virus in a similar way (Shearer *et al* 1997). In contrast, in a study of vertically HIV infected children in sub-Saharan Africa, mortality was significantly lower in children who were postnatally infected, predominantly via breastfeeding, and those infected perinatally or intra-partum (Newell *et al* 2004).

Using data from a large prospective cohort study, this analysis describes the short/medium-term natural and treated history and disease progression in parenterally HIV infected children. This chapter aims to document this progression and facilitates its comparison to existing literature on vertically HIV infected children to assess differences by mode of acquisition.

5.2 Methods specific to this chapter

The dataset of parenterally HIV infected children utilised for analyses in this Chapter were from the larger cohort of parenterally HIV and HIV/HCV infected children from the Libyan Follow-up Cohort Study (See section 2.3.4). Date of infection was estimated using the midpoint between the first positive HIV test and the earliest date at which infection was known to be possible, 1st January 1998. If the date of the first positive HIV test was unavailable, date of infection was assumed to be the 1st of January 1999 as this was the midpoint of the two year interval, 1st January 1998 to 31st December 1999, during which infection was known to have occurred. An undetectable viral load (UVL) is defined as the lower cut-off of the HIV RNA PCR assay.

5.2.1 Statistical analysis

HIV RNA viral load, CD4 count and progression to CDC immunological stage 3 and clinical stages B or C (CDC 1994) were used to indicate HIV-related progression in this analysis. Kaplan Meier survival analysis estimated the proportion of children progressing to moderate or severe clinical signs or severe immunosuppression over time and formal

comparisons were made using log-rank tests (Bland and Altman 1998). CDC clinical categories were based on clinician reporting, while CDC immunological categories were designated on the basis of CD4 cell counts and age at measurement (CDC 1994).

Standard linear mixed effect (LME) regression was used to examine HIV RNA viral load since infection, accounting for baseline CD4 count in the form of CDC immunological categories 1 vs 2/3. The final model included a random effect term for the intercept. The linear missed effect regression was performed using SAS software (v9.01, SAS Institute, Cary, North Carolina, USA).

5.3 Results

There was a total of 243 parenterally HIV infected children in the cohort and their follow-up profile, including age at entry to the cohort and age at infection are presented in Table 5.1. For 195 children (80%), date of infection was estimated to be the midpoint between 1st January 1998 and their first positive HIV test. For the remaining 48 children, date of infection was assumed to be the 1st January 1999. Follow-up began in all children within four years of infection, with 168 children (69%) first followed up within 12 months of presumed infection. The median number of follow-up visits was three, with ten children only having one follow-up visit and 212 (87%) having three or more visits.

Table 5.1 Follow-up profile of parenterally HIV infected children

	Parenterally HIV infected children (n=243)
Sex	
Male (%)	138 (58%)
Female (%)	101 (42%)
Missing	4
Median age at infection (range)	21.8 months (14 days - 15.8 years)
Median age follow-up start (range)	3.3 years (27 days - 17.2 years)
Median duration of follow-up (range)	2.1 years (1 month – 4.9 years)
Median time from infection to follow-up start (range)	8.2 months (range 14 days – 4.0 yrs)
Median follow-up visits (range)	3 (1-11)

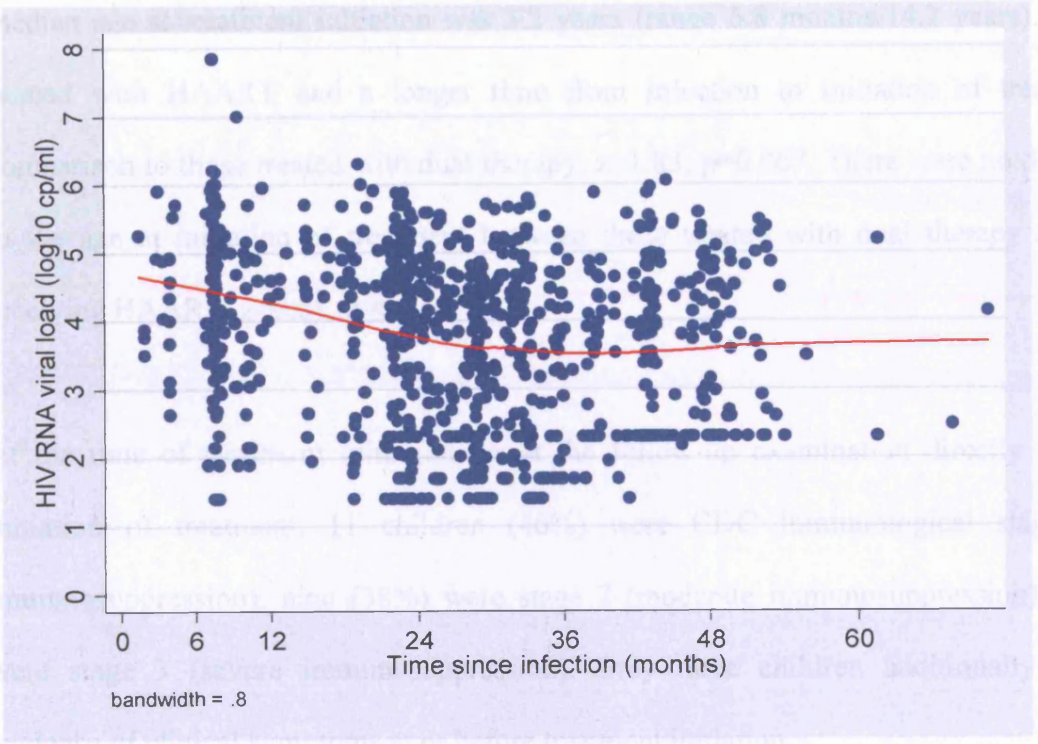
5.3.1 HIV RNA viral load

Eight hundred and eighty-nine HIV RNA viral load measurements were available from 236 parenterally HIV infected children with the median number of measurements per child three (range 1 – 10) (Figure 5.1). Twenty children (8%) only had one HIV RNA viral load measurement recorded during follow-up while 191 children (81%) had three or more measurements. In the linear mixed effect model, HIV RNA viral load was associated with time since infection, baseline CDC immunological stage, receiving treatment and gender but not with the age at infection. The mean HIV RNA viral load within 12 months of infection, adjusting for all the above factors, was 4.76 $\log_{10}$ cp/ml (95% CI 4.35 – 5.17 $\log_{10}$ cp/ml), from 153 HIV RNA measurements during this time. Mean HIV RNA level between 12 and 24 months after infection was significantly lower than in the first year after

infection, 4.25 log_{10} cp/ml (95% CI 3.64 – 4.86 log_{10} cp/ml). Mean HIV RNA viral load continued to decrease with increasing time since infection and the mean HIV RNA viral load between two and three years after infection was 3.78 log_{10} cp/ml (95% CI 3.18 – 4.37 log_{10} cp/ml). The viral load remained similar between three and four years after infection at 3.77 log_{10} cp/ml (95% CI 3.14 –4.39 log_{10} cp/ml) and decreased further after four years of infection but observations during this period were fewer and therefore the estimate is not as robust.

As expected, children with a baseline CDC immunological category of two or three had a significantly higher mean HIV RNA viral load than those with baseline category one, mean HIV RNA viral load in those with moderate or severe immunosuppression 5.11 log_{10} cp/ml (95% CI 4.45 – 5.77 log_{10} cp/ml, p=0.007). Children who received ART had a significantly lower HIV RNA viral load than those untreated, mean HIV RNA viral load in those treated was 0.74 log_{10} cp/ml lower than in those untreated, p<0.001 and girls had a lower HIV RNA viral load than boys, mean HIV RNA viral load in girls 0.23 log_{10} cp/ml lower than boys, p=0.061.

Figure 5.1 Unadjusted log_{10} HIV RNA viral load in 236 parenterally HIV infected children (889 measurements)



Running mean smoother calculating smoothed values for each data point

5.3.2 Treatment

No children received post-exposure prophylaxis following infection as diagnosis occurred too late after infection for this to be appropriate. Twenty-six (11%) parenterally infected children received antiretroviral therapy after confirmation of infection; 18 (69%) received highly active antiretroviral therapy (HAART) and eight dual therapy (Table 5.2). Eight (44%) of the 18 children given HAART received a combination of zidovudine, 3TC and nevirapine while 39% (7/18) received a combination of zidovudine, 3TC and Nelfinavir. Seven of the eight patients on dual therapy received Combivir (zidovudine and 3TC) while

one received zidovudine and zalcitabine. Among these 26 children, median time since infection at initiation of treatment was 15 months (range 3.4 months – 3.0 years) and the median age at treatment initiation was 3.2 years (range 6.8 months-14.2 years). Children treated with HAART had a longer time from infection to initiation of treatment in comparison to those treated with dual therapy, $z=1.83$, $p=0.067$. There were no differences in the age at initiation of treatment between those treated with dual therapy and those receiving HAART, $z=0.61$, $p=0.541$

At the time of treatment initiation, or at the follow-up examination directly preceding initiation of treatment, 11 children (46%) were CDC immunological stage 1 (no immunosuppression), nine (38%) were stage 2 (moderate immunosuppression) and four were stage 3 (severe immunosuppression). Only three children additionally had any evidence of clinical symptoms at or before treatment initiation.

Table 5.2 Treatment and disease progression in parenterally infected children

	Parenterally HIV infected children (n=243)
Ever treated	
No	217 (89%)
Yes	26 (11%)
Type of treatment (n=26)	
Dual therapy	8 (31%)
HAART	18 (69%)
Maximum CDC immunological stage during follow-up	
1	118 (49%)
2	98 (41%)
3	24 (10%)
missing	3
Maximum CDC clinical stage during follow-up	
N	29 (43%)
A	30 (45%)
B	4 (6%)
C	4 (6%)
missing	176

5.3.2.1 Response to treatment

Fourteen treated children (54%) had HIV RNA viral load measurements recorded before and after treatment. Of these 14 children, ten (71%) achieved an UVL between four and 18 months after initiation of treatment. The median HIV RNA viral load at initiation of treatment was 4.76 $\log_{10}$ cp/ml (range 3.04-5.70 $\log_{10}$ cp/ml) and decreased to a median of 1.77 $\log_{10}$ cp/ml four to 18 months after initiation of treatment. The median age at initiation in those who achieved an UVL was lower than in those who did not achieve an

undetectable viral load, 2.9 years (range 1.5 years – 7.2 years) compared to 6.5 years (range 6.8 months – 11.8 years), but not significantly so (Mann Whitney=0.14, p=0.888).

5.3.3 Clinical signs and symptoms of HIV-related disease

Sixty-seven (28%) children had information on CDC clinical category and 29 (43%) and 30 (45%) of these children had no or mild clinical symptoms respectively throughout follow-up. Only eight (12%) children progressed to moderate or severe clinical symptoms during follow-up (Table 5.2) (Figure 5.2). Kaplan Meier survival analysis estimated that 2% of children will have progressed to moderate or severe AIDS defining symptoms 12 months after infection and 3% 36 months after infection (Figure 5.3).

Figure 5.2 Centres for Disease Control immunological and clinical status of parenterally HIV infected children

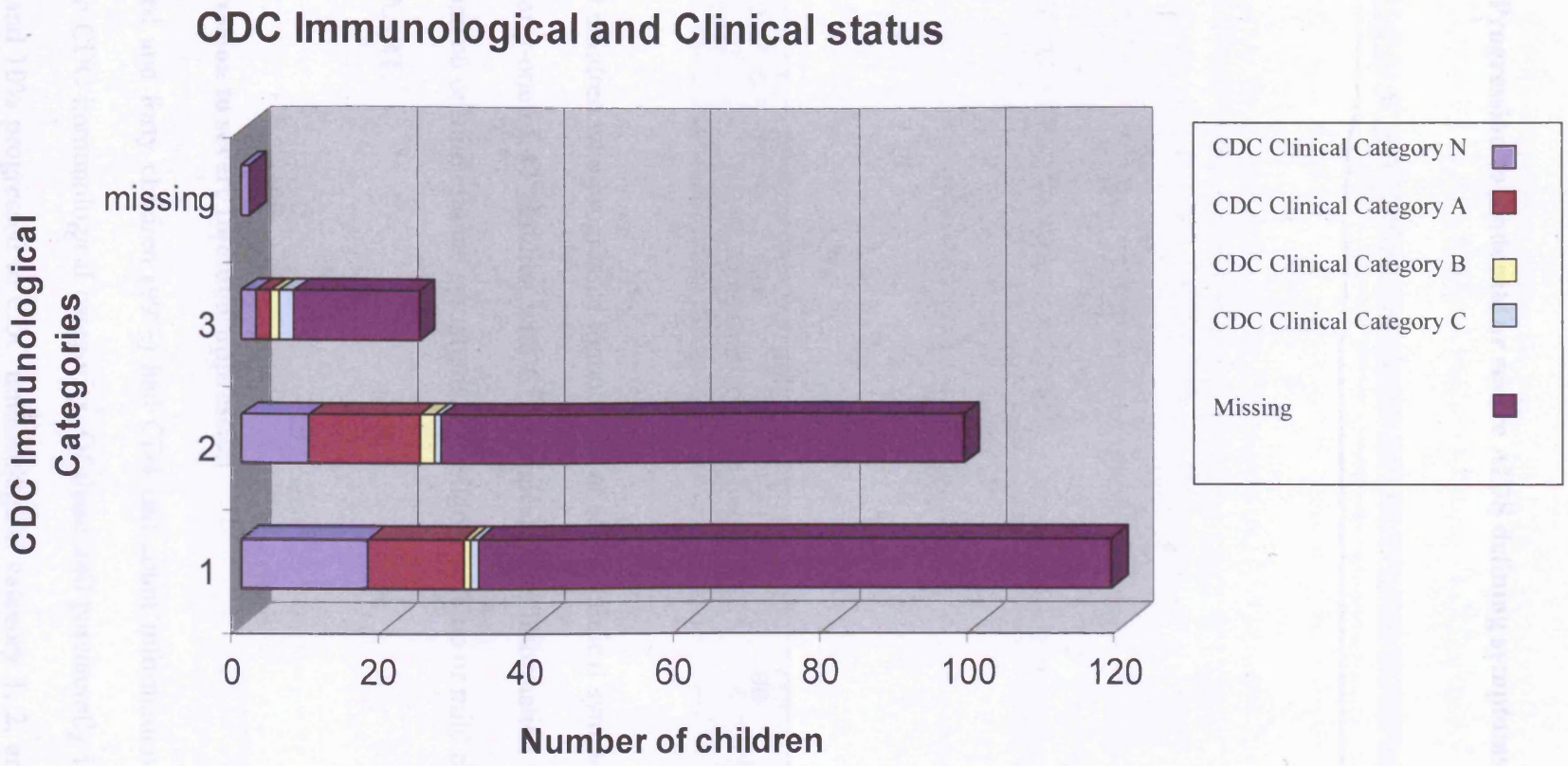
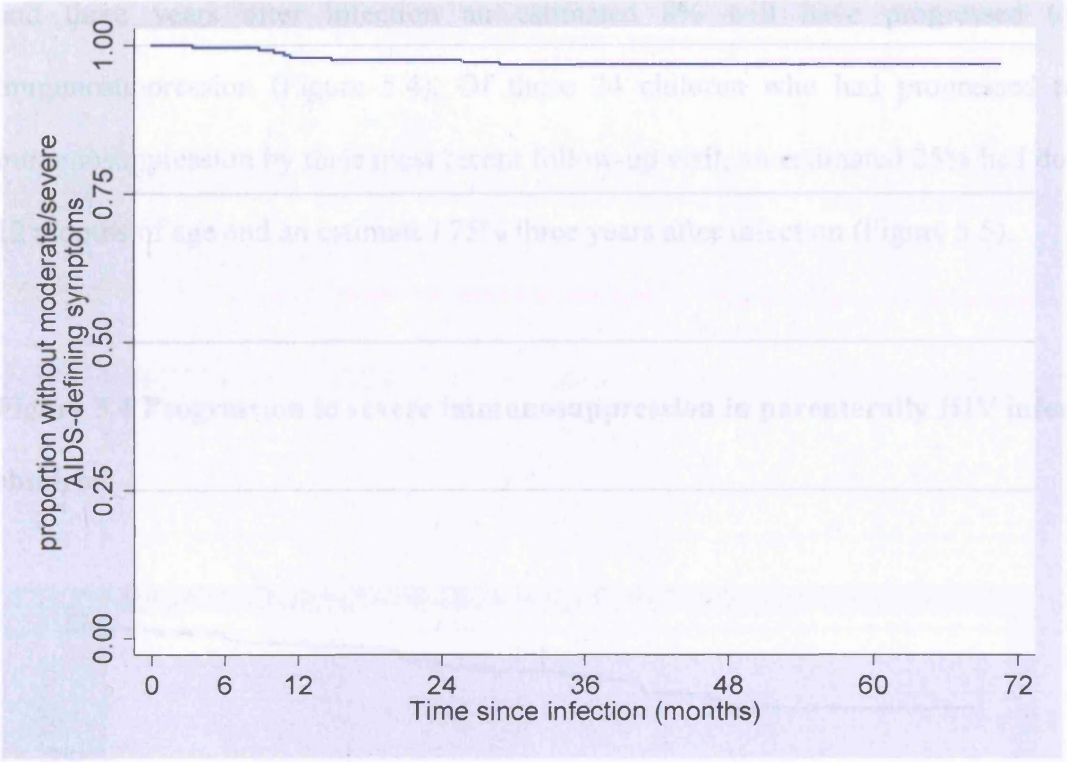


Figure 5.3 Progression to moderate or severe AIDS defining symptoms



Of the eight children who progressed to moderate or severe clinical symptoms, six received HAART. Forty-one of 43 children with CDC clinical stage information who received no treatment, had no or mild clinical symptoms. 11 children with no or mild clinical symptoms received HAART.

5.3.4 Progression to severe immunosuppression

Two hundred and forty children (99%) had CD4 cell count information which could be converted to CDC immunological categories. Of these 240 parenterally infected children, 49%, 41% and 10% progressed to CDC immunological category 1, 2, and 3 respectively

(Table 5.2) (Figure 5.2). Twelve months after infection, an estimated 2% of children will have progressed to severe immunosuppression (defined as CDC immunological category 3) and three years after infection an estimated 8% will have progressed to severe immunosuppression (Figure 5.4). Of those 24 children who had progressed to severe immunosuppression by their most recent follow-up visit, an estimated 25% had done so by 12 months of age and an estimated 75% three years after infection (Figure 5.5).

Figure 5.4 Progression to severe immunosuppression in parenterally HIV infected children

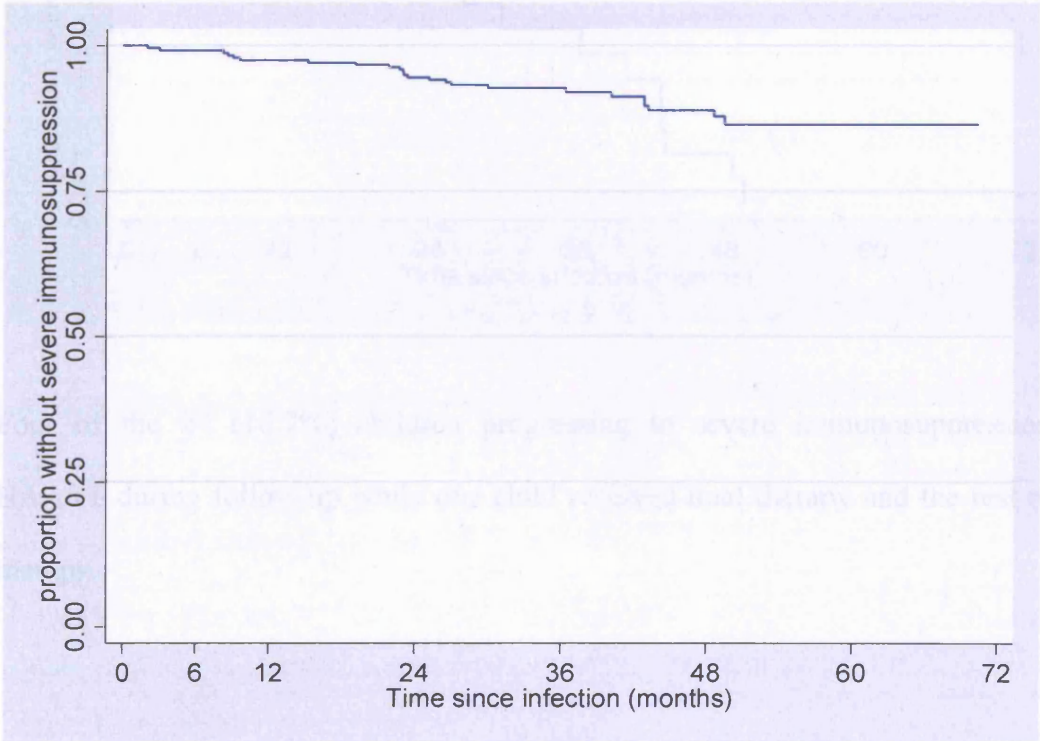
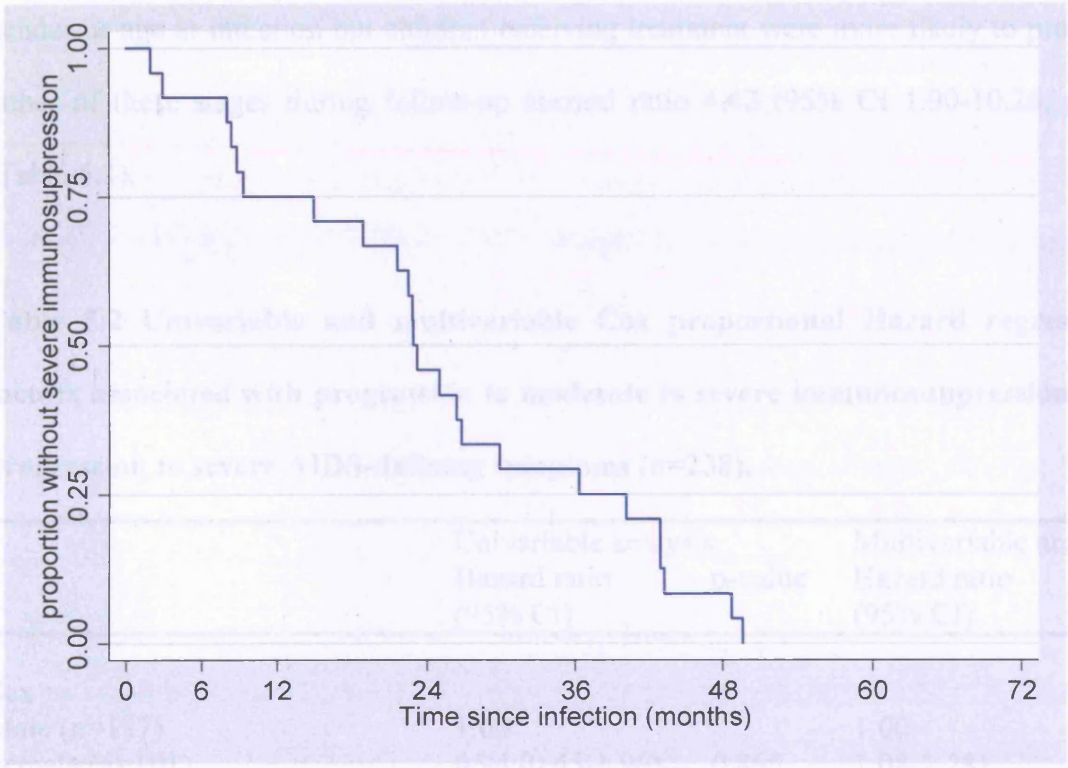


Figure 5.5 Time to severe immunosupression in parenterally HIV infected children progressing to this stage by their most recent follow-up visit



Four of the 24 (16.7%) children progressing to severe immunosuppression received HAART during follow-up while one child received dual therapy and the rest received no therapy.

5.3.5 Factors associated with progression to severe immunosuppression or moderate or severe AIDS-defining symptoms

In multivariable Cox proportional hazard analysis, progression to either moderate or severe immunosuppression or severe AIDS-defining symptoms (or both) was not associated with gender or age at infection but children receiving treatment were more likely to progress to either of these stages during follow-up Hazard ratio 4.42 (95% CI 1.90-10.26, p=0.001 (Table 5.3).

Table 5.2 Univariable and multivariable Cox proportional Hazard regression of factors associated with progression to moderate to severe immunosuppression and/or progression to severe AIDS-defining symptoms (n=238).

	Univariable analysis		Multivariable analysis*	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Sex				
Male (n=137)	1.00		1.00	
Female (n=101)	0.94 (0.45-1.96)	0.865	1.08-2.28)	0.842
Age at infection				
Younger than 22 months (n=111)	1.00		1.00	
Older than 22 months (n=127)	0.54 (0.25-1.14)	0.107	0.57 (0.27-1.22)	0.151
Received antiretroviral therapy				
No (n=212)	1.00		1.00	
Yes (n=26)	4.63 (2.00-10.69)	<0.001	4.42 (1.90-10.26)	0.001

5.4 Key Points

- The mean viral load in parenterally HIV-only infected children after adjustment for baseline CDC immunological category, age at infection (before or after the median of 2 months), sex and whether children received treatment or not, was 4.76 $\log_{10}$ cp/ml within 12 months of infection. The mean HIV RNA viral load significantly decreased in subsequent years following infection and began to level out between two and three years after infection.

- Twenty-six of 243 (11%) children received ART; 18 received HAART and eight dual therapy. ART was initiated at a median of 15 months after HIV infection and at a median age of 3.2 years.

- Ten of fourteen children (79%) who were treated had an undetectable HIV RNA viral load 4 to 18 months after treatment initiation. The mean decrease in viral load four to 18 months after infection was 3.04 $\log_{10}$ cp/ml and more children treated with HAART achieved an undetectable viral load than those treated with dual therapy.

- During a maximum of 4.9 years of follow-up, 88% (59/67) of parenterally HIV-only infected children had no or mild clinical symptoms with 12% (8/67) having moderate or severe clinical symptoms.

- An estimated 2% of parenterally infected children will have progressed to moderate or severe clinical symptoms 12 months after infection and an estimated 3% will have progressed to moderate or severe clinical symptoms 36 months after infection.

- During follow-up, 49% of children had no immunosuppression, 41% had moderate immunosuppression and 10% had severe immunosuppression.

- Twelve months after infection, an estimated 2% of children will have progressed to severe immunosuppression and 36 months after infection an estimated 8% will have progressed to severe immunosuppression.

- Of the 24 children progressing to severe immunosuppression, an estimated 25% did so within 12 months of infection and an estimated 75% within 36 months of infection.

- There was no association between gender or age at infection in those children progressing to moderate or severe AIDS-defining symptoms and/or severe immunosuppression during follow-up.

- Children who received treatment during follow-up were much more likely to have progressed to moderate or severe AID-defining symptoms and/or severe immunosuppression, likely reflecting confounding by indication and the initiation of treatment in children with worse disease progression.

6. HIV AND HCV COINFECTION: BIOLOGICAL MARKERS IN PARENTERALLY AND VERTICALLY INFECTED CHILDREN

6.1 Introduction

HIV/HCV coinfection and its effect on disease progression has become a more prominent issue in recent years due to the longer life-expectancy of HIV infected individuals and the subsequent increase in the effects of comorbidities. The extent to which HIV/HCV coinfection and HIV treatment impact on morbidity and mortality in children remains unknown and optimum clinical management and treatment for this group consequently remains uncertain (England *et al* 2006). To inform the management of coinfected children it is necessary that as much evidence on the natural and treated history of coinfection is gathered and to achieve this research in all areas of paediatric HIV/HCV coinfection must be prioritised.

It is unclear whether HIV or HCV disease progression in coinfected children differs by mode of acquisition and whether vertical infection during early immune development may lead to more severe or more rapid disease progression. If there are substantial differences in disease progression by mode of acquisition, evidence-based management of coinfected children should be tailored accordingly.

Using databases from three established and ongoing prospective cohort studies this chapter compares immunological, virological and clinical profiles of HIV/HCV coinfected children

to describe and identify predictors of disease progression and determine whether there are differences by mode of acquisition.

6.2 Methods specific to this chapter

Children vertically coinfected with HIV and HCV were identified from the European Collaborative Study (ECS) and the European Paediatric HCV Network (EPHN) (ECS 2002, EPHN 2005a). Parenterally HIV/HCV coinfected children were identified from the Libyan Follow-up Study (Yerly *et al* 2001). Date of infection in vertically infected children was assumed to be equivalent to date of birth. Given the evidence on timing of infection in mother-to-child transmission (See section 1.6) it is possible that infection occurred before birth but repeated PCRs in the first few days of life were unavailable and so it was not possible to formally allocate children into timing of infection groups. The date of HIV infection in parenterally infected children from the Libyan Cohort Follow-up Study was determined using the midpoint between 1st January 1998 (the first possible date of infection) and the date of their first positive HIV test or, if this was unavailable, the midpoint (1st January 1999) of the period that infection was known to have occurred – January 1998 to January 1999. It is feasible that HIV and HCV infection occurred at different times in those children who had multiple exposures, however, as the window of possible infection is relatively short (approximately two years) assuming that both infections occurred concurrently shouldn't lead to any substantial over- or under-estimates with regards timing of infection.

6.2.1 Statistical Analyses

ALT levels were converted to standard deviation (SD) z-scores to account for variations over age. This involved calculating ALT age-related standards using penalized maximum likelihood and LMS software, with HCV and HIV uninfected children from the EPHN as the reference group (Bunders *et al* 2005, Cole and Green 1992). ALT measurements from the HIV/HCV coinfected children were then compared to these standards to give age-standardised z-scores measuring the SD from the reference population. ALT z-scores above and below zero respectively indicate ALT levels above or below the reference median ALT level for that specific age. Elevated ALT levels were defined as greater than 2 SDs above the reference population for each age, corresponding to a value above the 95th centile of the standard population.

Similar methods were used to calculate age and sex adjusted CD4 cell count SD z-scores for HIV/HCV coinfected children in comparison to a standard population of HIV and HCV uninfected children from the ECS [Bunders *et al* 2005].

Comparisons between parenterally and vertically coinfected groups were carried out using Mann Whitney rank sum, Chi squared or Student t-tests as appropriate. Univariable and multivariable linear regression with a random effects estimator on the intercept determined the factors associated with ALT z-scores and CD4 count z-scores, accounting for within-child repeated measures.

Kaplan Meier survival analysis was used to estimate the proportion of parenterally and vertically coinfected children progressing to moderate or severe AIDS defining symptoms or immunosuppression over time since infection, with censoring occurring at the date a child first progresses to either CDC clinical stage B or C or CDC immunological stage 2 or 3. If progression to this stage did not occur then a child is censored at the date of their most recent follow-up visit. In addition, a further analysis was carried out among the sub-group of children who progressed to CDC clinical stage B or C or CDC immunological stage 2 or 3, to estimate the time taken for progression to occur from infection, again using Kaplan Meier survival analysis. Statistical comparisons between survival times in different groups were made using log-rank tests (Bland and Altman 1998).

Where indicated, follow-up data were limited to within 6.4 years of infection as this was the maximum duration of infection in parenterally coinfected children. Restricting the analysis in this way made the parenterally and vertically coinfected groups more equal in terms of the length of follow-up.

6.3 Results

Data on 160 parenterally and 32 vertically HIV/HCV coinfected children were analysed. Vertically coinfected children were infected between 1986 and 2001 with follow-up between 1986 and 2005. Parenterally coinfected children were all infected during 1998 and 1999 and follow-up occurred between 1999 and 2004. Significantly more vertically infected than parenterally infected children were male; vertically infected children had significantly more follow-up visits and a significantly longer duration of follow-up (Table

6.1). Significantly more vertically infected than parenterally infected children received antiretroviral treatment for HIV disease during follow-up and significantly more vertically than parenterally infected children were given HAART compared to dual therapy (Table 6.1).

Table 6.1 Summary of follow-up and HIV treatment in parenterally and vertically HIV/HCV coinfected children

	Parenteral coinfected (n=160)	**Vertical coinfected** (n=32)	**Comparison** (χ^2 or Ranksum)
Sex			
Male	97 (62.2%)	11 (39.3%)	χ^2=5.13, p=0.023
Female	59 (37.8%)	17 (60.7%)	
Missing	4	4	
Median age at infection	1.3 years (range 1month-15.9yrs)	At birth	N/A
Median age at start of follow-up	3.2 years (range 1month-17.3 yrs)	Follow-up started at birth	N/A
Median follow-up visits	4 visits (range 1-8)	11 visits (range 1-133)	z=-6.251, p<0.001
Median duration of follow-up	1.8 years (range 1 month -4.9 yrs)	4.6 years (range 1 month-19.0 yrs)	z=-3.434, p<0.001
ART during follow-up			
Not treated	127 (79.4%)	20 (62.5%)	$\chi^{2=}$4.232, p=0.040
Treated	33 (20.6%)	12 (37.5%)	
Type of ART			
Dual therapy	13 (39.4%)	1 (8.3%)	$\chi^{2=}$3.961, p=0.047
HAART	20 (60.6%)	11 (91.7%)	
Median age at initiation of ART	35.2 months (range 14.2 months-14.8 yrs)	54.2 months (range 7.5 months-14.3 yrs)	z=-1.360, p=0.174
Median duration of ART at last follow-up	1.3 yrs (1 month-5.0 years)	8.1 yrs (5.2 months-14.4 yrs)	z=-3.44, p<0.001

6.3.4 Treatment

No child received HCV treatment during follow-up. Of 45 (23%) coinfected children receiving HIV treatment, significantly more vertically than parenterally coinfected children were treated and most received HAART (Table 6.1). Eight (26%) children treated with HAART received a combination of Nelfinavir, Zidovudine and 3TC, Seven (23%) received a combination of Nevirapine, Zidovudine and 3TC, three (10%) received Nevirapine, Nelfinavir and D4T and a further three (10%) received Efavirenz, Tenofovir and 3TC. All children being treated with dual therapy received Zidovudine and 3TC.

HIV RNA viral load was available both before and six to 12 months after treatment initiation for ten of the 45 treated children, of whom seven were parenterally coinfected and three were vertically coinfected. Nine children were treated with HAART and one with dual therapy. At initiation, median HIV RNA viral load was 4.32 $\log_{10}$ copies/ml (range 1.69-6.32 $\log_{10}$ copies/ml) and six to 12 months after initiation, median HIV RNA viral load was 4.30 $\log_{10}$ copies/ml (range 1.69-5.43 $\log_{10}$ copies/ml). Six children showed a good virological response to treatment with a median decrease in HIV RNA viral load of 1.88 $\log_{10}$ copies/ml (range 0.28-3.56 $\log_{10}$ copies/ml). The median age at initiation of treatment was higher in those children who exhibited an increase in viral load in response to treatment compared to those who exhibited a decrease in viral load, median age at initiation 9.5 years (range 3.0 years – 14.3 years) compared to 3.6 years (range 2.0 years – 4.8 years), p=0.88.

6.3.1 ALT levels

Five hundred and twenty-eight ALT measurements were available from 156 parenterally coinfected children and 230 from 21 vertically coinfected children (Table 6.2) The 11 vertically coinfected children with no ALT measurements during follow-up were enrolled in the studies earlier with dates of birth ranging from 1987 to 1994 while those 21 who did have ALT measurements were born between 1986 and 2001. This suggests a change in the management of these children to include more ALT testing. The median measurements per child were three (range 1-7) and six (range 1-116) in the parenterally and vertically coinfected groups respectively. Mean ALT z-score was significantly higher in vertically than parenterally coinfected children, but when the analysis was restricted to measurements taken within 6.4 years of infection the difference was not statistically significant (Table 6.2), implying that this difference was a result of ALT levels collected beyond 6.4 years of infection where no comparable values from parenterally coinfected children were available. No significant differences were found in the proportion of parenterally and vertically infected children with either one or more elevated ALT levels or all elevated ALT levels (Table 6.2).

Table 6.2 ALT levels in parenterally and vertically HIV/HCV coinfected children.

	Parenteral acquisition (n=160)	**Vertical acquisition** (n=32) [comparison with parenteral group]	**Vertical acquisition limited to 6.4 yrs duration of infection** (n=32) [comparison with parenteral group]
Median ALT level (UI/L) (n= 758 obs)	38 (range 10-285)	49 (range 17-218) [z=-1.910, p=0.056]	52 (range 20-144) [z=-1.489, p=0.137]
Mean ALT z-score (n= 758 obs)	2.30 (SD 1.70)	3.32 (SD 1.76) [t=-2.52, p=0.013]	2.32 (SD 1.09) [t=0.005, p=0.996]
At least 1 elevated ALT (≥2SD above normal)	115 (73.7%)	17 (81.0%) [z=-0.739, p=0.460]	7 (77.8%) [z=-0.272, p=0.242]
All ALTs elevated (≥2SD above normal)	53 (34.0%)	11 (52.4%) [z=-1.647, p=0.100]	3 (14.3%) [z=0.043, p=0.966]

Linear regression analysis included 124 parenterally coinfected children with information available on all variables. These children did not differ from the whole group of parenterally coinfected children with respect to any variable (Table 6.3). There was an increase in ALT z-score of borderline statistical significance among parenterally coinfected children receiving HIV treatment and a significant decrease in ALT z-score with increasing duration of infection (Table 6.4). Both associations remained after adjusting for gender, age at infection, CD4 cell count z-score, HIV RNA viral load and HCV RNA viral load. For example, ALT z-scores were a mean of 0.831 standard deviations higher in treated children in comparison to untreated children. Additionally, in multivariable analysis, ALT z-score significantly increased in parenterally coinfected children with increasing age at infection, increasing HCV RNA viral load and in those who received HIV treatment compared with those who were untreated (Table 6.4). In 21 vertically coinfected children with information on all the variables included in the linear regression model, ALT z-score was significantly increased in children on HIV therapy and ALT z-score was significantly increased with duration of infection. Both associations remained in bivariable analysis (Table 6.4). Univariable analysis also showed a significant decrease in ALT z-score with increased CD4 count z-score in contrast to the parenteral group. There were too few vertically coinfected children with ALT levels recorded to carry out multivariable linear regression.

Table 6.3 Parenterally HIV/HCV coinfected children not included in multivariable analysis

	Parenterally HIV/HCV coinfected children not included in multivariable analysis n=36
Sex	
Male	16 (50%)
Female	16 (50%)
missing	4
Median age at infection	1.8 years (range 1 month-13.7 yrs)
Median duration of infection	10.7 months (1 month-3.8 yrs)
HIV treatment	
No	24 (66.7%)
Yes	12 (33.3%)
Mean CD4 cell count z-score	-0.216 (SD 1.20)
Mean ALT z-score	2.751 (SD 1.42)
Median HIV RNA viral load (log10 cp/ml)	4.03 (range 2.21-5.28)
Median HCV RNA viral load (log10 cp/ml)	5.55 (range 2.48-6.38)

Table 6.4 Factors affecting ALT z-score in parenterally and vertically coinfected children.

	Parenterally coinfected (n=124)		**Vertically coinfected** (n=21)	
	Univariable analysis Coefficient (95% CI), p-value	**Multivariable analysis Coefficient (95% CI), p-value**	**Univariable analysis Coefficient (95% CI), p-value**	**Bivariable analysis Coefficient (95% CI), p-value**
Sex				
Male	0.00	0.00	0.00	
Female	-0.027 (-0.712-0.658), p= 0.938	-0.109 (-0.769-0.542), p=0.747	-1.012 (-2.651-0.626), p=0.226	N/A
HIV treatment				
No	0.00	0.00	0.00	0.00
Yes	0.674 (0.180-1.529), p=0.122	0.831 (-0.021-1.683), p=0.056	2.50 (1.824-3.167), p<0.001	1.226 (0.460-1.992), p=0.002
Duration of infection (per one monthly increase)	-0.028 (-0.047 - -0.009), p=0.004	-0.022 (-0.042 - -0.003),p=0.026	0.019 (0.014-0.023), p<0.001	0.014 (0.009-0.019), p<0.001
Age at infection (per one monthly increase)	0.006 (-0.001-0.012), p=0.098	0.008 (-0.002-0.015), p=0.013	Collinear	N/A
CD4 cell count z-score (per standard deviation increase)	-0.083 (-0.257-0.091), p=0.348	-0.022 (-0.154-0.198), p=0.807	-0.225 (-0.433 - -0.018), p=0.033	N/A
HIV RNA viral load (per log10 increase)	0.078 (-0.093-0.248), p=0.372	0.069 (-0.110-0.249), p=0.449	0.072 (-0.656-0.799), p=0.847	N/A
HCV RNA viral load (per log10 increase)	0.256 (0.111-0.401), p=0.001	0.242 (0.096-0.387), p=0.001	0.270 (-0.116-0.656), p=0.171	N/A

6.3.2 CD4 cell count

Nine hundred and six CD4 cell counts from 158 parenterally and 15 vertically coinfected children were analysed. CD4 cell count SD z-scores were similar in both groups but lower than in the uninfected but HIV-exposed standard comparison group used to calculate the z-scores (See section 2.5) (Table 6.5). The median lowest recorded CD4 cell count during follow-up was significantly lower in vertically compared with parenterally coinfected children and this significant difference remained when limiting duration of infection to 6.4 years (Table 6.5). Median age at lowest CD4 cell count was 4.3 years (range 5.9 months–18 years) after a median time since infection of 2.6 years in the parenterally coinfected children compared to a median age at lowest CD4 cell count of 7.3 years (range 1.1 years-15.4 years) in vertically coinfected children.

Univariable and multivariable linear regression analyses were carried out separately for the parenterally and vertically coinfected groups to investigate factors associated with CD4 cell count z-scores. In parenterally coinfected children, CD4 cell count z-score was significantly increased with longer duration of infection, but only univariably (Table 6.6). CD4 cell count z-score decreased significantly with increasing HIV RNA viral load both univariably and multivariably (Table 6.6). In vertically coinfected children mean CD4 cell count z-score was significantly lower in children receiving HIV treatment than in those untreated and this association was strengthened by adjustment for duration of infection in bivariable analysis such that there was a mean decrease in CD4 z-score of 1.54 standard deviations in treated children compared with untreated children (Table 6.6). In bivariable analysis, there was a significant increase in CD4 z-score with increasing duration of infection but the

change in mean CD4 z-score was minimal despite the association being statistically significant (Table 6.6). There were too few vertically coinfected children with CD4 counts information to allow multivariable linear regression on this group.

Table 6.5 CD4 cell counts in parenterally and vertically HIV/HCV coinfected children (n=173)

	Parenteral acquisition (n=158)	**Vertical acquisition** (n=15) [comparison with parenteral group*]	**Vertical acquisition limited to 6.4 yrs duration of infection** (n=15) [comparison with parenteral group*]
Median CD4 cell count (cells/mm^3) (n=906 observations)	1018 (range 150-9423)	787 (range 260-3495) [z=1.478, p=0.139]	1060 (range 480-3495) [z=-0.239, p=0.811]
Mean CD4 cell count z-score (n=889 observations[†])	-0.570 (SD 1.285)	-0.607 (SD 1.188) [t=-0.104, p=0.917]	-0.943 (SD 1.281) [t=0.608, p=0.544]
Median nadir CD4 cell count (cells/mm^3) (n=173 children)	729 (range 62-2284)	368 (range 30-1850) [z=2.913, p=0.004]	368 (range 30-1850) [z=2.9139, p=0.004]

*Mann Whitney rank sum comparison of medians or Student t-test comparison of means

[†] CD4 cell count z-scores not calculated for those children with missing gender

Table 6.6 Factors affecting CD4 cell count z-score in parenterally and vertically coinfected children.

	Parenterally coinfected (n=124)		**Vertically coinfected** (n=15)	
	Univariable analysis Coefficient (95% CI), p-value	**Multivariable analysis* Coefficient (95% CI), p-value**	**Univariable analysis Coefficient (95% CI), p-value**	**Bivariable Coefficient (95% CI), p-value**
Sex				
Male	0.00	0.00	0.00	
Female	-0.134 (-0.636-0.367), p=0.600	-0.046 (-0.468-0.560), p=0.861	1.307 (0.189-2.426), p=0.022	N/A
HIV treatment				
No	0.00	0.00	0.00	0.00
Yes	-0.060 (-0.574-0.694), p=0.853	-0.362 (-1.015-0.292), p=0.278	-0.984 (-1.520- -0.447), p<0.001	-1.536 (-2.191- -0.881),p<0.001
Duration of infection (per one monthly increase)	0.020 (0.006-0.034), p=0.004	0.011 (-0.002-0.025), p=0.092	0.0002 (-0.004-0.004), p=0.905	0.006 (-0.002- -0.011), p=0.005
Age at infection (per one monthly increase)	-0.003 (-0.007-0.002), p= 0.307	-0.005 (-0.010-0.001), p=0.078	Collinear	N/A
ALT z-score (per one standard deviation increase)	-0.045 (-0.137-0.047), p=0.336	-0.004 (-0.084-0.092), p=0.934	-0.434 (-0.777 - -0.090), p=0.013	N/A
HIV RNA viral load (per log10 increase)	-0.338 (-0.451—0.224), p<0.001	-0.329 (-0.446—0.212), p<0.001	-0.131 (-0.342-0.080), p=0.225	N/A
HCV RNA viral load (per log10 increase)	-0.100 (-0.207-0.007), p=0.068	-0.073 (-0.176-0.030), p=0.863	0.001 (-0.053-0.056), p=0.446	N/A

*adjusting for all other variables

6.3.3 HIV and HCV RNA viral load

HIV RNA viral load was recorded 529 times from 142 parenterally and 196 times from 14 vertically coinfected children. Although there was no significant difference in median HIV RNA viral load by mode of acquisition (Table 6.7), in a sub-analysis of measurements taken within 6.4 years of infection, median HIV RNA viral load was higher in vertically than parenterally coinfected children, although not significantly so (Table 6.7). HCV RNA viral load was recorded 290 times from 139 parenterally and 50 times from 17 vertically coinfected children and was non-significantly higher in vertically than parenterally coinfected children (Table 6.7).

Table 6.7 HIV RNA and HCV RNA viral loads in parenterally and vertically HIV/HCV coinfected children (n=156)

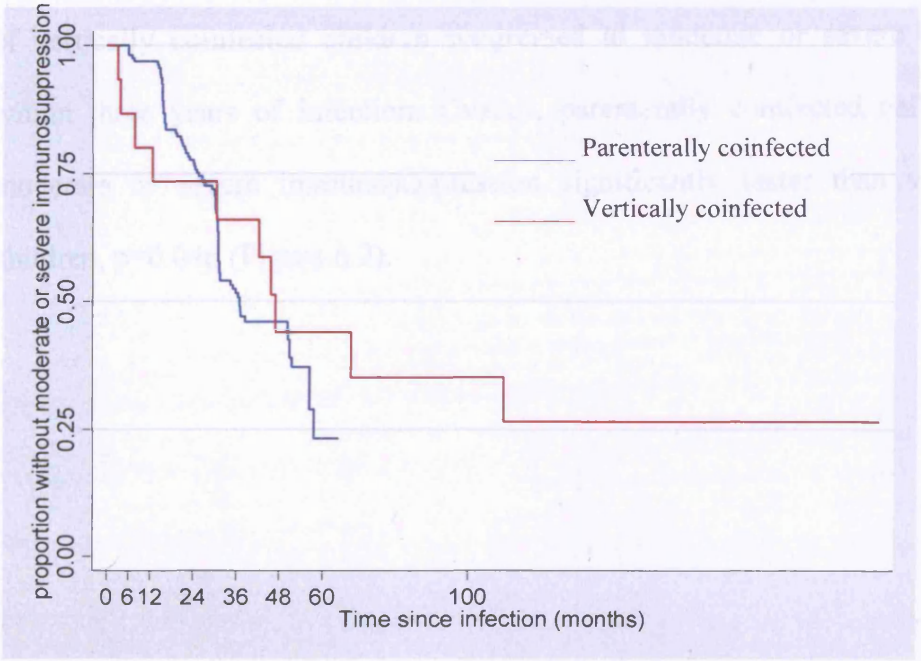
	Parenteral acquisition (n=142)	**Vertical acquisition** (n=14) [comparison with parenteral group]	**Vertical acquisition limited to 6.4 yrs duration of infection** (n=14) [comparison with parenteral group]
Baseline HIV RNA viral load (log10 cp/ml) (n=142)	4.48 (1.40-6.30)	4.52 (range 1.90-5.74)	4.52 (range 1.90-5.74)
Median HIV RNA viral load (log10 cp/ml) (n=725 obs)	3.79 (range 1.40-5.87)	3.46 (1.70-5.54) [z=0.465, p=0.642]	4.62 (1.93-5.54) [z=-1.390, p=0.165]
Baseline HCV RNA viral load (log10 cp/ml) (n=14)	5.40 (0.00-7.92)	5.46 (2.30-7.24)	5.46 (2.30-7.24)
Median HCV RNA viral load (log10 cp/ml) (n=340 obs)	5.35 (range 0.00-7.92)	5.76 (2.30-7.25) [z=-0.603, p=0.549]	Too few observations

6.3.5 Progression to moderate or severe immunosuppression

6.3.5.1 Proportion progressing to moderate or severe immunosuppression

CDC immunological stage was available for 172 children, 157 parenterally and 15 vertically coinfected. Ninety six children, 86 parenterally and 10 vertically coinfected, progressed to moderate or severe immunosuppression during follow-up (CDC immunological stage 2 or 3). From survival analysis, one year after infection, an estimated 4% of parenterally and 20% of vertically coinfected children will have progressed to moderate or severe immunosuppression, increasing to an estimated 48% and 34% respectively three years after infection (Figure 6.1). The overall difference during follow-up was not statistically significant, p=0.448.

Figure 6.1 Progression to moderate or severe immunosuppression in HIV/HCV coinfected children

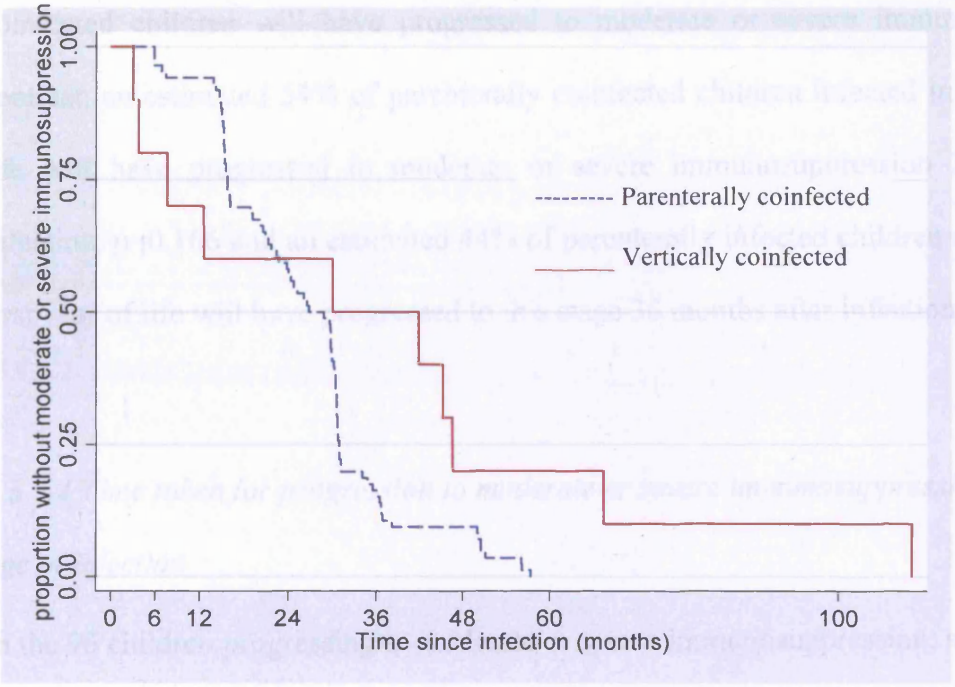


Forty-four of 172 children with immunological stage information were receiving HIV treatment, 32 parenterally and 12 vertically coinfected. A higher proportion of children who received HIV treatment (68%) progressed to moderate or severe immunosuppression during follow-up in comparison to those who did not receive treatment (60%), p=0.090, likely reflecting confounding by indication. Significantly more children treated with dual therapy versus HAART progressed to moderate or severe immunosuppression, p=0.049, with an estimated 40% and 26% of children treated with dual therapy and HAART respectively progressing to moderate or severe immunosuppression 36 months after infection.

6.3.5.2 Time taken for progression to moderate or severe immunosuppression to occur

Time from infection to progression to moderate or severe immunosuppression among the 96 children who progressed, showed that 12 months after infection progression was substantially slower in parenterally coinfected children, an estimated 6% compared with 30% of vertically coinfected children. However, an estimated 84% of parenterally and 50% of vertically coinfected children progressed to moderate or severe immunosuppression within three years of infection. Overall, parenterally coinfected children progressed to moderate or severe immunosuppression significantly faster than vertically coinfected children, p=0.046 (Figure 6.2).

Figure 6.2 Time taken for progression to moderate or severe immunosuppression to occur in parenterally and vertically HIV/HCV coinfected children



In parenterally and vertically coinfected children, there were no significant differences in the time it took children to progress to moderate or severe immunosupression among those receiving HIV treatment and those untreated (p=0.122), or between those receiving HAART and those receiving dual therapy (p=0.223).

6.3.5.3Proportion progressing to moderate or severe immunosuppression – age at infection

Progression to moderate or severe immunosupression in vertically coinfected children was compared with parenterally coinfected children infected before and after age one. Twelve months after infection, an estimated 20% of vertically coinfected children, an estimated 5% of parenterally coinfected children infected in the first year of life and an estimated 29%

infected after the first year of life will have progressed to moderate or severe immunosuppression. Thirty-six months after infection an estimated 34% of vertically coinfected children will have progressed to moderate or severe immunosupression. In contrast, an estimated 54% of parenterally coinfected children infected in the first year of life will have progressed to moderate or severe immunosuppression 36 months after infection, p=0.166 and an estimated 44% of parenterally infected children infected after the first year of life will have progressed to this stage 36 months after infection, p=0.030.

6.3.5.4 Time taken for progression to moderate or severe immunosuppression to occur – age at infection

In the 96 children progressing to moderate or severe immunosuppression, an estimated 87% of parenterally coinfected children infected after the first year of life had progressed 36 months after infection compared with an estimated 80% of those infected within the first year of life, p=0.850. Progression after 36 months was not significantly slower in vertically than parenterally coinfected children infected during the first year of life, p=0.100.

6.3.6 Progression to moderate or severe AIDS-defining symptoms

6.3.6.1 Proportion progressing to moderate or severe AID- defining symptoms

Information on CDC clinical category during follow-up was available for 43 coinfected children. Ten children progressed to moderate or severe AIDS-defining symptoms. An estimated 7% of the 43 children progressed to this stage 12 months after infection, 5% parenterally coinfected and 20% vertically coinfected. Three years after infection, an

estimated 19% of coinfected children with progression information available, will have progressed to moderate or severe AIDS-defining symptoms, 16% parenterally and 40% vertically coinfected, p=0.245. Two children (1 parenterally and 1 vertically) were receiving HIV treatment.

6.3.6.2 Time taken for progression to moderate or severe AIDS-defining symptoms to occur

Of the ten children who progressed to moderate or severe AIDS-defining symptoms, an estimated 30% had done so 12 months after infection and an estimated 80% by 36 months. Overall, there was no difference in the time to progression to moderate or severe AIDS-defining symptoms by mode of acquisition, p=0.582.

6.3.6.3Proportion progressing to moderate or severe AIDS-defining symptoms – age at infection

An estimated 26% of children parenterally coinfected after the first year of life will have progressed to moderate or severe AIDS-defining symptoms 36 months after infection compared to an estimated 15% of children parenterally coinfected before 12 months of age and 40% of vertically coinfected children, with no significant differences between the latter two groups, p=0.684 or the two parenterally coinfected groups, p=0.498. Too few children progressed to moderate or severe AIDS-defining symptoms during follow-up to assess differences in the speed of progression in groups infected at different ages or receiving different treatment.

6.4 Key points

- Vertically and parenterally HIV/HCV coinfected children differed in terms of some key population characteristics including duration of follow-up, proportion receiving ART for HIV disease and the type of ART being given.

- ALT z-score significantly increased with increasing duration of infection in both parenterally and vertically coinfected groups.

- ALT z-score was significantly higher among those children receiving ART for HIV disease in comparison to those untreated in both parenterally and vertically coinfected children.

- In vertically coinfected children, CD4 cell count z-score was significantly lower in those receiving ART than in those untreated. This association was not present in parenterally coinfected children.

- Six out of ten children had a decrease in HIV RNA viral load following initiation of antiretroviral treatment and this was similar in both parenterally and vertically coinfected groups.

- Three children achieved undetectable HIV RNA viral load after treatment; all were parenterally coinfected and treated with HAART.

- No difference was found in the proportions of vertically and parenterally coinfected children progressing to moderate or severe immunosuppression, but among those who did reach this stage, parenterally coinfected children did so faster.

- Few children progressed to moderate or severe AIDS-defining symptoms during follow-up and no differences were found in the proportions of vertically and parenterally coinfected children doing so.

7. POLICIES AND PRACTICES FOR THE CLINICAL MANAGEMENT OF CHIDREN DIAGNOSED WITH BOTH HIV AND HCV IN EUROPE

7.1 Introduction

To date no guidelines exist as to the most appropriate clinical management of children coinfected with HIV and HCV. The current guidelines from the British HIV Association and the Center for Disease Control on HIV/HCV coinfection in adults suggest that given the shared transmission route of both infections and the implications of coinfection on treatment in comparison to HIV infection alone, it is necessary to screen all HIV infected individuals for HCV infection (Nelson *et al* 2005, CDC 2004). Once coinfection has been established, the adult guidelines from BHIVA recommend carrying out a variety of liver function tests in addition to HCV genotyping and HCV RNA viral load testing. Liver biopsy can be used to asses the extent or the presence of any liver damage but uncertainty remains as to whether the benefits of this invasive procedure outweigh the costs, specifically in more vulnerable groups (Nelson *et al* 2005).

From a treatment perspective the current guidelines for HIV/HCV coinfection in adults vary depending on the stage at which HIV and or HCV is diagnosed. If patients are identified before HIV therapy is indicated or when CD4 cell count is greater than 500 cells/ml, then HCV treatment should be considered in order that a better response to HIV therapy might be elicited once necessary (as HCV treatment is a one-off six-month course it is anticipated that the infection could be eliminated before HIV progression indicated treatment) (Nelson *et al* 2005). If coinfection is diagnosed at a point where the patient

requires, or is currently receiving, HIV therapy or CD4 cell count is less than 500 cells/ml, then this treatment should be optimised in terms of increasing CD4 cell count before HCV treatment is considered (Nelson *et al* 2005, CDC 2004). Adding to the complexity of treatment in HIV/HCV coinfected individuals is the evidence that HAART is associated with a reduced likelihood of liver fibrosis and related disease progression in coinfected adults, implying that delaying treatment in coinfected patients could be detrimental in terms of both HIV and HCV disease progression (Quirishi *et al* 2003). The treatment of HIV/HCV coinfected children is made more complex still by the limited number of HIV drugs available for use in paediatric treatment, making it even harder to find paediatric ART that does not exhibit hepatotoxic properties (England *et al* 2006). The extent to which these issues identified as important in adult populations are relevant to HIV/HCV coinfected children remains unknown and thus no consensus on the treatment or management of coinfected children exists.

7.2 Methods specific to this chapter

A semi-structured questionnaire survey was developed to ascertain details about current European management and treatment of HIV/HCV coinfected children in the context of policies and practices for the treatment and management of HIV or HCV-only infected children. The survey was piloted on two clinicians who were asked to complete the questionnaire and provide feedback on ease of completion, content validity and whether any areas of specific clinical interest had been overlooked. The survey comprised the following sections; Background information, Policy for management of HIV/HCV coinfected children, Management of HIV/HCV coinfected children, Treatment of HIV-only

infected children, Treatment of HCV-only infected children and Treatment of HIV/HCV coinfected children (Appendix 8). The survey sections included a combination of closed and open ended questions in an attempt to gain as much information as possible about each area. Following revisions based on the pilot feedback, the survey was distributed in July 2006 via email to clinicians from eight European countries participating in either the European Collaborative Study of HIV-infected pregnant women and their children and/or the European Paediatric HCV Network of children born to HCV-infected mothers (ECS 2001, EPHN 2005a). Reminders were emailed to clinicians who had not responded four weeks after the original survey was circulated, together with another copy of the questionnaire. Clinicians were asked to respond even if they had no experience of treatment and or management of HIV/HCV coinfected children. As the number of HIV/HCV coinfected children seen by each clinician was assumed to be small, questionnaires were sent to all centres in the ECS and EPHN studies regardless of whether they were known to manage coinfected children in an effort to maximise the information on coinfected children collected. As a result of this more unselective distribution of questionnaires, only 16 responses were received from a total of 38 questionnaires distributed but it is likely that the majority of non-responders had no experience of managing HIV/HCV coinfected children and therefore deemed the questionnaire irrelevant.

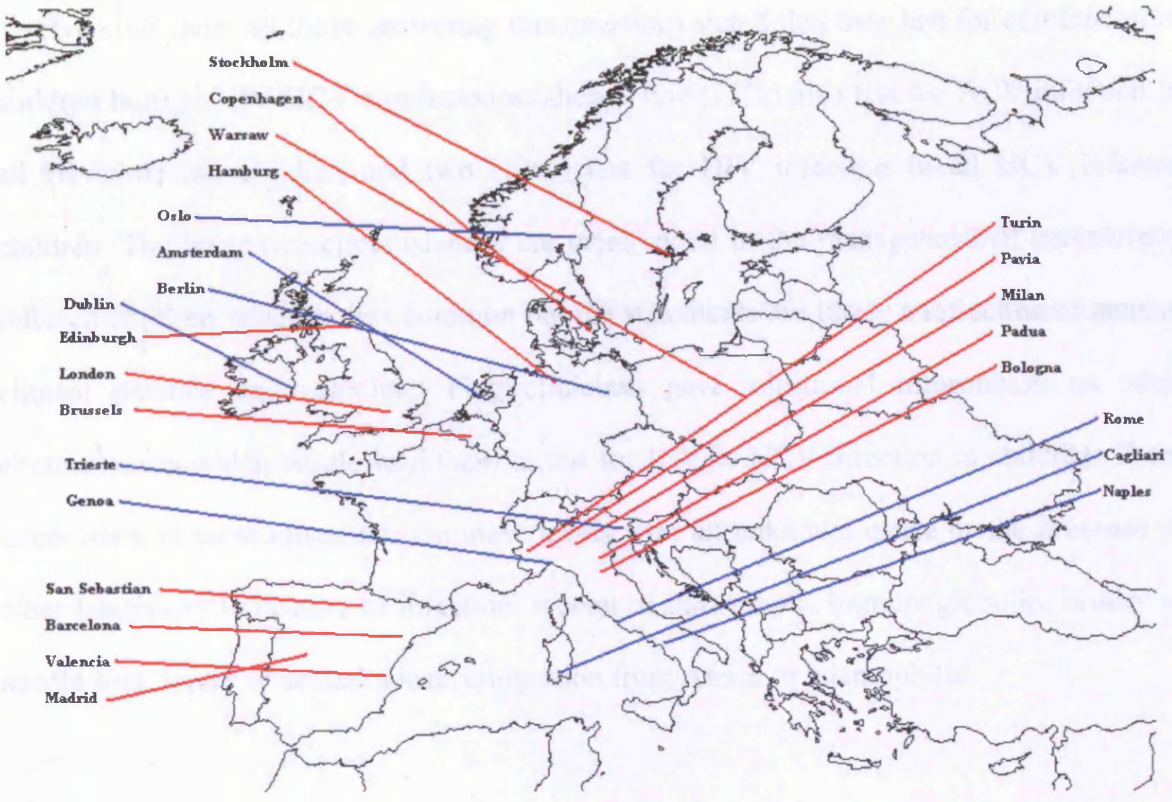
7.2.1 Data analysis

For the purposes of describing the results of this survey, the denominator will be the number of clinicians who completed any given question and this denominator may therefore differ for different questions.

7.3 Results

Survey responses were received from clinicians from 16 clinical centres in Italy, Spain, Sweden, UK, Belgium, Denmark, Poland and Germany (Figure 7.1). These clinicians were currently caring for a total of 44 HIV/HCV coinfected children (Appendix 4). Four centres (Bologna, Pavia, Edinburgh and London) were not currently following any coinfected children and had no experience of doing so in the past. Therefore, clinicians from these four centres did not complete the majority of the questionnaire.

Figure 7.1 Centres surveyed on the management and treatment of HIV/HCV coinfected children



7.3.1 Background information

Forty-four HIV/HCV coinfected children were currently being managed by the 16 clinicians contributing information to this survey. These 44 children ranged in age from three to 25 years old (the older patients having been managed by these clinicians since they were children) and 42 of the children were vertically infected while two were parenterally

infected. All the children were being followed up at university hospitals or tertiary referral centres and the respondents were all consultant paediatricians.

Twelve clinicians (all those answering this question) stated that they test for coinfection in children born to HIV/HCV coinfected mothers. Nine (75%) also test for HCV infection in all HIV infected children and two (17%) test for HIV infection in all HCV infected children. The latter two circumstances are more suited to the management of parenterally infected children which is less common but the statements are likely a reflection of general clinical practice and principle. Five clinicians gave additional information on other circumstances which would lead them to test for HIV or HCV infection in children. These circumstances were raised transaminase levels with an unknown cause or the presence of other laboratory indicators of infection, receipt of intravenous immunoglobulin, history of needle stick injury or sexual abuse, emigration from Africa or haemophilia.

7.3.2 Written policies for the management and treatment of HIV/HCV coinfected children

Only one centre (in Italy) had a written policy for the management and treatment of HIV/HCV coinfected children although one other centre (in Spain) was in the process of developing such a document. In most other centres who completed this section of the questionnaire, (8/11, 72%), decisions regarding the care of coinfected children were taken at the hospital or departmental levels with only three out of 11 centres relying on regional or national decision-making, although without the aid of written guidelines it is unclear

how decision-making on a regional or national level was communicated to specific clinical centres.

In five centres, the schedule for the management of HIV/HCV coinfected children was more frequent in children who were being treated for either HIV or HCV than those not treated. Two centres did not change their management schedule if children were being treated. One centre altered the management of coinfected children based on their age; specifically, HCV treatment would be delayed in younger coinfected children, but seven centres said that their management schedule was unaffected by the age of the child, the duration of infection or the mode of acquisition of infection.

The one centre with a written policy for the management of HIV/HCV coinfected children recommended a schedule of laboratory testing which included HCV RNA PCR testing every three months, IgA, IgM and IgG testing and hepatic ultrasound every six months and testing for cryoglobulins and a wide range of autoantibodies annually. This policy was written in the context of extra monitoring requirements for HIV infected children with HCV infection and therefore details of the HIV monitoring itself were not provided.

7.3.3 Laboratory testing

In addition to standard laboratory tests for monitoring HIV disease carried out in accordance with current guidelines (Helbert and Breuer 2000) 50% of 12 centres also performed HCV RNA PCR tests at least annually and 50% of 12 centres carried out ALT and AST tests at least every six months in coinfected children. Liver biopsy was rarely

performed unless HCV treatment was due to begin but liver ultrasound was performed on coinfected children in five out of 12 centres at least annually. Ten of eleven centres (91%) performed HCV genotyping on all HIV/HCV coinfected children and seven of nine centres (89%) monitored for evidence of hepatotoxicity in coinfected children receiving ART for HIV disease. This monitoring for hepatotoxicity predominantly took the form of ALT and AST testing approximately every three months.

7.3.4 Treatment of HIV/HCV coinfected children

Four centres (Milan, Warsaw, Madrid and Turin) followed a combination of CDC (CDC 1994), PENTA (Sharland *et al* 2004) and National guidelines for the treatment of HIV only infected children. Two centres (Padua and Barcelona) followed a combination of CDC and PENTA guidelines and four centres (Valencia, Brussels, Stockholm and Hamburg) relied upon National guidelines only. One centre (Copenhagen) adhered to PENTA guidelines only. Five centres had experience of treating HCV only infected children, of which four had never done so outside the context of a treatment trial.

Only one centre had ever treated coinfected children for HIV and HCV infections concurrently but four of seven other centres stated that they would consider treating both infections at the same time if a child met the criteria for both treatment of HIV and HCV at the same point in time. In the situation of an HIV/HCV coinfection diagnosis later in childhood, three out of seven centres responding stated that they would preferably treat HCV infection before initiating HIV therapy as suggested in the adult guidelines.

7.5 Key Points

- This survey represents a unique investigation of European clinical management practices for the care of HIV/HCV coinfected children by surveying clinicians actively involved in two of the largest paediatric HIV and HCV cohorts in Europe.

- In the absence of guidelines for the clinical management of children coinfected with HIV and HCV, practices in the European centres surveyed vary widely in terms of testing high risk groups for HIV/HCV coinfection, whether the hospital has a written policy for the management of treatment of coinfected children and what laboratory investigations were performed on children with coinfection in comparison to those with HIV infection only.

- Although a total of 44 HIV/HCV coinfected children were being cared for in the surveyed centres, individual centres see relatively few coinfected children and therefore a lack of experience in the management of this group and the lack of evidence-based policy may be a barrier to achieving optimal care and treatment.

- This survey highlights the importance of research focussed on HIV/HCV coinfected children to inform guidelines for their best possible care.

- The development of such guidelines can only be achieved with consensus among clinicians on the optimal management of HIV/HCV coinfected children.

8. DISCUSSION AND CONCLUSIONS

8.1 Introduction

In this thesis a diverse range of topics related to paediatric HIV and HCV infection have been investigated and in doing so much needed information on the wider areas of natural history and disease progression and on more specific areas of coinfection, disease monitoring methodologies and the impact of mode of acquisition of infection has been provided.

Given the asymptomatic nature of paediatric HCV infection there is a need for accurate biological markers of infection to monitor progression. One of the main markers used in the management of HCV, especially in children, is **ALT levels** and in this thesis the first reference ranges for normal ALT levels in young children stratified by sex and age and based on an appropriate, defined reference population are provided. This work from Chapter three suggests that an ALT level of 60 U/l or greater in boys and 55 U/l or greater in girls under 18 months of age and an ALT level of 40U/l or greater in boys and 35 U/l or greater in girls over 18 months of age should be regarded as elevated. The fact that these ALT levels are lower than previously reported or used in a clinical or research setting (Behrman 2003, EPHN 2005a), may explain why children with what were assumed to be normal ALT levels have been found to have mild or moderate liver abnormalities and may consequently have implications for the referral of children with elevated ALT levels to specialist hepatic services and the management and monitoring of HCV infected children.

The impact of **mode of acquisition** of HIV or HCV infection on disease progression was the focus of Chapters four, five and six. Parenterally HCV-only infected children were one and a half times more likely to have evidence of hepatomegaly during a similar duration of follow-up than vertically HCV-only infected children but no differences were found between mode of acquisition groups in the genotype profile, proportion with consistent viraemia, proportion with consistently elevated ALT levels or the proportion with evidence of two or more markers of infection. These results provide evidence on the natural and treated history of parenterally acquired HCV infection which is lacking in the literature and also suggest predominantly similar characteristics in terms of biological markers of infection between mode of acquisition groups. This is a vital tool in informing the management and treatment of parenterally HCV infected children and confirms the likely appropriateness of guidelines, developed on the basis of information from vertically HCV-only infected children, for their parenterally infected counterparts.

The **disease progression** of vertically HIV-only infected children is well described in the literature and the results presented in this thesis show for the first time, patterns of HIV RNA viral load in parenterally HIV-only infected children similar to those seen in vertically infected children, with HIV RNA viral load decreasing with increasing time since infection. In parenterally infected children, HIV RNA viral load increased by 0.35 $\log_{10}$ cp/ml with decreasing immune competency, as expected. In the 11% of parenterally HIV infected children treated, response to ART was high with 71% achieving an UVL four to 18 months after initiation. This is higher than the response to treatment in some studies of vertically HIV infected children (ECS 2006) and may be higher still given the small numbers of parenterally infected children receiving treatment and the even smaller number,

6%, with information on response to treatment. During a maximum of five years follow-up, 88% of parenterally HIV-only infected children had no or mild clinical symptoms and 12% had moderate or severe clinical symptoms. Three years after infection, only 3% of children were estimated to have progressed to moderate of severe clinical symptoms. Similarly, only 10% of parenterally HIV-only infected children progressed to severe immunosuppression during follow-up with 8% having done so three years after infection. Given the lack of ART in this group of children, their disease progression thus appears to be slower than that of vertically HIV-only infected children reported in the literature (Gray 2001). The natural and treated history of parenterally acquired HIV infection has not been described in such a large group of children previously and this thesis therefore provides insight into the most appropriate clinical management and treatment of this group. The relative lack of ART in comparison to what has been the norm for vertically infected children for the last decade provides a rare look at true *natural* history and demonstrates that even without therapy, this group of children are predominantly well with no or mild clinical symptoms or evidence of immunosuppression, as was found for the parenterally HCV infected children. These results support delayed initiation of treatment in this group and may fuel the debate on delayed treatment in vertically infected children although further research in this area would be necessary.

Both vertically and parenterally **HIV/HCV coinfected children** exhibited an increase in ALT z-score with increasing duration of infection and in those treated versus untreated. There is the possibility that this increase in ALT z-score in treated children is confounded by treatment indication; it is plausible that children requiring HIV treatment will be those with worse HIV progression and consequently may have worse HCV progression and

consequently higher ALT levels. It may also be possible that the hepatotoxicity of the ART regimes being given are manifesting in increased ALT levels. No vertically coinfected children achieved an undetectable HIV RNA viral load following HIV treatment in comparison to 50% of parenterally coinfected children. Additionally parenterally HIV/HCV coinfected children progressed to severe immunosuppression faster than vertically infected children. There were no differences in the proportions of vertically and parenterally HIV/HCV coinfected children with severe immunosuppression of clinical symptoms. Information on all aspects of paediatric HIV/HCV coinfection is lacking despite recent increases in the importance of coinfection in adult populations. This thesis provides the most detailed description of parenterally coinfected children to date and in the context of mode of acquisition of infection demonstrates possible detrimental effects of ART on ALT levels and the possible need for differential management of children infected via different routes. Additionally, this work helps clarify differences between coinfected and singly infected children as differences between parenterally and vertically infected children seem to be more evident in coinfected children than singly HIV or HCV infected children. This may be a result of the interaction of the HIV and HCV viruses which perhaps are more detrimental for HIV disease progression in parenterally coinfected children due to the source of their infection. It is possible that infection transmitted through donor blood or which remains on un-sterilised equipment may have a higher level of virus and therefore the effects of infection in this way may be more than from mothers who by nature of the fact that they are pregnant are a reasonably well group. This difference may not be seen in singly parenterally and vertically infected children purely due to the bodies possible increased ability to respond to single infection compared to coinfection

The issue of the management of HIV/HCV coinfected children in the context of the lack of paediatric guidelines specifically for this group is explored in Chapter seven of this thesis by the survey of current practices and policies for care of these children by European clinicians. The fact that only one centre had a working policy for the care of coinfected children and that in general the management practices varied widely in terms of testing high risk groups for coinfection, which laboratory tests to carry out in comparison to those performed on HIV-only and HCV-only infected children and the opinions on optimal treatment for this group emphasise the importance of research in this area to inform clinical guidelines. The lack of coinfected children seen at individual centres further underlines the need for clinical consensus among those caring for HIV/HCV coinfected children in order to identify the optimum management and treatment for this group.

8.2 Context of results

The abundance of information on vertically acquired paediatric HIV and HCV infection has created evidence-based knowledge on MTCT of these viruses and the natural and treated history of infected children and has led to paediatric treatments diminishing the effects of infection and, in the case of HIV, interventions vastly reducing vertical transmission. This body of information also means that the majority of HIV or HCV infected children are managed and treated according to clinical guidelines developed on the basis of evidence from large cohorts of vertically infected children. However, it remains unknown whether children who are parenterally HIV or HCV infected via exposure to contaminated blood or blood products follow a similar natural and treated history as their vertically infected counterparts and therefore, whether managing and treating parenterally infected children

according to guidelines for vertically infected children is appropriate. In light of potential differences in children infected early or late in childhood, it is possible that parenterally infected children, who are receiving care based on evidence from vertically infected children, are not receiving optimal clinical management. Additionally, there may be differences in disease progression by age at infection in parenterally infected children which have further implications for the management of this group. As well as informing clinical management, clarification of these issues will provide more detailed information for families in terms of the prognosis of an infected child and for health services with regards planning for the burden of infection given the ever evolving HIV and HCV epidemics globally.

As the numbers of parenterally HIV or HCV infected children increase, or their reporting and diagnostic identification increases, specifically in less developed countries, it becomes progressively more important to understand disease progression in these children and determine differences to the vertically infected population.

This thesis goes some way to informing knowledge regarding these potential differences and provides an evidence base upon which clinical decisions regarding parenterally HIV or HCV infected children can be confidently made, further research in specific areas can be carried out and ultimately, guidelines can be expanded to include topics relevant to parenterally HIV or HCV infected children or developed specifically in relation to this group alone if necessary. Additionally, this thesis addresses some issues inextricably linked to the more general area of paediatric HIV or HCV infection in terms of methodologies for analysing specific types of data, the development and improvement of some disease

progression monitoring tools and the more qualitative assessment of clinical practices. As a whole, this thesis expands on existing evidence and indicates where further research in more under-studied but increasingly important areas of paediatric HIV and HCV infection is needed, particularly as the paediatric epidemics continue to present challenges for the care of infected children.

8.3 Monitoring of disease progression in HCV infected children

Monitoring progression of paediatric HCV infection and associated diseases or abnormalities via the identification of clinical symptoms of disease is difficult and often inappropriate due largely to the asymptomatic nature of the infection and the presentation of symptoms often several decades after infection, despite early establishment of chronicity and lasting abnormalities. Consequently, the use of biological markers that can be related to disease outcomes, which can help to predict disease progression in HCV infected children and can help monitor treatment response are ever more valuable.

For these biological markers to be of use in a clinical setting, it is vital to optimise the way in which they are used. One example of a marker about which conflicting views and evidence of usefulness exist is ALT levels which serve as a marker of liver-related abnormalities in HCV infected individuals. Moreover, there is some doubt about the validity of ALT as a marker of liver disease, especially in the case of chronic HCV infection where patients with normal ALT levels have been found to have minimal to mild liver abnormalities (Prati *et al* 2002). As has recently been suggested for adult studies, this may be partly explained by the current definitions of normal or abnormal ALT levels based

on data from undefined populations. In Chapter 3 I investigated the extent to which this was true for paediatric ALT levels and identified more accurate reference ranges for ALT levels in children.

8.3.1 Age and sex related reference ranges of ALT levels in children

I analysed data on serum ALT levels from a large group of healthy, HCV uninfected children and described ALT levels during a maximum of five years follow-up from birth, subsequently presenting ALT centiles and reference ranges for early childhood for the first time stratified by sex and updating previous studies (Lockitch *et al* 1988, Siest *et al* 1975, Behrman *et al* 2003). The study population from which these references were calculated were children born to HCV infected mothers but who were themselves uninfected with HCV. There is no evidence to suggest that ALT levels are affected by maternal HCV infection status in HCV uninfected children, although this cannot be totally excluded, and there is also no evidence to suggest that HCV exposure in utero affects the health of uninfected children, especially given the lack of anti-HCV therapy during pregnancy, but information and follow-up of HCV exposed but uninfected infants is short and underreported in the literature (EPHN 2005d, Mast et al 2005). Additionally, these children had been followed up from birth according to a standard protocol with intense reporting of clinical signs and symptoms of disease, both liver-related and otherwise, and there is nothing to suggest that children in this group were more morbid than the general population. Thus, any increases in ALT level as a result of underlying morbidity are likely to be representative of the general paediatric population and not because this group had a higher prevalence of any liver-related abnormalities. This is further supported by the lack of

consistently elevated ALT levels in any individual child. Moreover, this group of children was followed from birth and there are thus no children included in the uninfected group who were HCV infected but spontaneously cleared the virus. As a result, these children represent the largest group of HCV uninfected children with no underlying disease and regular ALT measurements, between birth and age five years, carried out within the standardised protocol of a cohort study. As ALT levels are not generally available for healthy children, it is unlikely that a more representative group of children would be available.

Although the few outlying ALT levels are one-off events and do not coincide with clinical events, there is the possibility that they are a result of infection with other viruses, for example non-A, non-B or non-C hepatitis viruses or possibly SEN-V which can temporarily elevate ALT levels without evidence of concurrent clinical manifestations. There is also the possibility that children who cleared the virus very early in life, before their first HCV RNA test result, were diagnosed as uninfected when actually they are clearers and therefore possibly have higher residual ALT levels. It is not possible to isolate the reason for these outlying high ALT levels, simply to minimise the possibility that they occurred as a result of any underlying liver disease, which has been achieved. Additionally, as these outliers account for less than 1% of all ALT measurements available, it is unlikely that their presence has influenced the resulting ALT centiles and reference ranges.

ALT assays were carried out at local hospital laboratories according to standardised study protocol but inter-laboratory assay variation is possible. However, variations in assays are likely to be random or lab-based rather than child-based, and not associated with age, sex or

weight (Pembrey *et al* 2003). Furthermore, as these centiles are intended to be used as population based reference ranges, calculating them based on ALT assays from a number of laboratories and with potential inter-laboratory variations minimises any bias which might arise from specific techniques had only one laboratory been used, making these reference ranges more generalisable to the broad paediatric population and in particular of relevance for vertically and parenterally HCV infected children. Additionally, there is the possibility that different laboratory machines were being used to analyse samples for ALT levels, again causing inter-laboratory variation. However, there are a limited number of machines in use throughout Europe and similarly to the possible variation in assays, any differences in the results obtained using different machines are likely to be random and not child-based and not associated with age, sex or weight.

The analyses presented in Chapter three showed that ALT levels significantly decrease with increasing age after a peak in the first six months of life and that mean ALT levels in boys are 1.5 U/l higher than in girls up to five years of age. Additionally this investigation demonstrated an increase in mean ALT levels of 1.1 U/l with each increase in weight-for-age standard deviation. Despite this, weight-for-age above the 95th centile compared with below the 95th centile was not associated with an increase in ALT levels leaving no clinically relevant measure of weight which could be applied to the ALT centiles. The small number of children with weight-for-age above the 95th centile (23 children in total) most likely limited the power to detect a difference in ALT levels between groups. However, in children under five years of age, weight will vary less than in older children or adolescents and therefore the effect of weight-for-age on ALT levels here is likely to be smaller than in studies including older children and adolescents (Quiros-Tejeira *et al* 2007,

Bedogni *et al* 2004). Previous studies have investigated the effect on ALT levels of sex and weight in children and adolescents, however, none have presented sex and age specific ALT centiles.

The finding that ALT levels were higher in boys than in girls under five years of age strengthens the evidence that similar sex differences in adult studies are at least in part due to biological mechanisms and not purely due to environmental factors as has been suggested (Piton *et al* 1998). Higher ALT levels in males have been previously reported to some extent in children but not at such young ages (Lockitch *et al* 1988) and so this investigation provides the first evidence of such a difference in children younger than five years old and confirms the similarity of ALT differences by sex found in adult studies.

Previous reports of appropriate upper limits of ALT levels in children suggested that ALT levels greater than 40U/l should be considered as elevated (Mohamadnejad *et al* 2003, Behrman *et al* 2003). There are also a number of other studies which used a variety of values, such as greater than 55U/l or greater than 80U/l, representing the upper limit of normal ALT reference ranges but these rarely refer to the population from which the reference range originated or they refer to previous studies which have used inappropriate populations for comparison purposes (Lockitch *et al* 1988, Siest *et al* 1975). The investigation presented here endorses the need for different cut-offs for different ages, as recommended by previous studies, but provides a more detailed, age-specific account of these variations. Some previous studies have suggested that ALT cut-offs should differ in those younger and older than 12 months of age (EPHN 2005a, Berhman *et al* 2003), however, the patterns of ALT levels we present indicate that 18 months would be a more appropriate age at which to offer a clinically-relevant cut-off in terms of ALT level centiles

as this is the point at which Figure 1 shows ALT to level off indicating the end of the peak seen early in life. The results from this investigation therefore recommend that an ALT level of greater than 60U/l in boys and 55U/l in girls should be regarded as elevated in the first 18 months of life while in older children the upper limits of normal ALT levels are lower: 40U/l in boys and 35U/l in girls. While the difference of 5U/l between boys and girls is small, it is a crucial difference if defined cut-offs are being used to identify elevated ALT levels because of the implications of an elevated ALT level in terms of further investigation and possible treatment.

It is important for appropriate clinical care that patients' ALT levels are correctly classified as normal or elevated. The implications of revising paediatric ALT reference ranges, i.e. the lowering of upper limits, requires careful consideration given to the potential increase in children with elevated ALT levels being referred to hepatology services. The benefits of a revised (i.e. reduced) upper limit of normal in terms of identifying liver abnormalities which would otherwise go undetected may be small in comparison to the extra costs, both economic and psychological, for health services, patients and families. However, this finding that ALT levels in children vary significantly by sex is an important consideration and clinicians should be aware of these differences when categorising ALT levels as normal or elevated.

The frequency with which ALT is used as a marker of liver abnormalities, in clinical and research settings, highlights the importance of using reference ranges for normality which are appropriate for the specific population under investigation, rather than relying on arbitrarily assigned reference ranges which do not account for differences by age or sex.

This study represents the largest, most detailed and, to date, the only investigation of the appropriateness of ALT reference ranges in children under five years of age and consequently provides an evidence base for the identification of elevated ALT levels in children under five years of age.

8.4 Challenges in the investigation of mode of acquisition of parenteral and vertical HIV and/or HCV infection in children

There are three major components to unravelling the issue of whether mode of acquisition impacts on the biological and clinical markers of paediatric HIV and HCV which complicates comparison of parenterally and vertically infected groups in general and which have proved challenging throughout this thesis: 1) accurately estimating the date of infection in parenterally infected children, 2) dealing with differing ages at infection between parenterally and vertically infected groups and within parenterally infected groups and 3) accounting for the different durations of infection when measurements are recorded. These, in combination with the differences in the timing of commencement of follow-up, the schedule of these follow-up examinations, exacerbated by the exclusive analysis of vertically infected children from birth cohorts where information during primary infection is collected, and the often different population characteristics of children who acquired infection parenterally in comparison to vertically made deciphering whether differences between these groups were truly due to mode of acquisition of infection increasingly complex.

8.4.1 Accurately estimating the timing of infection

In the case of many parenterally infected children, it is difficult to record a definitive source and timing of HIV or HCV infection due to multiple exposure, exposure many years before diagnosis of infection or inadequate recording of medical procedures. To compare the natural/treated history and disease progression of parenterally infected children with that in vertically infected children requires information on the timing of infection as the effect of age on many markers of HIV and HCV infection and subsequent prognosis is substantial and may additionally be related to age at infection.

In this thesis, the uncertainty surrounding date of infection has been dealt with in different ways for the two parenterally infected cohorts. Children in the UK National HCV Register had been identified primarily from retrospective studies specifically aiming to identify children who received blood or blood products from a source which has since been confirmed as contaminated (Department of Health 1995, Gibb *et al* 2000). The medical records for these children were comprehensive and for many children (approximately 60%) the date of infection was accurately estimated as the date at which a blood transfusion or other medical procedure occurred. However, for 40% of children in the Register it was impossible to accurately estimate the timing of infection from medical notes, predominantly because these children received multiple transfusions. For these children, and for others with no known date of infection because they were exposed while living abroad and medical notes are not available or incomplete, the infection date either has to be regarded as missing, and the implications in terms of reduced sample size and possible bias dealt with, or has to be estimated. For the purposes of this thesis I explored estimating the date of infection using two methods. Firstly, using the mid point of an interval during which

infection is known to have occurred is a common statistical methods but posed problematic when applied to the HCV only infected children in Chapter four. The only point at which the children were known to be negative was birth and the only point at which they were known to be infected was when they were reported to the Register or diagnosed within specific retrospective studies (Department of Health 1995, Gibb *et al* 2000). For many children the interval between these two events represented a period of many years (as many as 17 years for the oldest child) and this was deemed too wide to make the use of mid points appropriate. Secondly, I explored the possibility of using interval censoring (as with Turnbull analysis (Alioum *et al* 2003, EPHN 2005e)) to model a likely infection date based on the distribution of infection dates from those children who did have a known date of infection. However, as the children without a known infection date seemed to be random in terms of their differences to those who did have a known infection date there were no covariates indicative of a missing infection date and so this methods was not possible. As a result, analyses in Chapter four were limited to only include those with a known date of infection where this piece of information was a vital component of the specific analysis being carried out, i.e. a denominator of 76. Results may therefore be less generalisable or have less ability to demonstrate statistically significant results, although 76 is still a large number of parenterally HCV infected children in comparison to other studies. There are however, many analyses in Chapter four where a known date of infection was not necessary and data from the whole group of children were utilised, e.g. the analysis of factors associated with being consistently viraemic, having consistently elevated ALT levels or two or more markers of infection. Moreover a univariable comparison of key characteristics of those with and without a known date of infection showed no differences in the terms of

gender, HCV genotype or treatment profiles and confirmed that dates of infection were most likely missing at random.

The parenterally HIV and HIV/HCV infected children from the Libyan Cohort Follow-up Study posed different problems in terms of their dates of infection. For this cohort the window of possible infection was very short – the nosocomial source was reasonably defined - and so infection was known to have occurred during 1998 and 1999. This is in contrast to the children in the UK National HCV Register where exposure was via multiple sources in the UK and abroad. Some children had a defined date at which a procedure was carried out and infection was known to have occurred but again, for many children, multiple exposures occurred and an infection date was undefined. However, unlike the parenterally HCV infected children from the UK National HCV Register, the children from the Libyan cohort were all infected during a period of two years and additionally a large number have the date of their first positive HIV test recorded. It was therefore decided to estimate the date of infection in these children using the midpoint between the earliest date at which infection could have occurred (1st January 1998) and the date of the child's first positive HIV test result. For those children where no date of first positive HIV test was recorded, the midpoint during the two year interval of possible infection (1st January 1999) was taken as the date of infection. In this way, the accuracy of the estimated dates of infection were ensured and all analyses in Chapters five and six could utilise all data from the parenterally HIV and HIV/HCV coinfected children.

8.4.2 Analytical challenges relating to different ages at infection

One of the greatest challenges in the comparison of parenterally and vertically HIV or HCV infected groups was trying to separate differences due to mode of acquisition from those attributable to differing ages at infection. Accounting for different ages at infection was therefore a vital part of assessment of differences by mode of acquisition in this thesis. The most straightforward way to do this was to stratify by different ages at infection whereby all the vertically infected children were included in the youngest age group along with those parenterally infected children who acquired infection early in childhood. The more children being analysed and the greater spread of infection ages, the more specific these age groups could be. Specifically, the most important comparison was between vertically infected children and those parenterally infected children who were infected early in infancy as this allowed a comparison of mode at acquisition while limiting the confounding effects of age at infection. For the purposes of this thesis, the distribution of the date of infection were such that parenterally infected children were categorised as those infected before and after 12 months of age so that a sufficient number of children were included in each category to allow meaningful analyses. By comparing clinical and biological markers of HIV or HCV infection in parenterally and vertically infected children and then making the same comparison according to age at infection (before or after 12 months of age), it was possible to tease out whether and to what extent mode of acquisition or age at infection or both were influential in the nature of specific markers of infection.

8.3.3 Accounting for differing durations of infection at the time of measurements

In trying to account for differences in age at infection, it was demonstrated that measurements taken from parenterally infected children were often more sparse immediately after infection than they were for vertically infected children. This was due to the fact that information was not collected from parenterally infected children from the point of infection because diagnosis was made on the basis of symptoms leading to HCV or HIV testing or due to retrospective testing of children exposed to a contaminated source as was the case for children subsequently enrolled in the UK National HCV Register and the Libyan Cohort Follow-up study. This delay between acquisition and diagnosis of infection meant that follow-up data and biological/clinical information for children in both these cohorts but especially for those enrolled in the UK Register for whom retrospective testing occurred after a longer duration of infection, was unavailable during this period of undiagnosed, primary infection.

There were a number of solutions to these problems which needed to be tailored to the analysis being performed but for the purposes of this thesis it was first necessary to make sure that comparisons between parenterally and vertically infected groups were accounting for different ages at which measurements were taken. This was done by calculating age-standardised z-scores for variables that vary with age, specifically, ALT levels and CD4 cell counts, so that an ALT z-score represented the same raw ALT level regardless of the age at which it was measured (See section 6.2.1). However, this only goes part of the way to accounting for differences in the distribution of measurements; to illustrate, for a measurement taken at three years, for a vertically infected child this would be three years after infection but for a parenterally infected child this might have been taken a matter of

months after infection. This has implications for the resultant measurements taken from vertically infected children during primary infection where some markers of infection, specifically viral load, are known to be high for this short period of time. This is especially relevant for the vertically infected children included in this thesis who are all enrolled in birth cohorts meaning measurements are available from birth. To avoid the use of measurement-specific data when z-scores were not appropriate or did not fully account for biases in terms of duration of infection, summary variables were created to form a measure of the proportion of test results per child which met specific criteria. There is the possibility that children with more measurements available, had a higher proportion of measurements that met specific criteria but these children had not necessarily been infected for a longer duration.

8.4.4 Incorporating differences in population characteristics

There were many differences between parenterally and vertically HCV and/o HIV infected groups in terms of key population characteristics. Specifically, the setting of parenteral and vertical HIV cohorts were very different as all parenterally HIV infected children were Libyan and were infected from a defined hospital source. Additionally, by nature of the way in which parenterally infected children acquired their infection, they were in general a more morbid group at the time of infection and possibly remained so throughout follow-up. This is problematic if the symptoms or biological markers associated with these comorbidities are similar to, or have an effect on, those symptoms or markers of HIV or HCV infection. Accounting for these in specific analyses was complex and the numbers of children with individual comorbidities were small. Therefore the issue of comorbidities was

dealt with at the time of interpretation of results, bearing in mind the feasibility of any differences in the presence or levels of biological markers of HIV or HCV infection being influenced by the excess of comorbidities in the parenterally infected group.

Analysis of these cohorts of parenterally infected children highlight just some of the difficulties in analysing data from this group and provide guidance on how these difficulties can be overcome or accounted for. Obviously, in addition to these particular areas, there are individual challenges specific to each analysis which are dealt with in this discussion chapter under the subject-specific headings.

8.5 Paediatric HCV infection

In Chapter four, the impact of mode of acquisition on biological markers of HCV infection in the largest comparison of vertically and parenterally infected children to date was investigated. A significantly higher mean ALT z-score in vertically versus parenterally infected and a significantly higher mean ALT z-score in children infected before 12 months of age compared with those infected later, regardless of mode of acquisition, was found. This latter association did not remain when only parenterally infected children were investigated which may be due to the small numbers (29 infected before 12 months of age and 24 infected after 12 months of age) but may also indicate that the differences between parenterally and vertically infected groups are not completely explained by age at infection but may be related more directly to the mode of acquisition of infection itself.

Comparing biological markers of HCV infection in vertically and parenterally infected children is problematic in light of the often substantial differences in populations in terms of age at infection, age at study entry, treatment profile, likelihood of clearance of viraemia or genotype. In the children from the cohorts studied in Chapter four, four times as many parenterally than vertically infected children received HCV treatment; this may be due to more severe disease in parenterally infected children due to their age or mode of acquisition, as suggested by the finding here of an increased proportion with two or more markers of disease progression. Alternatively their older age at diagnosis (median age 19 months) may make them more eligible for treatment; anti-HCV therapy is contraindicated in children under three years of age, therefore vertically infected children who were followed from birth, will have started on a regime of clinical monitoring without treatment and if they remain asymptomatic at three years of age clinicians and parents may be reluctant to treat. Anti-HCV therapy has substantial side-effects and many clinicians only treat in circumstances where clinical symptoms compel them to do so. Although HCV therapy is well adhered to in the paediatric population the unpleasant side-effects and the potential impact on growth make the decision to treat a complex one (Fischler 2007). There could also be bias in terms of the individual clinic or national treatment policies, especially given the continually evolving nature of information on paediatric HCV treatment and the ongoing debate about when to initiate treatment (EPHN 2005c).

This debate on when to initiate treatment is partly fuelled by knowledge that some children spontaneously clear viraemia without treatment. In this study there was no effect of mode of acquisition on clearance of viraemia, 30% and 34% cleared the virus in vertically and parenterally infected children respectively, in contrast to some previous studies

(Rerksuppaphol *et al* 2004). However, parenterally infected children in the UK National HCV Register were not followed up from the time of infection and possibly a large number of those who cleared viraemia did so before diagnosis or study entry. Therefore, any estimate of clearance here and elsewhere, likely underestimates the true proportion clearing viraemia in parenterally infected groups and it was therefore considered inappropriate to compare clearance in vertically and parenterally infected children. Of note, however, is the high clearance rates in parenterally infected groups reported in some studies (Vogt *et al* 1999, O'Riordan *et al* 1998, Matsuoka *et al* 1994) which would presumably be even higher if those who cleared viraemia prior to diagnosis could be included. Similarly, in Chapter four, it is assumed that some parenterally HCV infected children cleared the virus prior to diagnosis the rate would be substantially higher than in the vertically infected group. It may therefore be the case that parenterally infected children are more likely to clear the virus than vertically infected children. It is likely that this can be substantiated as follow-up from infection in parenterally infected children is so difficult.

Quantitative levels of HCV RNA viral load could not be examined in this thesis as they were only available for the children from the EPHN and not those from the UK National HCV Register. As the natural history of children enrolled in the EPHN is already well described, any analysis of viral load here was restricted to quantitative measurements.

No differences were found in the HCV genotype profiles of vertically and parenterally infected children in contrast to Jara *et al* who found genotype 1b more frequently in children with transfusion-acquired HCV and genotype 3 more frequently in vertically infected children from seven European countries (Jara *et al* 2003). It is possible that the

differences in the years at which infection occurred, infection in the studies investigated in Chapter four having occurred more recently than in Jara *et al*'s study, may reflect changes in the genotype profile of the HCV epidemic in Europe as suggested recently (Bortolotti 2007). A higher proportion of children with genotype 3 achieved a SVR to anti-HCV therapy, as has been reported from a number of other paediatric studies (Bortolotti 2005). Additionally, in univariable logistic regression, children with genotype 1 were more likely to have consistently elevated ALT levels and consistently positive HCV RNA PCR results, although the associations did not reach statistical significance likely due to small numbers. This finding does however support those of Harris *et al* who suggested that type 1 infections may be more aggressive than types 2 or 3 (Harris *et al* 2007) and those of a previous EPHN study which found that intrauterine vertical transmission was more likely to occur from mothers with genotype 1 (Mok *et al* 2005). As no differences in the genotype profile of vertically and parenterally infected children were found here, it is unlikely that any differences in biological or clinical markers of HCV infection between groups can be attributed to the possible differences in HCV progression by genotype. In the analyses presented in Chapter 4, only 39% of those children receiving anti-HCV therapy had genotype information recorded which may be underreporting, especially in the vertically infected group, but could also be a genuine lack of genotype testing prior to treatment initiation, which is more likely in the parenterally infected group where data collection is more complete. These results confirm the importance of genotype testing in all children regardless of treatment status, to individualise monitoring and provide information for patients and families in terms of prognosis and likely treatment outcome. They also demonstrate the need for management and treatment guidelines to stress the importance of this which this analysis suggests is not being carried out in practice.

The investigation carried out in Chapter four reduced biases by accounting for treatment, genotype, age at infection and study entry, to a minimum and demonstrated the persistence of some differences by mode of acquisition of infection regardless of age at infection or age at which measurements were taken. In multivariable logistic regression no association between consistently raised ALT z-scores and mode of acquisition was found, although the odds ratio remained below one, indicating higher ALT z-scores in vertically infected children. This could be due to a lack of power as only 29 parenterally infected and 47 vertically infected children had ALT z-scores consistently above 2 SD. ALT levels have been shown to peak in the first two years of life in vertically infected children (Tovo *et al* 2000, Palomba *et al* 1996, Paccagnini *et al* 1995) and to adjust for this peak and any other differences resulting from age at measurement, ALT z-scores were used. This ensured that the bias resulting from the fact that by nature of the age distribution, ALT levels recorded during this peak would be more common in vertically infected children. However, the finding of increased ALT z-scores in vertically infected children adjusted for age is similar to an Australian study in which significantly higher geometric mean ALT levels in 16 vertically versus 15 parenterally infected children in the first five years of life were found, again after accounting for the early peak in ALT levels (Rerksuppaphol *et al* 2004).

Significant positive associations were found between consistently high ALT z-scores and both consistent HCV RNA viraemia and ever having evidence of hepatomegaly. There was also a higher odds of having consistently positive HCV RNA PCRs in children ever having evidence of hepatomegaly but not significantly so. The associations between these three markers of HCV-related disease progression support previous studies indicating that they

may define a group of children with evidence of chronic progressive HCV or who are at increased risk of rapid or more severe progression (EPHN 2005a). In this analysis, similar proportions of parenterally and vertically infected children had evidence of two or more of these markers of infection. Similarly, no difference was found in the proportion with two or more markers and age at infection, in either all children or just the parenterally infected group. This lack of association with mode of or age at acquisition may have been due to combining the markers of infection into this summary variable and also the small number (15 children) of parenterally and vertically infected children with evidence of two or more markers of infection.

The prevalence of comorbidities in parenterally infected children in this and other populations is high given the nature of their infection during receipt of medical treatment (Popova *et al* 1999) and this may have been influential in terms of the child's ability to mount an initial or continued immune response to HCV infection. In contrast, vertically infected children, although acquiring infection during immune development, may benefit from persistence of maternal antibodies, although it has been suggested that vertical acquisition of HCV may lead to a greater anti-HCV immune response and higher ALT levels soon after infection (Rerksuppaphol *et al* 2004, Murakami 2000). These mechanisms require specific investigation and although evidence from Chapter four doesn't support substantial differences between vertically and parenterally infected group, until they can be further defined it is important that the potential differences between vertically and parenterally HCV infected children are recognised in a clinical setting.

8.6 Parenterally acquired paediatric HIV infection

The work presented in Chapter five was a unique study of the natural and treated history of parenterally acquired HIV infection. To date, no such detailed or large investigation of this understudied group exists and given the increasing number of parenterally infected children being diagnosed, specifically in resource limited settings, this research provides and invaluable resource and informs evidence-based management of these children. Specifically, this work describes the disease progression of paediatric HIV acquired in this way, the natural history of parenterally acquired infection, the treated history of parenterally acquired infection and additionally allows comparisons to be made between parenterally and vertically infected groups reported in the literature to ultimately assess whether management and treatment of children infected via contact with contaminated blood, blood products or medical instruments should differ from that of vertically HIV infected children.

Information on parenterally HIV infected children in the literature is sparse and access to data on these children is rare. This is due to the geographical distribution of children with parenterally acquired infection and the fact that the majority of parenteral infections occur in resource limited countries were the diagnosis of parenterally infected children and the collection and recording of clinical and epidemiological data on these groups is incomplete. The result is a lack of epidemiological cohorts from which information can be obtained and a subsequent lack of evidence-based management and treatment of these children in all parts of the world. The work presented in Chapter five is therefore a very exclusive insight into the natural history and disease progression of parenterally HIV infected children not available elsewhere. Furthermore, the Libyan Cohort Follow-up Study database is

comprehensive and complete and the analyses in Chapter five are therefore based on a group of children for whom follow-up began within 12 months of infection for 69% and for whom the majority (87%) have measurements recorded from three or more follow-up visits.

The work presented in Chapter five shows that the mean HIV RNA viral load within 12 months of infection was high, 4.76 $\log_{10}$ cp/ml, regardless of receiving treatment, baseline CDC immunological stage, gender or age at infection. As so few children received treatment (11%) and in those who did, treatment was initiated at a median time from infection of 15 months, the majority of measurements contributing to this mean HIV RNA viral load calculation are pre-treatment. After 12 months of infection the mean HIV RNA viral load decreased with each increasing age after infection until it began to level out around three years after infection. This pattern is similar to that seen in vertically HIV infected children although more vertically infected children receive treatment early in infection, suggesting a possible better virological response to HIV infection in this parenterally infected group (ECS 2002). As expected, the mean HIV RNA viral load increased with decreasing immune competency and those few children receiving treatment had a mean HIV RNA viral load 0.74 $\log_{10}$ cp/ml lower than those untreated. Interestingly, girls had a mean HIV RNA viral load 0.23 $\log_{10}$ cp/ml lower than boys, in contrast to an earlier ECS analysis which reported higher HIV RNA viral loads in vertically infected girls than boys, although only in the initial period after infection (ECS 2002). In vertically HIV infected children this may be a reflection of the increased susceptibility of girls to vertical transmission (Galli etal 2005) and their subsequent disease progression. This may also be

relevant for parenterally infected children in a different way as it indicates an innate difference in girls' and boys' immune response to HIV infection.

Only 11% of this parenterally infected group received any treatment for HIV disease which is a low proportion in light of recent studies on the benefits of early treatment initiation in vertically infected children (ECS 2006, Violari 2007). This suggests that this group of parenterally infected children were being managed in a way more similar to HIV infected adults than vertically infected children whereby treatment is initiated when clinical signs and symptoms indicate it is necessary. As this group has a low prevalence of clinical symptoms and immunosuppression, this seems appropriate. However, further analysis showed that those with CDC clinical or immunological statuses which would be indicative of the need for treatment were not always those receiving treatment and similarly, 46% of children with no or mild clinical symptoms and immunosuppression were being treated. Moreover, 31% of those being treated were receiving dual therapy despite treatment initiation after 1999 when HAART use was widespread. These inconsistencies with paediatric or adults guidelines suggest that the management of these children was extremely individualised, perhaps as a result of concerns about adherence and resistance, the cost of or access to ART drugs in Libya – which would explain why a relatively high proportion of children were receiving dual therapy, or perhaps due to the lack of clinical guidelines or consensus on optimal management and treatment of parenterally infected children and uncertainties about when best to initiate treatment.

Despite the small proportion of children receiving treatment, it was possible to assess the response to treatment in those 14 children with enough information available. Given the

lack of information in the literature on parenterally infected children this still represents a significant group. 71% of parenterally infected children with information available achieved an UVL four to 18 months after treatment initiation. Nine out of these ten received HAART while of those who did not achieve an undetectable viral load, two were treated with HAART and two were treated with dual therapy. The 71% UVL response rate is high and indicates a promising treatment response in parenterally infected children treated with HAART. Additionally it is higher than that found in a recent analysis of vertically infected children (ECS 2006) and may be even higher if a larger number of treated children were available or if fewer children had received dual therapy.

Only 12% of parenterally infected children progressed to moderate or severe AIDS-defining symptoms but only 28% of the whole group had CDC clinical category information recorded. It is likely that this is due to the low prevalence of clinical symptoms but could also be an underreporting of clinical symptoms and therefore the low prevalence found in this analysis could be an underestimation of the true clinical wellbeing of this group. In contrast, CDC immunological category was available for 99% of this group and similarly to their clinical status, only 10% progressed to severe immunosuppression during follow-up. This low prevalence of immune deficiency likely confirms the genuine low prevalence of AIDS defining symptoms in the group.

Twelve months after infection, progression to severe immunosuppression had only occurred in 2% of parenterally infected children which is lower than would be expected from a vertically infected group with a similarly low prevalence of treatment (Gray *et al* 2001). Additionally, 75% of all children who progressed to severe immunosuppression

during follow-up had done so by three years after infection, supporting the idea that delaying treatment in this group may be appropriate given the low prevalence of disease progression. There were no effects of gender or age at infection on progression to immunosuppression or AIDS-defining symptoms. The lack of effect of age at infection may be a result of the way this was categorised (those progressing before and after the median age at infection of 22 months) due to the distribution of measurements and an effect may have been seen if those parenterally infected at earlier ages, perhaps before 12 or 6 months of age, could have been compared with those infected after this point, as has been seen with vertically infected children (Newell *et al* 2006).

The findings of this investigation of parenterally HIV infected children suggest a favourable disease progression and response to treatment and provide for the first time a detailed description of natural history and disease progression in this population. Additionally, the fact that disease progression in parenterally infected children with such a low prevalence of treatment seems to be better than in vertically infected children is a positive finding for the well being of children infected under difficult circumstances and often in parts of the world where access to clinical expertise and treatment is lacking.

8.7 Paediatric HIV/HCV coinfection

Little is known about HIV/HCV coinfection in childhood, especially the impact of mode of acquisition of infection on disease progression and its markers. Utilising data from three distinct paediatric cohorts, analyses in Chapter six described immunological, virological and clinical aspects of HIV and HCV infection in the context of coinfection and mode of

acquisition. ALT levels in HIV treated versus untreated coinfected children were significantly higher. In children receiving HIV treatment, HIV RNA viral load decreased in two-thirds of parenterally and vertically coinfected children 6-12 months after initiation. Although no differences were apparent in the overall proportion of parenterally and vertically coinfected children progressing to moderate or severe immunosuppression, parenterally coinfected children progressed to this stage more rapidly than vertically coinfected children. No differences were seen in the proportions progressing or the time to progression to moderate or severe AIDS-defining symptoms during follow-up, by mode of acquisition.

At the most recent follow-up visit, vertically and parenterally HIV/HCV coinfected children had been infected for significantly different periods of time, median duration 9.6 years and 2.6 years respectively and this had the potential to introduce bias in terms of the time since infection at which measurements were recorded. This bias was minimised primarily by limiting the maximum duration of infection in vertically infected children to 6.4 years which was equivalent to the maximum duration of infection in parenterally coinfected children. Comparison of ALT z-score measurements initially implied more severely impaired liver function in vertically than parenterally coinfected children. However, limiting duration of infection to account for the longer follow-up in vertically coinfected children showed this difference to be non-significant, highlighting the importance of accounting for duration of infection when assessing differences by mode of acquisition.

No children received HCV treatment, likely due to the asymptomatic nature of HCV infection in the first decade of infection and reflecting recent guidelines whereby IFN treatment is contraindicated in children younger than three years of age (EPHN 2005a, EPHN 2005b). Nearly a quarter of children were receiving ART for HIV disease and the decrease in HIV RNA viral load in approximately 66% of these children after treatment initiation was similar to the 58% with a decreased viral load six months after treatment initiation in a recent European study of vertically HIV-only infected children (ECS 2006). Children receiving ART had a higher ALT z-score than those untreated, significantly so in vertically coinfected children possibly because of the higher proportion of treated children in the vertically coinfected group. This may be indicative of a detrimental effect of HIV treatment on liver function due to the hepatotoxic nature of some drugs commonly used in paediatric HIV therapy (England *et al* 2006, Nelson *et al* 2005). Specific problems associated with nevirapine use in HIV/HCV coinfected children have recently been highlighted (England *et al* 2006, Nelson *et al* 2005) and the treatment profiles of the coinfected children here reveal that 25% of 45 children were receiving nevirapine with a further 10% Efavirenz, another non-nucleoside reverse transcriptase inhibitor known to have hepatotoxic properties (Alberti 2005). However, no serious hepatic effects directly related to NNRTIs can be confirmed in this group that would oppose recent WHO guidelines (WHO 2006). Of note is that the median age at treatment initiation in parenterally coinfected children was approximately three years, similar to the median age at start of follow-up and so more of the z-score measurements from parenterally infected children will have been while on treatment and this was there the group where the effect of treatment on ALT z-scores was less pronounced.

Progression to moderate or severe immunosuppression occurred in similar proportions of parenterally and vertically coinfected children but more children receiving ART therapy progressed to moderate or severe immunosuppression than those untreated, although this difference only reached borderline statistical significance. This result likely reflects confounding by indication as the number of children receiving treatment limited any analysis of timing of symptoms and timing of ART initiation. Among those children who did progress to moderate or severe immunosuppression during follow-up, progression in parenterally infected children was significantly faster. Differential management might be considered a key reason for this finding but no differences in the rate of clinical progression in treated versus untreated children were found although those on HAART progressed significantly slower than those on dual therapy, confirming the superiority of HAART in a coinfected population (Tedaldi *et al* 2003). An alternative explanation is that the persistence of passively acquired maternal antibodies (Thorne and Newell 2000) in vertically coinfected children gives a protective effect such that their initial progression is slower. Additionally, neonatal ART prophylaxis in vertically infected children is a previously reported predictor of slower HIV progression (ECS 2006). It is also possible that this difference relates to thymic function as vertically coinfected children are younger at infection with a larger thymus which could mean they have a higher CD4 count and lower HIV RNA viral load (Vigano *et al* 1999). The finding that clinical progression was not significantly slower in those treated versus untreated, is likely a reflection of the small numbers of children receiving treatment rather than an indication of no treatment effect.

More parenterally coinfected children infected during the first year of life progressed to moderate or severe immunosuppression than vertically coinfected children indicating a

difference relating to mode of acquisition rather than age at infection. The prevalence of progression to moderate or severe immunosuppression in parenterally coinfected children infected after the first year of life was less than those infected in the first year of life but not as low as in vertically infected children indicating an additional difference relating to age at infection and a possible relationship between early parenteral infection and more severe disease progression. This suggests that thymic function and infection during early immune maturation may be important influences of disease progression in parenterally coinfected children while vertically coinfected children are in some way additionally protected possibly due to persistence of maternal antibodies or neonatal ART prophylaxis.

The group of coinfected children studied in Chapter six progressed more slowly than vertically HIV-only infected children from a European multi-centre study, 50% of whom had progressed to moderate or severe immunosupression by one year of age [ECS 2001]. These differences may be due to the small numbers of vertically coinfected children progressing to moderate or severe immunodeficiency in this group or, more likely, may be due to differences in treatment profiles between the two studies. The possibility exists that the interaction of the HIV and HCV viruses in coinfected children provide some kind of protective effect in terms of HIV progression but the data here were inadequate to explore this further.

The analysis of progression to moderate or severe AIDS-defining symptoms was limited by small numbers and the lack of differences by mode of acquisition or age at infection may have been due to a lack of statistical power. The findings on progression to symptomatic HIV disease were similar to those reported by the ECS on vertically HIV-only infected

children, in which approximately 75% of children progressed to moderate or severe AIDS-defining symptoms by 24 months of age (ECS 2001).

Any differences in disease progression between parenterally and vertically coinfected children could be due to varying durations of infection. However, this investigation has shown that accounting for this, aspects of HIV and HCV disease progression differ between parenterally and vertically HIV/HCV coinfected children. The findings suggest that the HIV therapeutic management of HIV/HCV parenterally coinfected children, infected during the first year of life should be more aggressive than for their older counterparts or vertically coinfected children. This result ties in with the debate on when to initiate treatment in vertically HIV-only infected children and suggests that early initiation may be appropriate for vertically and parenterally coinfected children who acquire infection early in infancy but may not be necessary for those infected later in childhood (ECS 2006, Violari *et al* 2007). Furthermore, the findings also point towards a difference in disease progression according to age at infection and clinical management decisions must consider possible differences in children infected via similar routes but at different ages. ALT levels were significantly higher in coinfected children receiving HIV treatment emphasising the importance of monitoring and individualising HIV treatment in coinfected children to account for possible hepatotoxicity in HIV drugs and their impact on liver function in HIV/HCV coinfected children. This however, if complicated by the limited paediatric HIV drugs available and requires a balance between the possible detrimental effects of HIV treatment and the effect of not treating HIV disease with the most appropriate HIV drugs. Issues relating to possible structured HIV treatment interruption to allow for a concentrated period of HCV treatment need to be explored with an emphasis on optimising the

management of both infections and the possible implications for drug resistance. Further research involving larger numbers of vertically coinfected children would help clarify the differences found in this study, investigate further the possibility of coinfection with HCV reducing disease progression in comparison to HIV only infected children and inform individual clinical management and treatment of this group of HIV/HCV coinfected children.

8.8 Management survey of HIV/HCV coinfection

In the absence of guidelines for the clinical management of children coinfected with HIV and HCV, practices in the European centres surveyed varied widely. Only one centre surveyed had a working policy for the management of HIV/HCV coinfected children and therefore most centres were relying on guidelines based on HIV or HCV-only infected children. As none of the published guidelines on paediatric HIV or HCV management make reference to coinfection, clinicians are forced to rely on personal clinical experience rather than the consensus of global clinical speciality on the treatment and management of HIV and HCV singly infected children that has been pooled together and is constantly updated.

This survey highlights that individual centres see relatively few coinfected children and therefore a lack of experience in the management of this group and the lack of evidence-based policy may be a barrier to achieving optimal care and treatment. The centre which did have a HIV/HCV coinfection management policy and the centre who was in the process of compiling a policy were in Italy and Spain respectively. Both Italy and Spain have higher rates of HIV and HCV infection than other parts of Western Europe and therefore it

is likely that they also have a higher prevalence of HIV/HCV coinfection which may explain why written policies for coinfection management are available in these centres and not in others.

The low response rate for the survey is a limitation in terms of the generalisability of results but the geographical distribution of responses was wide and both Italy and Spain were overrepresented which is appropriate given the likely higher prevalence of HIV/HCV coinfection in these countries.

The circumstances under which clinicians test for HIV/HCV coinfection vary but it is apparent that they would ascertain most cases of coinfection in the paediatric population by current methods. Some centres however did report more thorough testing in specific groups, such as those with less common risk factors for coinfection, and a consensus on the circumstances for coinfection testing would be helpful to ensure that all risk groups are targeted.

The schedule for laboratory testing for specific HCV-related markers varied between centres and there seemed little agreement in terms of which tests were appropriate and at what intervals these should be carried out. In terms of monitoring HCV progression in coinfected children based on the evidence from long-term multi-centre studies, a lack of available information on biological markers of infection would make this problematic and therefore it is possible that the monitoring of HCV infection in this group of coinfected children is inadequate.

The treatment of HIV/HCV coinfected children is very complex and as yet no definition of the most appropriate treatment regimes exists other than to provide information on specific drugs used to treat HIV infection which are inappropriate or which carry risks for a coinfected population. This lack of consensus is reflected in the survey in Chapter seven and is compounded by the lack of experience in treating HCV infection alone, as well as treating coinfected children. Treatment of HCV singly infected children remains relatively rare but the possibility exists that it may be more necessary in coinfected children and therefore the concurrent treatment of HIV and HCV disease needs to be explored. Very few clinicians stated that they would happily administer HIV and HCV treatment concurrently and it seems more appropriate that a scheduled HIV treatment break may be more suitable. Issues regarding optimal treatment regimes are usually resolved by clinical treatment trials and this may be the most appropriate course of action for coinfected children as this survey highlights that management and treatment practices vary widely and therefore retrospectively looking at treatment outcomes in coinfected children is unlikely to provide definitive answers. However, the small number of HIV/HCV coinfected children may be a barrier to treatment trials.

In the absence of guidelines for the clinical management of children coinfected with HIV and HCV, practices in the European centres surveyed varied widely. Individual centres see relatively few coinfected children and therefore a lack of experience in the management of this group and the lack of evidence-based policy may be a barrier to achieving optimal care and treatment. This survey highlights the importance of research focussed on this group of children to inform guidelines for their best possible care and the need for a consensus among clinicians on the optimal management of these children. The survey is limited by

small numbers and subsequently in its generalisability but it is a much needed first step in emphasising this little studied area and highlighting issues for further investigation specifically with a view to developing treatment guidelines for HIV/HCV coinfected children.

8.9 Recommendations for future research

Mode of acquisition of infection – parenterally infected children

The results of this thesis highlight the scarcity of information regarding parenterally HIV infected children, largely due to the countries where parenteral infection occurs and subsequent difficulties in access to clinical information. Those countries where the parenteral epidemic is still important are to some extent in Eastern Europe, although the situation is greatly improved there, and to a greater extent central Asian countries where acquisition of HIV infection is largely among injecting drug users and blood safety is not yet a priority for economic and logistical reasons (Hauri et al 2004). In these countries the infrastructure in terms of diagnosis and monitoring of HIV infected individuals must improve before any epidemiological study would be feasible. Resources also need to be expended on gathering sufficient epidemiological data to clarify the natural history of parenterally acquired HIV infection which appears from this research to result in slower disease progression than vertically infected children and therefore may inform more individualised treatment recommendations and help resource-limited settings prioritise their treatment programs. As surveillance and HIV prevention programs are established or improve in these Eastern European and Central Asian countries it would be ideal for

epidemiological research programs on parenteral acquisition of infection to run along side prevention and treatment programs to achieve this.

HIV/HCV coinfected children

The ideal future research recommendation for HIV/HCV coinfected children would be the establishment of a cohort of HIV/HCV coinfected children infected vertically or parenterally with data collected from as wide an area as possible. This would inform knowledge in a number of ways. Firstly, it would increase the amount of data available on HIV/HCV coinfected children, minimise the limitations encountered in this thesis with regards the small numbers of children, specifically vertically infected, available for analysis and improve reliability of the estimates. Secondly, a cohort specifically dedicated to coinfected children could collect all necessary HIV and HCV related information from all coinfected children, thus overcoming the problem encountered in this thesis whereby the coinfected children enrolled in HIV cohorts had limited HCV-related information and vice-versa for those enrolled in HCV cohorts. Now that a picture has been built by this thesis and other studies on what may be the differences between HIV or HCV only infected children and coinfected children and speculation about the optimum treatment for coinfected children has been made, it is timely to study a group of coinfected children who are followed prospectively within a specifically designed cohort study in order to clarify many of these still speculative results and provide an evidence base for management and treatment guidelines for coinfected children. The survey presented in this thesis showed that there are a number of confected children being cared for by Western and Central European clinicians whose expertise on management and countries' infrastructure would be useful in the establishment of such a cohort. In addition, many Eastern European countries

continue to report a great number of both HIV and HCV infected children to birth cohorts and while MTCT rates are falling the HIV infected populations in these countries are so large that even a 4% transmission rate as recently reported from Ukraine, results in a great many potentially coinfected children being born each year, information about whom would greatly benefit a cohort of HIV/HCV coinfected children. Additionally, the infrastructure for follow-up of coinfected children in many of these countries is already in place thanks to groups such as the ECS who expanded into Eastern Europe in 2000.

REFERENCES

Aceijas, C., Stimson, G.V., Hickman, M., and Rhodes, T. (2004). Global overview of injecting drug use and HIV infection among injecting drug users. *AIDS*; 18: 2295-2303.

Aceijas, C. and Rhodes, T. (2007). Global estimates of prevalence of HCV infection among injecting drug users. *Int.J Drug Policy*; 18: 352-358.

Al Sherbiny, M., Osman, A., Mohamed, N., Shata, M.T., Abdel-Aziz, F., Abdel-Hamid, M., Abdelwahab, S.F., Mikhail, N., Stoszek, S., Ruggeri, L., Folgori, A., Nicosia, A., Prince, A.M., and Strickland, G.T. (2005). Exposure to hepatitis C virus induces cellular immune responses without detectable viremia or seroconversion. *Am.J.Trop.Med.Hyg.*; 73: 44-49.

Alberti, A., Clumeck, N., Collins, S., Gerlich, W., Lundgren, J., Palu, G., Reiss, P., Thiebaut, R., Weiland, O., Yazdanpanah, Y., and Zeuzem, S. (2005). Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. *J Hepatol.*; 42: 615-624.

Alioum, A., Cortina-Borja, M., Dabis, F., Dequae-Merchadou, L., Haverkamp, G., Hughes, J., Karon, J., Leroy, V., Newell, M.L., Richardson, B.A., van Weert, L., and Weverling, G.J. (2003). Estimating the efficacy of interventions to prevent mother-to-child transmission of human immunodeficiency virus in breastfeeding populations: comparing statistical methods. *Am.J.Epidemiol.*; 158: 596-605.

Alter, M.J. (2007). Epidemiology of hepatitis C virus infection. *World J Gastroenterol.*; 13: 2436-2441.

Babiker, A., Darbyshire, J., Pezzotti, P., Porter, K., Prins, M., Sabin, C., and Walker, A.S. (2003). Short-term CD4 cell response after highly active antiretroviral therapy initiated at different times from seroconversion in 1,500 seroconverters. *J.Acquir.Immune.Defic.Syndr.*; 32: 303-310.

Bagchi, S. (2007). Kazakh medical workers guilty of causing HIV outbreak. *Lancet Infect.Dis.*; 7: 512.

Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*; 220: 868-871.

Bedogni, G. and Bellentani, S. (2004). Fatty liver: how frequent is it and why? *Ann.Hepatol.*; 3: 63-65.

Behrman R, Kliegman R, and Jenson H. (2003). Nelson Textbook of Pediatrcs 17th Edition.

Bica, I., McGovern, B., Dhar, R., Stone, D., McGowan, K., Scheib, R., and Snydman, D.R. (2001). Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin.Infect.Dis.*; 32: 492-497.

Blanche, S., Newell, M.L., Mayaux, M.J., Dunn, D.T., Teglas, J.P., Rouzioux, C., and Peckham, C.S. (1997). Morbidity and mortality in European children vertically infected by HIV-1. The French Pediatric HIV Infection Study Group and European Collaborative Study. *J.Acquir.Immune.Defic.Syndr.Hum.Retrovirol.*; 14: 442-450.

Bland, J.M. and Altman, D.G. (1998). Survival probabilities (the Kaplan-Meier method) 620. *British Medical Journal*; 317: 1572-1580.

Bonacini, M. and Puoti, M. (2000). Hepatitis C in patients with human immunodeficiency virus infection: diagnosis, natural history, meta-analysis of sexual and vertical transmission, and therapeutic issues. *Arch.Intern.Med.*; 160: 3365-3373.

Bortolotti, F., Resti, M., Giacchino, R., Azzari, C., Gussetti, N., Crivellaro, C., Barbera, C., Mannelli, F., Zancan, L., and Bertolini, A. (1997). Hepatitis C virus infection and related liver disease in children of mothers with antibodies to the virus. *J.Pediatr.*; 130: 990-993.

Bortolotti, F., Resti, M., Marcellini, M., Giacchino, R., Verucchi, G., Nebbia, G., Zancan, L., Marazzi, M.G., Barbera, C., Maccabruni, A., Zuin, G., Maggiore, G., Balli, F., Vajro, P., Lepore, L., Molesini, M., Guido, M., Bartolacci, S., and Noventa, F. (2005). Hepatitis C virus (HCV) genotypes in 373 Italian children with HCV infection: changing distribution and correlation with clinical features and outcome. *Gut*; 54: 852-857.

Bortolotti, F., Iorio, R., Resti, M., Camma, C., Marcellini, M., Giacchino, R., Marazzi, M.G., Verucchi, G., Zancan, L., Barbera, C., Maggiore, G., Vajro, P., Giannattasio, A., and

Bartolacci, S. (2007). Epidemiological profile of 806 Italian children with hepatitis C virus infection over a 15-year period. *J Hepatol.*; 46: 783-790.

Braitstein, P., Palepu, A., Dieterich, D., Benhamou, Y., and Montaner, J.S. (2004). Special considerations in the initiation and management of antiretroviral therapy in individuals coinfected with HIV and hepatitis C. *AIDS*; 18: 2221-2234.

Brinkmann, T., Dreier, J., Diekmann, J., Gotting, C., Klauke, R., Schumann, G., and Kleesiek, K. (2003). Alanine aminotransferase cut-off values for blood donor screening using the new International Federation of Clinical Chemistry reference method at 37 degrees C. *Vox Sang.*; 85: 159-164.

Bunders, M., Cortina-Borja, M., and Newell, M.L. (2005). Age-related standards for total lymphocyte, CD4+ and CD8+ T cell counts in children born in Europe. *Pediatr.Infect.Dis.J.*; 24: 595-600.

Carlos, M.J., Castilla, J., Lopez, M., Arranz, R., Gonzalez-Lahoz, J., and Soriano, V. (2004). Impact of chronic hepatitis C on HIV-1 disease progression. *HIV.Clin.Trials*; 5: 125-131.

Carre, N., Deveau, C., Belanger, F., Boufassa, F., Persoz, A., Jadand, C., Rouzioux, C., Delfraissy, J.F., and Bucquet, D. (1994). Effect of age and exposure group on the onset of AIDS in heterosexual and homosexual HIV-infected patients. SEROCO Study Group. *AIDS*; 8: 797-802.

Ceci, O., Margiotta, M., Marello, F., Francavilla, R., Lerardi, E., Loizzi, P., Impedovo, L., and Francavilla, A. (2001). High rate of spontaneous viral clearance in a cohort of vertically infected hepatitis C virus infants: what lies behind? *J.Hepatol.*; 35: 687-688.

Centres for Disease Control (1994). 1994 revised classification system for HIV infection in children less than 13 years of age. *Morbidity and Mortality Weekly Report*; 43: 1-10.

Chisari, F.V. (2005). Unscrambling hepatitis C virus-host interactions. *Nature*; 436: 930-932.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., and Houghton, M. (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*; 244: 359-362.

Chung, R.T., Andersen, J., Volberding, P., Robbins, G.K., Liu, T., Sherman, K.E., Peters, M.G., Koziel, M.J., Bhan, A.K., Alston, B., Colquhoun, D., Nevin, T., Harb, G., and van der, H.C. (2004). Peginterferon Alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. *N.Engl.J.Med.*; 351: 451-459.

Cole, T.J. and Green, P.J. (1992). Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat.Med.*; 11: 1305-1319.

Cole, T.J., Freeman, J.V., and Preece, M.A. (1998). British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat.Med.*; 17: 407-429.

Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action.Concerted Action on SeroConversion to AIDS and Death in Europe. (2000). Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. *Lancet*; 355: 1131-1137.

Connor, E.M., Sperling, R.S., Gelber, R., Kiselev, P., Scott, G., O'Sullivan, M.J., VanDyke, R., Bey, M., Shearer, W., Jacobson, R.L., and . (1994). Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N.Engl.J.Med.*; 331: 1173-1180.

Darby, S.C., Ewart, D.W., Giangrande, P.L., Spooner, R.J., and Rizza, C.R. (1996). Importance of age at infection with HIV-1 for survival and development of AIDS in UK haemophilia population. UK Haemophilia Centre Directors' Organisation. *Lancet*; 347: 1573-1579.

Davaalkham, D., Ojima, T., Nymadawa, P., Uehara, R., Watanabe, M., Oki, I., and Nakamura, Y. (2006). Prevalence and risk factors for hepatitis C virus infection in Mongolian children: Findings from a nationwide survey. *J Med.Virol.*; 78: 466-472.

Davison, S.M., Mieli-Vergani, G., Sira, J., and Kelly, D.A. (2006). Perinatal hepatitis C virus infection: diagnosis and management. *Arch.Dis.Child*; 91: 781-785.

Department of Health. (1995). Hepatitis C and blood transfusion Lookback London: HMSO.

Dorrucci, M., Pezzotti, P., Phillips, A.N., Lepri, A.C., and Rezza, G. (1995). Coinfection of hepatitis C virus with human immunodeficiency virus and progression to AIDS. Italian Seroconversion Study. *J.Infect.Dis.*; 172: 1503-1508.

Dunn, D. (2003). Short-term risk of disease progression in HIV-1-infected children receiving no antiretroviral therapy or zidovudine monotherapy: a meta-analysis. *Lancet*; 362: 1605-1611.

Dusheiko, G., Schmilovitz-Weiss, H., Brown, D., McOmish, F., Yap, P.L., Sherlock, S., McIntyre, N., and Simmonds, P. (1994). Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology*; 19: 13-18.

El Raziky, M.S., El Hawary, M., El Koofy, N., Okasha, S., Kotb, M., Salama, K., Esmat, G., El Raziky, M., Abouzied, A.M., and El Karaksy, H. (2004). Hepatitis C virus infection in Egyptian children: single centre experience. *J.Viral Hepat.*; 11: 471-476.

England, K., Thorne, C., and Newell, M.L. (2006). Vertically acquired paediatric coinfection with HIV and hepatitis C virus. *Lancet Infect.Dis.*; 6: 83-90.

Esteban, J.I., Sauleda, S., and Quer, J. (2007). The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol.*; 48: 148-62.

EuroHIV (2007). HIV/AIDS surveillance in Europe; mid-year report 2007. http://www.eurohiv.org/reports/report_76/pdf/report_eurohiv_76.pdf. Accessed 10.02.2008.

European Collaborative Study. (2002). Level and pattern of HIV-1-RNA viral load over age: differences between girls and boys?. *AIDS*; 16: 97-104.

European Collaborative Study. (2004). Gender and race do not alter early-life determinants of clinical disease progression in HIV-1 vertically infected children. *AIDS*; 18: 509-516.

European Collaborative Study. (2006). The mother-to-child HIV transmission epidemic in Europe: evolving in the East and established in the West. *AIDS*; 20: 1419-1427.

European Paediatric HCV Network, Tovo, P.-A., Pembrey, L., and Newell, M.-L. (2000). Persistence rate and progression of vertically acquired hepatitis C infection. *Journal of Infectious Diseases*; 181: 419-424.

European Paediatric HCV Network, Pembrey, L., Tovo, P.-A., and Newell, M.-L. (2001). Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. *British Journal of Obstetrics and Gynaecology*; 108: 371-377.

European Paediatric HCV Network, England, K., Pembrey, L., Tovo, P.A., and Newell, M.L. (2005d). Growth in the first 5 years of life is unaffected in children with perinatally-acquired hepatitis C infection. *J.Pediatr.*; 147: 227-232.

European Paediatric HCV Network, England, K., Pembrey, L., Tovo, P.A., and Newell, M.L. (2005e). Excluding hepatitis C virus (HCV) infection by serology in young infants of HCV-infected mothers. *Acta Paediatr.*; 94: 444-450.

European Paediatric HCV Network, Pembrey L, Tovo, P.-A., and Newell, M.-L. (2005a). Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. *Clin.Infect.Dis.*; 41: 45-51.

European Paediatric HCV Network (Tovo PA, Pembrey L, and Newell ML). (2005b). A significant sex--but not elective cesarean section--effect on mother-to-child transmission of hepatitis C virus infection. *J Infect.Dis.*; 192: 1872-1879.

European Paediatric HCV Network, Pembrey L, Newell, M.-L., Tovo, P.-A., and the EPHN Collaborators. (2005c). The management of HCV infected pregnant women and their children. *Journal of Hepatology*; 43: 515-525.

Ferrero, S., Lungaro, P., Bruzzone, B.M., Gotta, C., Bentivoglio, G., and Ragni, N. (2003). Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990-2000). *Acta Obstet.Gynecol.Scand.*; 82: 229-234.

Fischler, B. (2007). Hepatitis C virus infection. *Semin.Fetal Neonatal Med.*; 12: 168-173.

Frank, C., Mohamed, M.K., Strickland, G.T., Lavanchy, D., Arthur, R.R., Magder, L.S., El Khoby, T., Abdel-Wahab, Y., Aly Ohn, E.S., Anwar, W., and Sallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet*; 355: 887-891.

Fuster, D., Planas, R., Muga, R., Ballesteros, A.L., Santos, J., Tor, J., Sirera, G., Guardiola, H., Salas, A., Cabre, E., Ojanguren, I., Barluenga, E., Rey-Joly, C., Clotet, B., and Tural, C. (2004). Advanced liver fibrosis in HIV/HCV-coinfected patients on antiretroviral therapy. *AIDS Res.Hum.Retroviruses*; 20: 1293-1297.

Galli, L., Puliti, D., Chiappini, E., Gabiano, C., Tovo, P.A., Pezzotti, P., and de Martino, M. (2005). Lower mother-to-child HIV-1 transmission in boys is independent of type of delivery and antiretroviral prophylaxis: the Italian Register for HIV Infection in Children. *J Acquir.Immune.Defic.Syndr.*; 40: 479-485.

Garcia-Monzon, C., Jara, P., Fernandez-Bermejo, M., Hierro, L., Frauca, E., Camarena, C., Diaz, C., De, l., V, Larrauri, J., Garcia-Iglesias, C., Borque, M.J., Sanz, P., Garcia-Buey, L., Moreno-Monteagudo, J.A., and Moreno-Otero, R. (1998). Chronic hepatitis C in children: a clinical and immunohistochemical comparative study with adult patients. *Hepatology*; 28: 1696-1701.

Giacchino, R., Tasso, L., Timitilli, A., Castagnola, E., Cristina, E., Sinelli, N., Gotta, C., Giambartolomei, G., Moscatelli, P., and Picciotto, A. (1998). Vertical transmission of hepatitis C virus infection: usefulness of viremia detection in HIV-seronegative hepatitis C virus-seropositive mothers. *J.Pediatr.*; 132: 167-169.

Gibb, D.M., Neave, P.E., Tookey, P.A., Ramsay, M., Harris, H., Balogun, K., Goldberg, D., Mieli-Vergani, G., and Kelly, D. (2000). Active surveillance of hepatitis C infection in the UK and Ireland. *Arch.Dis.Child*; 82: 286-291.

Gibb, D.M., Goodall, R.L., Dunn, D.T., Healy, M., Neave, P., Cafferkey, M., and Butler, K. (2000). Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. *Lancet*; 356: 904-907.

Giovannini, M., Tagger, A., Ribero, M.L., Zuccotti, G., Pogliani, L., Grossi, A., Ferroni, P., and Fiocchi, A. (1990). Maternal-infant transmission of hepatitis C virus and HIV infections: a possible interaction. *Lancet*; 335: 1166-

Goedert, J.J., Kessler, C.M., Aledort, L.M., Biggar, R.J., Andes, W.A., White, G.C., Drummond, J.E., Vaidya, K., Mann, D.L., Eyster, M.E., and . (1989). A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. *N.Engl.J Med.*; 321: 1141-1148.

Gottlieb, G.J., Ragaz, A., Vogel, J.V., Friedman-Kien, A., Rywlin, A.M., Weiner, E.A., and Ackerman, A.B. (1981). A preliminary communication on extensively disseminated Kaposi's sarcoma in young homosexual men. *Am.J Dermatopathol.*; 3: 111-114.

Granovsky, M.O., Minkoff, H.L., Tess, B.H., Waters, D., Hatzakis, A., Devoid, D.E., Landesman, S.H., Rubinstein, A., Di Bisceglie, A.M., and Goedert, J.J. (1998). Hepatitis C virus infection in the mothers and infants cohort study. *Pediatrics*; 102: 355-359.

Gray, L., Newell, M.L., Thorne, C., Peckham, C., and Levy, J. (2001). Fluctuations in symptoms in human immunodeficiency virus-infected children: the first 10 years of life. *Pediatrics*; 108: 116-122.

Greub, G., Ledergerber, B., Battegay, M., Grob, P., Perrin, L., Furrer, H., Burgisser, P., Erb, P., Boggian, K., Piffaretti, J.C., Hirschel, B., Janin, P., Francioli, P., Flepp, M., and Telenti, A. (2000). Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet*; 356: 1800-1805.

Guido, M., Rugge, M., Jara, P., Hierro, L., Giacchino, R., Larrauri, J., Zancan, L., Leandro, G., Marino, C.E., Balli, F., Bagni, A., Timitilli, A., and Bortolotti, F. (1998). Chronic hepatitis C in children: the pathological and clinical spectrum. *Gastroenterology*; 115: 1525-1529.

Guido, M., Bortolotti, F., Leandro, G., Jara, P., Hierro, L., Larrauri, J., Barbera, C., Giacchino, R., Zancan, L., Balli, F., Crivellaro, C., Cristina, E., Pucci, A., and Rugge, M. (2003). Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? *Am.J.Gastroenterol.*; 98: 660-663.

Harris, H.E., Ramsay, M.E., Heptonstall, J., Soldan, K., and Eldridge, K.P. (2000). The HCV National Register: towards informing the natural history of hepatitis C infection in the UK *J.Viral Hepat.*; 7: 420-427.

Harris, H.E., Eldridge, K.P., Harbour, S., Alexander, G., Teo, C.G., and Ramsay, M.E. (2007). Does the clinical outcome of hepatitis C infection vary with the infecting hepatitis C virus type?. *J Viral Hepat*; 14: 213-220.

Hauri, A.M., Armstrong, G.L., and Hutin, Y.J. (2004). The global burden of disease attributable to contaminated injections given in health care settings. *Int.J STD AIDS*; 15: 7-16.

Hersh, B.S., Popovici, F., Apetrei, R.C., Zolotusca, L., Beldescu, N., Calomfirescu, A., Jezek, Z., Oxtoby, M.J., Gromyko, A., and Heymann, D.L. (1991). Acquired immunodeficiency syndrome in Romania. *Lancet*; 338: 645-649.

Hersh, B.S., Popovici, F., Jezek, Z., Satten, G.A., Apetrei, R.C., Beldescu, N., George, J.R., Shapiro, C.N., Gayle, H.D., and Heymann, D.L. (1993). Risk factors for HIV infection among abandoned Romanian children. *AIDS*; 7: 1617-1624.

Hershow, R.C., Riester, K.A., Lew, J., Quinn, T.C., Mofenson, L.M., Davenny, K., Landesman, S., Cotton, D., Hanson, I.C., Hillyer, G.V., Tang, H.B., and Thomas, D.L. (1997). Increased vertical transmission of human immunodeficiency virus from hepatitis C virus-coinfected mothers. Women and Infants Transmission Study. *J.Infect.Dis.*; 176: 414-420.

Hladik, W., Kataaha, P., Mermin, J., Purdy, M., Otekat, G., Lackritz, E., Alter, M.J., and Downing, R. (2006). Prevalence and screening costs of hepatitis C virus among Ugandan blood donors. *Trop.Med.Int.Health*; 11: 951-954.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., and Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*; 373: 123-126.

Hoofnagle, J.H. (2002). Course and outcome of hepatitis C. *Hepatology*; 36: S21-S29.

Jacobson, K.R., Murray, K., Zellos, A., and Schwarz, K.B. (2002). An analysis of published trials of interferon monotherapy in children with chronic hepatitis C. *J.Pediatr.Gastroenterol.Nutr.*; 34: 52-58.

Jamal, M.M. and Abdelkarim, B.Z. (2003). Chronic hepatitis C with persistently normal aminotransferase levels: do we have an adequate definition? *Am.J.Gastroenterol.*; 98: 1455-1456.

Jara, P., Resti, M., Hierro, L., Giacchino, R., Barbera, C., Zancan, L., Crivellaro, C., Sokal, E., Azzari, C., Guido, M., and Bortolotti, F. (2003). Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. *Clin.Infect.Dis.*; 36: 275-280.

Jones, P. (1995). Haemophilia and HIV infection: some lessons learned. In Mok, J. and Newell, M-L. HIV infection in Children. Cambridge University Press.

Kamili, S., Krawczynski, K., McCaustland, K., Li, X., and Alter, M.J. (2007). Infectivity of hepatitis C virus in plasma after drying and storing at room temperature. *Infect.Control Hosp.Epidemiol.* ; 28: 519-524.

Kane, A., Lloyd, J., Zaffran, M., Simonsen, L., and Kane, M. (1999). Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: model-based regional estimates. *Bull.World Health Organ*; 77: 801-807.

Kariv, R., Leshno, M., Beth-Or, A., Strul, H., Blendis, L., Kokia, E., Noff, D., Zelber-Sagie, S., Sheinberg, B., Oren, R., and Halpern, Z. (2006). Re-evaluation of serum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study. *Liver Int.*; 26: 445-450.

Kerkar, N. (2005). Hepatitis B in children: complexities in management. *Pediatr.Transplant.*; 9: 685-691.

Kettaneh, A., Marcellin, P., Douvin, C., Poupon, R., Ziol, M., Beaugrand, M., and de, L., V. (2007). Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol.*; 46: 628-634.

Kirkwood, B. and Sterne, J. (2003). Essential Medical Statistics.

Kumar, R.M. and Shahul, S. (1998). Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. *J Hepatol.*; 29: 191-197.

Langford, S.E., Ananworanich, J., and Cooper, D.A. (2007). Predictors of disease progression in HIV infection: a review. *AIDS Res.Ther.*; 4: 11-

Lin, H.H., Kao, J.H., Hsu, H.Y., Ni, Y.H., Chang, M.H., Huang, S.C., Hwang, L.H., Chen, P.J., and Chen, D.S. (1995). Absence of infection in breast-fed infants born to hepatitis C virus-infected mothers. *J Pediatr.*; 126: 589-591.

Little, K., Thorne, C., Luo, C., Bunders, M., Ngongo, N., McDermott, P., and Newell, M.L. (2007). Disease progression in children with vertically-acquired HIV infection in sub-Saharan Africa: reviewing the need for HIV treatment. *Curr.HIV Res.*; 5: 139-153.

Lockitch, G., Halstead, A.C., Albersheim, S., MacCallum, C., and Quigley, G. (1988). Age- and sex-specific pediatric reference intervals for biochemistry analytes as measured with the Ektachem-700 analyzer. *Clin.Chem.*; 34: 1622-1625.

Mast, E.E., Hwang, L.Y., Seto, D.S., Nolte, F.S., Nainan, O.V., Wurtzel, H., and Alter, M.J. (2005). Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. *J Infect.Dis.*; 192: 1880-1889.

Mathews, G. and Bhagani, S. (2003). The epidemiology and natural history of HIV/HBV and HIV/HCV co-infections. *J.HIV.Ther.*; 8: 77-84.

Matsuoka, S., Tatara, K., Hayabuchi, Y., Nii, M., Mori, K., and Kuroda, Y. (1994). Post-transfusion chronic hepatitis C in children. *J.Paediatr.Child Health*; 30: 544-546.

Micheloud, D., Berenguer, J., Bellon, J.M., Miralles, P., Cosin, J., Lopez-Bernaldo de Quiros JC, Conde, M.S., Munoz-Fernandez, M.A., and Resino, S. (2008). Negative influence of age on CD4(+) cell recovery after highly active antiretroviral therapy in naive HIV-1-infected patients with severe immunodeficiency. *J Infect.*; 56: 130-136.

Mofenson, L.M. (2003). Advances in the prevention of vertical transmission of human immunodeficiency virus. *Semin.Pediatr.Infect.Dis.*; 14: 295-308.

Mohamadnejad, M., Pourshams, A., Malekzadeh, R., Mohamadkhani, A., Rajabiani, A., Asgari, A.A., Alimohamadi, S.M., Razjooyan, H., and Mamar-Abadi, M. (2003). Healthy ranges of serum alanine aminotransferase levels in Iranian blood donors. *World J Gastroenterol.*; 9: 2322-2324.

Mohsen, A.H., Easterbrook, P., Taylor, C.B., and Norris, S. (2002). Hepatitis C and HIV-1 coinfection. *Gut*; 51: 601-608.

Mok, J., Pembrey, L., Tovo, P.A., and Newell, M.L. (2005). When does mother to child transmission of hepatitis C virus occur? *Arch.Dis.Child Fetal Neonatal Ed*; 90: F156-F160.

Morris K. (2006). Transfusion-related HIV outbreak in Kazakhstan children. *Lancet Infect.Dis.*; 6: 689.

Munoz, A., Wang, M.C., Bass, S., Taylor, J.M., Kingsley, L.A., Chmiel, J.S., and Polk, B.F. (1989). Acquired immunodeficiency syndrome (AIDS)-free time after human immunodeficiency virus type 1 (HIV-1) seroconversion in homosexual men. Multicenter AIDS Cohort Study Group. *Am.J Epidemiol.*; 130: 530-539.

Murakami, J., Okamoto, M., Miyata, H., Nagata, I., Shiraki, K., and Hino, S. (2000). Evolution in the hypervariable region of hepatitis C virus in infants after vertical transmission. *Pediatr.Res.*; 48: 450-456.

Nduati, R., John, G., Mbori-Ngacha, D., Richardson, B., Overbaugh, J., Mwatha, A., Ndinya-Achola, J., Bwayo, J., Onyango, F.E., Hughes, J., and Kreiss, J. (2000). Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA*; 283: 1167-1174.

Nelson, K.E. and Thomas, D.L. (2001). Reciprocal interaction of human immunodeficiency virus and hepatitis C virus infections. *Clin.Diagn.Lab Immunol.*; 8: 867-870.

Nelson, M., Matthews, G., Brook, M.G., and Main, J. (2005). BHIVA guidelines on HIV and chronic hepatitis: coinfection with HIV and hepatitis C virus infection (2005). *HIV.Med.*; 6 Suppl 2: 96-106.

Newell, M.L. (1998). Mechanisms and timing of mother-to-child transmission of HIV-1. *AIDS*; 12: 831-837.

Newell, M.L. and Pembrey, L. (2002). Mother-to-child transmission of hepatitis C virus infection. *Drugs Today (Barc.)*; 38: 321-337.

Newell, M.L., Coovadia, H., Cortina-Borja, M., Rollins, N., Gaillard, P., and Dabis, F. (2004). Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet*; 364: 1236-1243.

Ni, Y.H., Chang, M.H., Lue, H.C., Hsu, H.Y., Wang, M.J., Chen, P.J., and Chen, D.S. (1994). Posttransfusion hepatitis C virus infection in children. *J.Pediatr.*; 124: 709-713.

Nigro G, D'Orio F, Catania S, Badolato MC, Livadiotti S, Bernardi S, and Argenio PD. (1997). Mother to infant transmission of coinfection by human immunodeficiency virus and hepatitis C virus: prevalence and clinical manifestations. *Archives of Virology*; 142: 453-457.

O'Riordan, J.M., Conroy, A., Nourse, C., Yap, P.L., McDonald, G.S., Kaminski, G., Leong, K., Lawlor, E., Davoren, A., Strong, K., Davidson, F., Lloyd, A., and Power, J. (1998). Risk of hepatitis C infection in neonates transfused with blood from donors infected with hepatitis C. *Transfus.Med.*; 8: 303-308.

Paccagnini, S., Principi, N., Massironi, E., Tanzi, E., Romano, L., Muggiasca, M.L., Ragni, M.C., and Salvaggio, L. (1995). Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr.Infect.Dis.J.*; 14: 195-199.

Palomba, E., Manzini, P., Fiammengo, P., Maderni, P., Saracco, G., and Tovo, P.A. (1996). Natural history of perinatal hepatitis C virus infection. *Clin.Infect.Dis.*; 23: 47-50.

Palumbo, P.E., Raskino, C., Fiscus, S., Pahwa, S., Fowler, M.G., Spector, S.A., Englund, J.A., and Baker, C.J. (1998). Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. *JAMA*; 279: 756-761.

Papaevangelou, V., Pollack, H., Rochford, G., Kokka, R., Hou, Z., Chernoff, D., Hanna, B., Krasinski, K., and Borkowsky, W. (1998). Increased transmission of vertical hepatitis C virus (HCV) infection to human immunodeficiency virus (HIV)-infected infants of HIV- and HCV-coinfected women. *J.Infect.Dis.*; 178: 1047-1052.

Patel PR, Larson AK, Castel AD, Ganova-Raeva LM, Myers RA, Roup BJ, Farrell KP, Edwards L, Nainan O, Krick JP, Blythe D, Fiore AE, and Rochford, G. (2006). Hepatitis C virus infection from a contaminated radiopharmaceutical used in Myocardial perfusion studies. *JAMA*; 296: 2005-2011.

Pembrey, L., Newell, M.L., Tovo, P.A., van Drimmelen, H., Quinti, I., Furlini, G., Galli, S., Meliconi, M.G., Burns, S., Hallam, N., Sonnerborg, A., Cilla, G., Serrano, E., Laccetti, P., Portella, G., Polywka, S., Icardi, G., Bruzzone, B., Balbo, L., and Alfarano, A. (2003). Inter-laboratory comparison of HCV-RNA assay results: implications for multi-centre research. *J.Med.Virol.*; 69: 195-201.

Pietrobelli, A., Faith, M.S., Allison, D.B., Gallagher, D., Chiumello, G., and Heymsfield, S.B. (1998). Body mass index as a measure of adiposity among children and adolescents: a validation study. *J Pediatr.*; 132: 204-210.

Piton, A., Poynard, T., Imbert-Bismut, F., Khalil, L., Delattre, J., Pelissier, E., Sansonetti, N., and Opolon, P. (1998). Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. *Hepatology*; 27: 1213-1219.

Pliner, V., Weedon, J., Thomas, P.A., Steketee, R.W., Abrams, E.J., Lambert, G., Greenberg, B., Bamji, M., Thea, D.M., and Matheson, P.B. (1998). Incubation period of HIV-1 in perinatally infected children. New York City Perinatal HIV Transmission Collaborative Study Group. *AIDS*; 12: 759-766.

Pokrovski, V. (1992). Localization of nosocomial outbreak of HIV infection in southern Russia in 1988-89. *Proceedings of the Eighth International Conference on AIDS; July 1992.*

Popova, I.A., Burova, N.V., Fomin, I., Rakhmanova, A.G., Voronin, E.E., and Galkina, M.V. (1999). The clinical course of HIV infection in children who were parenterally infected. *Zh.Mikrobiol.Epidemiol.Immunobiol.*; 75-78.

Powis, J., Peltekian, K.M., Lee, S.S., Sherman, M., Bain, V.G., Cooper, C., Krajden, M., Deschenes, M., Balshaw, R.F., Heathcote, E.J., and Yoshida, E.M. (2008). Exploring differences in response to treatment with peginterferon alpha 2a (40kD) and ribavirin in chronic hepatitis C between genotypes 2 and 3. *J Viral Hepat*; 15: 52-57.

Poynard, T., Ratziu, V., McHutchison, J., Manns, M., Goodman, Z., Zeuzem, S., Younossi, Z., and Albrecht, J. (2003). Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology*; 38: 75-85.

Prati, D., Taioli, E., Zanella, A., Della, T.E., Butelli, S., Del Vecchio, E., Vianello, L., Zanuso, F., Mozzi, F., Milani, S., Conte, D., Colombo, M., and Sirchia, G. (2002). Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann.Intern.Med.*; 137: 1-10.

Prati, D. (2006). Transmission of hepatitis C virus by blood transfusions and other medical procedures: a global review. *J Hepatol.*; 45: 607-616.

Prendergast, A., Tudor-Williams, G., Jeena, P., Burchett, S., and Goulder, P. (2007). International perspectives, progress, and future challenges of paediatric HIV infection. *Lancet*; 370: 68-80.

Prins, M. and Veugelers, P.J. (1997). Comparison of progression and non-progression in injecting drug users and homosexual men with documented dates of HIV-1 seroconversion.

European Seroconverter Study and the Tricontinental Seroconverter Study. *AIDS*; 11: 621-631.

Quiros-Tejeira, R.E., Rivera, C.A., Ziba, T.T., Mehta, N., Smith, C.W., and Butte, N.F. (2007). Risk for nonalcoholic fatty liver disease in Hispanic youth with BMI > or =95th percentile. *J Pediatr.Gastroenterol.Nutr.*; 44: 228-236.

Qurishi, N., Kreuzberg, C., Luchters, G., Effenberger, W., Kupfer, B., Sauerbruch, T., Rockstroh, J.K., and Spengler, U. (2003). Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection. *Lancet*; 362: 1708-1713.

Rerksuppaphol, S., Hardikar, W., and Dore, G.J. (2004). Long-term outcome of vertically acquired and post-transfusion hepatitis C infection in children. *J.Gastroenterol.Hepatol.*; 19: 1357-1362.

Resti, M., Azzari, C., Mannelli, F., Moriondo, M., Novembre, E., de Martino, M., and Vierucci, A. (1998). Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. *British Medical Journal*; 317: 437-441.

Resti, M., Jara, P., Hierro, L., Azzari, C., Giacchino, R., Zuin, G., Zancan, L., Pedditzi, S., and Bortolotti, F. (2003). Clinical features and progression of perinatally acquired hepatitis C virus infection. *J.Med.Virol.*; 70: 373-377.

Rezza, G. (1998). Determinants of progression to AIDS in HIV-infected individuals: an update from the Italian Seroconversion Study. *J Acquir. Immune. Defic. Syndr. Hum. Retrovirol.*; 17 Suppl 1: S13-S16.

Rockstroh, J.K. and Spengler, U. (2004). HIV and hepatitis C virus co-infection. *Lancet Infect.Dis.*; 4: 437-444.

Rosenthal, E., Poiree, M., Pradier, C., Perronne, C., Salmon-Ceron, D., Geffray, L., Myers, R.P., Morlat, P., Pialoux, G., Pol, S., and Cacoub, P. (2003). Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study). *AIDS*; 17: 1803-1809.

Rothenberg, R., Woelfel, M., Stoneburner, R., Milberg, J., Parker, R., and Truman, B. (1987). Survival with the acquired immunodeficiency syndrome. Experience with 5833 cases in New York City. *N.Engl.J Med.*; 317: 1297-1302.

Rouet, F., Sakarovitch, C., Msellati, P., Elenga, N., Montcho, C., Viho, I., Blanche, S., Rouzioux, C., Dabis, F., and Leroy, V. (2003). Pediatric viral human immunodeficiency virus type 1 RNA levels, timing of infection, and disease progression in African HIV-1-infected children. *Pediatrics*; 112: e289.

Ruiz-Extremera, A., Salmeron, J., Torres, C., De Rueda, P.M., Gimenez, F., Robles, C., and Miranda, M.T. (2000). Follow-up of transmission of hepatitis C to babies of human immunodeficiency virus-negative women: the role of breast-feeding in transmission. *Pediatr.Infect.Dis.J.* ; 19: 511-516.

Sasaki, N., Matsui, A., Momoi, M., Tsuda, F., and Okamoto, H. (1997). Loss of circulating hepatitis C virus in children who developed a persistent carrier state after mother-to-baby transmission. *Pediatr.Res.*; 42: 263-267.

Scarlatti, G. (2004). Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. *AIDS Rev.*; 6: 67-78.

Seef, L.B. (1997). Natural history of Hepatitis C. *Hepatology*; 26: 21S-28S.

Sharland, M., Blanche, S., Castelli, G., Ramos, J., and Gibb, D.M. (2004). PENTA guidelines for the use of antiretroviral therapy, 2004. *HIV Med.*; 5 Suppl 2: 61-86.

Shearer, W.T., Quinn, T.C., LaRussa, P., Lew, J.F., Mofenson, L., Almy, S., Rich, K., Handelsman, E., Diaz, C., Pagano, M., Smeriglio, V., and Kalish, L.A. (1997). Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. *N.Engl.J.Med.*; 336: 1337-1342.

Siest, G., Schiele, F., Galteau, M.M., Panek, E., Steinmetz, J., Fagnani, F., and Gueguen, R. (1975). Aspartate aminotransferase and alanine aminotransferase activities in plasma: statistical distributions, individual variations, and reference values. *Clin.Chem.*; 21: 1077-1087.

Staples, C.T., Jr., Rimland, D., and Dudas, D. (1999). Hepatitis C in the HIV (human immunodeficiency virus) Atlanta V.A. (Veterans Affairs Medical Center) Cohort Study (HAVACS): the effect of coinfection on survival. *Clin.Infect.Dis.*; 29: 150-154.

Stauber, R.E. and Lackner, C. (2007). Noninvasive diagnosis of hepatic fibrosis in chronic hepatitis C. *World J Gastroenterol.*; 13: 4287-4294.

Sulkowski, M.S., Moore, R.D., Mehta, S.H., Chaisson, R.E., and Thomas, D.L. (2002). Hepatitis C and progression of HIV disease. *JAMA*; 288: 199-206.

Sulkowski, M.S., Mehta, S.H., Torbenson, M., Afdhal, N.H., Mirel, L., Moore, R.D., and Thomas, D.L. (2005). Hepatic steatosis and antiretroviral drug use among adults coinfected with HIV and hepatitis C virus. *AIDS*; 19: 585-592.

Tedaldi, E.M., Baker, R.K., Moorman, A.C., Alzola, C.F., Furhrer, J., McCabe, R.E., Wood, K.C., and Holmberg, S.D. (2003). Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. *Clin.Infect.Dis.*; 36: 363-367.

Thomas, D.L., Villano, S.A., Riester, K.A., Hershow, R., Mofenson, L.M., Landesman, S.H., Hollinger, F.B., Davenny, K., Riley, L., Diaz, C., Tang, H.B., and Quinn, T.C. (1998). Perinatal transmission of hepatitis C virus from human immunodeficiency virus type 1-infected mothers. Women and Infants Transmission Study. *J.Infect.Dis.*; 177: 1480-1488.

Thomas, S.L., Newell, M.L., Peckham, C.S., Ades, A.E., and Hall, A.J. (1998). A review of hepatitis C virus (HCV) vertical transmission: risks of transmission to infants born to mothers with and without HCV viraemia or human immunodeficiency virus infection. *Int.J.Epidemiol.*; 27: 108-117.

Thorne, C. and Newell, M.L. (2000). Epidemiology of HIV infection in the newborn. *Early Hum.Dev.*; 58: 1-16.

Thorne, C., Newell, M.L., Botet, F.A., Bohlin, A.B., Ferrazin, A., Giaquinto, C., de, J.G., I, Mok, J.Y., Mur, A., and Peltier, A. (2002). Older children and adolescents surviving with vertically acquired HIV infection. *J Acquir.Immune.Defic.Syndr.*; 29: 396-401.

Torres-Puente, M., Cuevas, J.M., Jimenez-Hernandez, N., Bracho, M.A., Garcia-Robles, I., Wrobel, B., Carnicer, F., Del Olmo, J., Ortega, E., Moya, A., and Gonzalez-Candelas, F. (2008). Genetic variability in hepatitis C virus and its role in antiviral treatment response. *J Viral Hepat*; 15: 188-199.

Tovo, P.A., Palomba, E., Ferraris, G., Principi, N., Ruga, E., Dallacasa, P., and Maccabruni, A. (1997). Increased risk of maternal-infant hepatitis C virus transmission for women coinfected with human immunodeficiency virus type 1. Italian Study Group for HCV Infection in Children. *Clin.Infect.Dis.*; 25: 1121-1124.

Tovo, P.A., Pembrey, L.J., and Newell, M.L. (2000). Persistence rate and progression of vertically acquired hepatitis C infection. European Paediatric Hepatitis C Virus Infection 15. *J.Infect.Dis.*; 181: 419-424.

UNAIDS (2007). UNAIDS Annual Report 2007: Knowing your epidemic. http://data.unaids.org/pub/Report/2008/jc1535_annual_report07_en.pdf. Accessed 10.02.2008.

Vigano', A., Vella, S., Principi, N., Bricalli, D., Sala, N., Salvaggio, A., Saresella, M., Vanzulli, A., and Clerici, M. (1999). Thymus volume correlates with the progression of vertical HIV infection. *AIDS*; 13: F29-F34.

Violari, A., Cotton, D., Gibb, D.M., and and the CHER Study Team. (2007). Antiretroviral therapy initiated before 12 weeks of age reduces early mortality in young HIV-infected infants: evidence from the Children with HIV Early Antiretroviral Therapy (CHER) Study. *4th IAS Conference.Sydney, AU.*

Visco-Comandini, U., Cappiello, G., Liuzzi, G., Tozzi, V., Anzidei, G., Abbate, I., Amendola, A., Bordi, L., Budabbus, M.A., Eljhawi, O.A., Mehabresh, M.I., Girardi, E.,

Antinori, A., Capobianchi, M.R., Sonnerborg, A., and Ippolito, G. (2002). Monophyletic HIV type 1 CRF02-AG in a nosocomial outbreak in Benghazi, Libya. *AIDS Res.Hum.Retroviruses*; 18: 727-732.

Vogt, M., Lang, T., Frosner, G., Klingler, C., Sendl, A.F., Zeller, A., Wiebecke, B., Langer, B., Meisner, H., and Hess, J. (1999). Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N.Engl.J.Med.*; 341: 866-870.

Warszawski, J., Tubiana, R., Le Chenadec, J., Blanche, S., Teglas, J.P., Dollfus, C., Faye, A., Burgard, M., Rouzioux, C., and Mandelbrot, L. (2008). Mother-to-child HIV transmission despite antiretroviral therapy in the ANRS French Perinatal Cohort. *AIDS*; 22: 289-299.

World Health Organisation (2005). Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance. http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf. Accessed 10.02.2008.

World Health Organisation (2007) Hepatitis C fact sheet. http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed 10.02.2008.

Wilfert, C.M. and Stringer, J.S. (2004). Prevention of pediatric human immunodeficiency virus. *Semin.Pediatr.Infect.Dis.*; 15: 190-198.

Wirth, S., Pieper-Boustani, H., Lang, T., Ballauff, A., Kullmer, U., Gerner, P., Wintermeyer, P., and Jenke, A. (2005). Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. *Hepatology*; 41: 1013-1018.

Yerly, S., Quadri, R., Negro, F., Barbe, K.P., Cheseaux, J.J., Burgisser, P., Siegrist, C.A., and Perrin, L. (2001). Nosocomial outbreak of multiple bloodborne viral infections. *J.Infect.Dis.*; 184: 369-372.

Yoo, T.W., Donfield, S., Lail, A., Lynn, H.S., and Daar, E.S. (2005). Effect of hepatitis C virus (HCV) genotype on HCV and HIV-1 disease. *J.Infect.Dis.*; 191: 4-10.

Zaba, B., Whiteside, A., and Boerma, J.T. (2004). Demographic and socioeconomic impact of AIDS: taking stock of the empirical evidence. *AIDS*; 18 Suppl 2: S1-S7.

Zacharakis, G., Koskinas, J., Kotsiou, S., Pouliou, E., Papoutselis, M., Tzara, F., Vafeiadis, N., Maltezos, E., Archimandritis, A., and Papoutselis, K. (2007). Natural history of chronic hepatitis B virus infection in children of different ethnic origins: a cohort study with up to 12 years' follow-up in northern Greece. *J Pediatr.Gastroenterol.Nutr.*; 44: 84-91.

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APPENDIX 4 CLINICIANS RESPONDING TO THE SURVEY OF CLINICAL MANAGEMENT PRACTICES AND POLICIES OF CHILDREN COINFECTED WITH HIV AND HCV

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APPENDIX 5 EUROPEAN PAEDIATRIC HCV NETWORK DATA COLLECTION FORMS

FORM A

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

MATERNAL ENROLLMENT INFORMATION

EPHN ID number __ __ / __ __ __ / __ __ __
centre no. mother no. child no.

Mother's date of birth __ __/__ __/__ __ __ __

Most likely mode of maternal acquisition of HCV infection

- blood transfusion/blood products ☐
- injecting drug use ☐
- other ☐ *please specify* _____
- not known ☐

Reason for HCV testing

- mother's request ☐
- routine antenatal policy ☐
- identified as high risk group (selective screening policy) ☐
- clinical (e.g. signs of hepatitis) ☐
- other ☐ *please specify* _____

Has the mother used intravenous drugs?

- yes, during this pregnancy ☐
- yes, but not during this pregnancy ☐
- never ☐
- don't know ☐

Is the mother HIV infected? Yes ☐ No ☐ Don't know ☐

If yes, CDC stage: (please circle) *A1 A2 A3 B1 B2 B3 C1 C2 C3*

Has this mother been previously reported to the Network? Yes ☐ No ☐

If yes, please give EPHN ID number(s): __ __ /__ __ __ /__ __ __

FORM B

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

MATERNAL CLINICAL AND LABORATORY INFORMATION DURING PREGNANCY

EPHN ID number ____/____/____
centre no. mother no. child no.

HCV test results
Please give details of ALL tests carried out during **this pregnancy and at delivery**, starting with most recent.
If available, please also give details of earlier tests.

ELISA	Date					
	Result					
RIBA	Date					
	Result					
PCR-RNA	Date					
	Result					
RNA copies per ml	Date					
	Result					
ALT IU/L	Date					
	Result					

Has the mother's HCV genotype been determined? Yes ☐
No ☐

If yes, please specify genotype:_____

Has the mother received treatment during pregnancy or during delivery for HCV or HIV infection?

Yes ☐ If yes, name of drug dosage
No ☐ ....

....

date treatment started __ __/__ __/__ __ __ __

date treatment stopped __ __/__ __/__ __ __ __

Was a 1 ml serum sample taken and stored from the mother at or near delivery?

Yes ☐ Date: __ __/__ __/__ __ __ __
No ☐ *If not, why not:*.....

FORM C

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

DELIVERY INFORMATION

EPHN ID number ___/___/___
centre no. / mother no. / child no.

Date of delivery ___/___/_____
day / month / year

Mode of delivery

vaginal, spontaneous ☐
vaginal, instrumented ☐
unknown ☐
emergency Caesarean section ☐
elective Caesarean section* ☐

*i.e. before onset of labour and rupture of membranes

Total duration of labour: __ __ hrs __ __ mins

Time from rupture of membranes to delivery: __ __ hrs __ __ mins

Were any obstetric interventions (e.g. fetal scalp monitors, forceps) used during delivery? Yes ☐ *If yes, please specify*.....
No ☐
Don't know ☐

NEONATE INFORMATION

Birth weight (grams) .....

Gestational age (weeks) .....

Gender male ☐
female ☐

Was any anti-retroviral treatment to reduce the risk of transmission of HIV administered to the neonate?

		If yes,	*name of drug*	*dosage*
Yes	☐		.....	.....
No	☐			

FORM D1

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

PAEDIATRIC FOLLOW-UP: CLINICAL EXAMINATION
For examinations at birth, 6 weeks and 3 months of age

EPHN ID number ___ ___ / ___ ___ / ___ ___ (centre no. / mother no. / child no.) Date of examination ___ / ___ / ___ (day / month / year)

Date of birth ___ / ___ / ___ (day / month / year)

Weight (kg) ____ . ___

Height (cm) _____

Is this child HIV infected? Yes ☐ No ☐ Indeterminate ☐

If yes, CDC stage (please circle): N1 N2 N3 A1 A2 A3 B1 B2 B3 C1 C2 C3

Clinical manifestations
Has this child developed any of the following symptoms since the last visit?

		Please give details
Growth failure	yes / no	.....
Hepatomegaly	yes / no	.....
Splenomegaly	yes / no	.....
Nephropathy	yes / no	.....
Cardio-pulmonary	yes / no	.....
Skin disorders	yes / no	.....
Haematological disorders	yes / no	.....
Auto-immune disease	yes / no	.....
Other	yes / no	.....

Was this child ever breast-fed? Yes ☐ No ☐ Don't know ☐ *If yes, age stopped (weeks)* ____

FORM D2

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

PAEDIATRIC FOLLOW-UP: CLINICAL EXAMINATION
For examinations at 6 months of age and older

EPHN ID number ___/___/___ Date of examination ___/___/_____
centre no. mother no. child no. day month year

Date of birth ___/___/_____
day month year

Weight (kg) ____ . ___

Height (cm) _____

Is this child HIV infected? Yes ☐
No ☐
Indeterminate ☐

If yes, CDC stage (please circle): N1 N2 N3 A1 A2 A3 B1 B2 B3 C1 C2 C3

Clinical manifestations
Has this child developed any of the following symptoms since the last visit?
Please give details

Growth failure	yes / no	.....
Hepatomegaly	yes / no	.....
Splenomegaly	yes / no	.....
Nephropathy	yes / no	.....
Cardio-pulmonary	yes / no	.....
Skin disorders	yes / no	.....
Haematological disorders	yes / no	.....
Auto-immune disease	yes / no	.....
Other	yes / no	.....

FORM D2

Has this child been vaccinated against:

hepatitis B? Yes ☐ *If yes, date of first dose* __ __/__ __/__ __ __ __
No ☐

hepatitis A? Yes ☐ *If yes, date of first dose* __ __/__ __/__ __ __ __
No ☐

Has this child had an ultrasound since the last visit? Yes ☐
No ☐

If yes, date of ultrasound __/__/____

result .....
.....
.....

Has this child had a liver biopsy since the last visit? Yes ☐
No ☐

If yes, date of biopsy __/__/____

reason for biopsy .....

result .....
.....
.....

Is this child receiving treatment for HCV infection? Yes ☐
No ☐

Date treatment started __ __/__ __/__ __ __ __

Reason for starting treatment .....
.....

Drug ..... Dosage.....

If child is no longer being treated, date treatment stopped _ _/_ _/_ _ _ _

Reason for stopping treatment .....
.....

FORM E1

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

PAEDIATRIC FOLLOW-UP - LABORATORY TEST INFORMATION
For tests done at birth, 6 weeks, and 3 months of age

EPHN ID number __ __/__ __ __/__ __ __
centre no. mother no. child no.

Details of laboratory tests carried out since the last visit:

HCV PCR tests

Date of test	*Result (positive/negative)*	*Viral load* - copies per ml*

*method used: .....

HCV Antibody tests

Date of test	*Type of test*	*Result*	
	ELISA or RIBA, 2nd or 3rd generation	*positive/negative/ indeterminate?*	*no.of bands*

ALT levels

Date of test	*ALT level (IU/l)*

Samples to be stored

Was a Guthrie card (filter paper) sample taken and stored (birth & 3 mths)?

Yes ☐ Date: __ __/__ __/__ __ __ __

No ☐ If not, why not: .....

Was a 1 ml serum or plasma sample taken and stored (3 mths)?

Yes ☐ Date: __ __/__ __/__ __ __ __

No ☐ If not, why not:.....

Other tests

	Date of test	Result
haemoglobin		g/dl
		g/dl
		g/dl
WBC		10^6/ml
		10^6/ml
		10^6/ml
lymphocytes		%
		%
		%
neutrophils		%
		%
		%
platelets		/ml
		/ml
		/ml
coagulation screen *normal or abnormal:* *if abnormal please specify*		
IgG		mg/dl
		mg/dl
		mg/dl
IgM		mg/dl
		mg/dl
		mg/dl
IgA		mg/dl
		mg/dl
		mg/dl
IgE		IU/ml
		IU/ml
		IU/ml
total bilirubin		mg/dl
		mg/dl
		mg/dl
albumin		g/dl
		g/dl
		g/dl
alpha fetoprotein		ng/ml
		ng/ml
		ng/ml

FORM E1

Is the HCV genotype of this child known?	Yes ☐ If yes, genotype ____ No ☐

Have any other tests been carried out for this child?	Yes ☐ No ☐

If yes, please give details below (date, type of test, result):

Date	*Type of test*	*Result*

FORM E2

EUROPEAN PAEDIATRIC HCV NETWORK
Prospective Study

PAEDIATRIC FOLLOW-UP - LABORATORY TEST INFORMATION
For tests done at 6 months of age and older

EPHN ID number __ __ / __ __ __ / __ __ __
centre no. mother no. child no.

Details of laboratory tests carried out since the last visit:

HCV PCR tests

Date of test	*Result (positive/negative)*	*Viral load* - copies per ml*

*method used: .....

HCV Antibody tests

Date of test	*Type of test* *ELISA or RIBA, 2nd or 3rd generation*	*Result*	
		positive/negative/ indeterminate?	*no.of bands*

ALT levels

Date of test	*ALT level (IU/l)*

Samples to be stored
Was a 1 ml serum or plasma sample taken and stored (at 12 mths, 24 mths, then once a year)?

Yes ☐ Date: __ __ / __ __ / __ __ __ __

No ☐ If not, why not:.....

FORM E2

Other tests

	Date of test	Result
haemoglobin		g/dl
		g/dl
		g/dl
WBC		10^6/ml
		10^6/ml
		10^6/ml
lymphocytes		%
		%
		%
neutrophils		%
		%
		%
platelets		/ml
		/ml
		/ml
coagulation screen *normal or abnormal:* *if abnormal please specify*		
IgG		mg/dl
		mg/dl
		mg/dl
IgM		mg/dl
		mg/dl
		mg/dl
IgA		mg/dl
		mg/dl
		mg/dl
IgE		IU/ml
		IU/ml
		IU/ml
total bilirubin		mg/dl
		mg/dl
		mg/dl
albumin		g/dl
		g/dl
		g/dl
alpha fetoprotein		ng/ml
		ng/ml
		ng/ml

Has this child been tested for the presence of auto-antibody since the last visit?

Yes ☐ If yes, result _____

No ☐

Is the HCV genotype of this child known? Yes ☐ If yes, genotype ____

No ☐

Have any other tests been carried out for this child? Yes ☐

No ☐

If yes, please give details below (date, type of test, result):

Date	*Type of test*	*Result*

APPENIDIX 6 EUROPEAN COLLABORATIVE STUDY DATA COLLECTION FORMS

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ECS3
INTENSIVE PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS

MATERNAL INFORMATION AT DELIVERY

Centre
Mothers Study Number
Child Study Number

Mother's date of birth (day, month, year)
Country of birth .....

Marital Status

Single (1), Married (2), Divorced, Separated, Widowed (3), Cohabiting (4)

Ethnic Group

Asian (1), White (2), Black (3), Oriental (4), Other (5)
Age when leaving full-time education, years .....

Obstetric History

Number of previous livebirths .....
Number of previous stillbirths .....
Number of previous miscarriages .....
Number of previous terminations .....

Mothers Risk Group

History of intravenous Drug Abuse (Y/N)
Trimester of last use: pre-conception (0), 1st (1), 2nd (2), 3rd (3), unknown (9)
Needle sharing? never (1) past (2) present (3) unknown (9)
Sexual partner of Bisexual (Y/N)
Sexual partner of Haemophiliac (Y/N)
Sexual partner of Intravenous Drug Abuser (Y/N)
Sexual partner of Other high risk group (Y/N)
(Specify) .....
Other .....

Mothers HIV History

Date of first HIV+ test (day, month, year)

Current clinical status

Current HIV staging (CDC) .....
Specify symptoms .....
Date of onset .....

Details of treatment during pregnancy

Has the woman received any antiretroviral therapy at any time during this pregnancy? Y/N
Please give details of both ART and other prophylaxis (eg. TMP-SMX)

Drug	Date started	Date stopped	Currently taken? (yes/no)

ECS 3
PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS

Page 2

MATERNAL INFORMATION

Laboratory investigations during pregnancy and at delivery:

Centre Number 1-2
Mothers Study Number 3-5
Child Study Number 6

Virology

	Date:	Date:	Date:
HIV-DNA PCR	Pos / Neg	Pos / Neg	Pos / Neg

HIV-RNA PCR	copies/ml	copies/ml	copies/ml
Sample type	Plasma / Serum	Plasma / Serum	Plasma / Serum
Assay used			

Other laboratory investigations

	Date:	Date:	Date:
Total lymphocytes			
CD4 (10^9/litre)			
CD8 (10^9/litre)			
IgG (gm/litre)			
IgA (gm/litre)			
IgM (gm/litre)			
p24 Ag			
HIV Elisa			

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ECS3

INTENSIVE PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS
MULTI CENTRE EEC STUDY

MEDICAL EXAMINATION

Please circle or complete as appropriate

Assessment at : 3w, 6w, 3m, 4.5m and 6 m

Centre 1-2

Mothers Study Number 3-5

Child Study Number 6

Date of Examination 7-12

Weight (kg) 13-16

Height (cm) 17-20

OFC (cm) 21-23

24 **For office use only**

Recurrent fever of unknown origin requiring medical attention .....Y/N 25

Chronic or Recurrent diarrhoea requiring medical attention .....Y/N 26

Specify organism 27

Bacterial infection .....Y/N

If yes, specify: 28-30

Septicaemia, Meningitis, Urinary tract infection, Pneumonia, Other 31-33

Communicable Disease .....Y/N

Measles (1) Mumps (2) Rubella (3) Varicella (4) Zoster (5) Other (6) 34-35

Complications .....

Skin Infection requiring medical attention .....Y/N 36-38

Staph (1) Strep (2) Herpes (3) Candida (4) Other (5) .....

Non-infectious skin eruption .....Y/N 39

Petechiae/Purpura (1) Eczema (2) Kaposi Sarcoma (3) Other (4) 40

Palpable Lymph Nodes .....Y/N 41-43

Axillary (1) Postoccipital (2) Cervical (3) Inguinal (4) Epitrochlear (5) Other (6) 44-46

Chronic parotid swelling .....Y/N 47

Oral Candida persistent or recurrent despite therapy .....Y/N 48

Upper respiratory tract infection .....Y/N

Chronic otitis media (1) Sinusitis (2) Chronic purulent rhinitis (3) Other (4)..... 49-50

Lower respiratory tract disease confirmed by X-ray.....Y/N

Lymphocytic intersitial pneumonitis or Pulmonary lymphoid hyperplasia (1)

Pneumonia (2) Bronchiolitis (3) Other (4) 51-53

specify organism, if known.....

Opportunistic Infection .....Y/N 54

PCP (1) CMV (2) Toxo (3) Candida (4) Mycobacterium (5) Other (6) .....

Hepatomegaly.....Y/N 55

Splenomegaly.....Y/N 56

7 1 57-58

Please circle or complete as appropriate

Medical Examination

Date of Examination ___ / ___ / ___

Centre 1-2

Mothers Study Number 3-5

Child Study Number 6 7

For office use onl

Neurological abnormality ..... Y/N 8-10

encephalopathy (static/progressive) (1) 11-13

seizures (2) paresis (3) pathologic reflexes (4) increased tone (5)

decreased tone (6) abnormal gait (7) other (8) 14-16

Other Findings on exam Specify ..... Y/N 17

Developmental Asessment

Gross motor	Pass (1) Fail (2) Suspicious (3)	18
Fine motor/adaptive	Pass (1) Fail (2) Suspicious (3)	19
Language	Pass (1) Fail (2) Suspicious (3)	20
Personal/social	Pass (1) Fail (2) Suspicious (3)	21

Loss of developmental milestones ..... Y/N

specify 22-23

Neonate

Has the baby received any anti-retroviral therapy to reduce

the risk of vertical transmission? ..... Y/N 24

If yes: which drug(s)? 25-26

for how long? 27

Treatment

Has this child been enrolled in an anti-retroviral treatment trial ..... Y/N 28

If yes: which trial? 29-31

Current treatment (excluding the above)

IVGG, AZT, DDi, Other 32-36

37-41

Hospital Admission(s) ..... Y/N 42

(Indicate dates of admission/discharge and diagnoses for each hospitalization)

..... 43-46

..... 47-50

Immunisations given since last visit ..... Y/N

DPT (1) DT(2) Oral Polio (3) Killed Polio (4) Measles (5) MMR (6)

Hepatitis B (7) Other (8) 51-52

Abnormal reactions ..... Y/N 53-54

55

Child care

mother / father / other relative / fostered / adopted / hospital / institution

Breast Feeding ..... Y/N 56

If stopped, when 57-58

Health of Mother .....

Is mother alive / dead? 59

if dead, was death HIV-related? ..... Y/N 60

cause of death 61

Mother's current HIV staging (CDC) 62-63

defining symtoms 64-68

date of diagnosis 69-73

current treatment? 74-75

8 2 76-77

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ECS.1

PROSPECTIVE STUDY OF CHILDREN BORN TO HIV +VE MOTHERS

<u>Assessment:</u> 9, 12, 18 and 24 months; thereafter annually for antibody -ve, uninfected children and 6-monthly for infected children

Please circle or tick as appropriate

Centre number 1-3
Mother Study number 4-6
Child Study number 7
Weight (kg) 8-11
Height (cm) 12-15
Head circumference (cm) 16-18

Is child alive? Y/N 19 **For office use only**
Date of assessment (day, month, year) 20-25

Name of paediatrician (initials) 26-27

Is this child HIV infected? . . . . . Y/N 28
Has this child developed AIDS? (CDC def) . . . . . Y/N 29
If AIDS has been diagnosed since previous report,
specify date of diagnosis 30-35
AIDS indicator disease 36-38

Care
mother / father / other relative / fostered / adopted / hospital / institution 39

Is mother alive / dead?
if dead, was death HIV-related? . . . . . Y/N 40
cause of death 41

Preschooling/Schooling
Does this child require special educational provisions . . . . . Y/N 42
if yes, specify 43-44

Treatment
Has this child been enrolled in an anti-retroviral treatment trial . . . . . Y/N 45
if yes, which trial? 46-48
Current treatment (excluding the above) 49-53
Intravenous gammaglobulin/AZT/DDi/other, specify 54-58
. . . . . .

Communicable diseases . . . . . Y/N 59
if yes, specify: measles / whooping cough / varicella / tuberculosis / mumps / zoster

G 2 1 60-62

Oral Candida since last visit (persisting > 2m or recurring despite therapy) Y / N

if yes, date on onset (day, month, year) .....

Hospital admission(s) Y / N

(indicate dates of admission/ discharge and diagnoses for each hospitalization)

.....

Since the last visit, has this child had any of the following abnormalities:

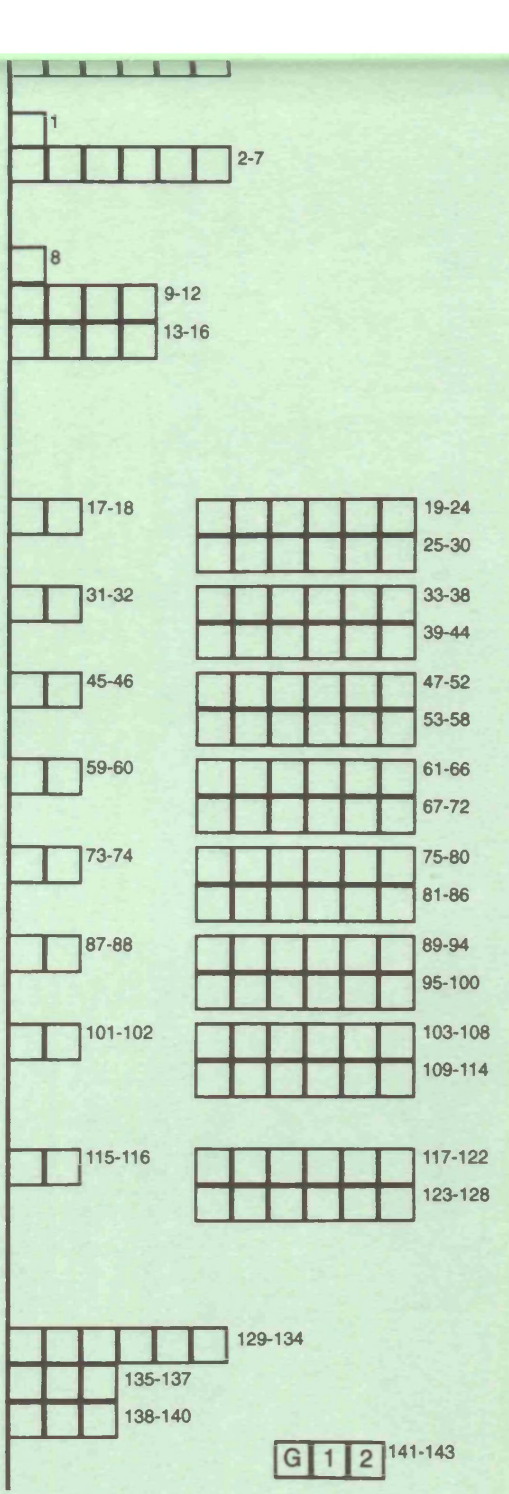
	yes/no	date	clinical presentation	method of diagnosis	diagnosis
renal	Y / N	.....			
hepatic	Y / N	.....			
gastro-intestinal	Y / N	.....			
cardio-vascular	Y / N	.....			
central nervous system	Y / N	.....			
respiratory	Y / N	.....			
malignancy	Y / N	.....			
other, specify	Y / N	.....			

Please complete this section, if the child has died

Date of death .....

Cause of death .....

Post mortem results .....



PROSPECTIVE STUDY OF CHILDREN BORN TO HIV POSITIVE MOTHERS

Centre 1-2
Mothers Study Number 3-5
Child Study Number 6

LABORATORY INVESTIGATIONS

Assessment at:
0-7 days, 3w, 6w, 4.5m, 6m, 9m, 12m, 18m, 24m, and then annually if child presumed not infected, or 6 monthly if infected

Ring findings and specify as appropriate:

for office use only

Date blood drawn: _____ / _____ / _____ 7-12
day month year

HIV / ELISA + / - Specify system used 13-14
antibodies 15-16

Western blot + / - 17-18

Virus culture 19-20
+ / - Specify identification system(s) 21-22
+ / - Specify identification system(s) .....

Viral load 23-24
DNA - PCR 25-26
RNA - PCR .....

Antigen assay + / - 27-29
Specify identification system .....

Other tests (eg IVAP, PCR, IgM) Specify method and result 30-32
+ / - 33-35
+ / - .....

IgG (gm/litre) 36-38
IgA (gm/litre) 39-41
IgM (gm/litre) 42-44
T4 (10^9/litre) 45-48
T8 (10^9/litre) 49-52
Absolute lymphocyte (10^9/litre) 53-56
Neutrophil (10^9/litre) 57-60
Platelet (10^9/litre) 61-64
Haemoglobin (gm/dl) 65-67

Toxo IgG Latex (at 9 months to exclude congenital infection) (+/-) 68
Tetanus IgG (at least 1 month after third DT/DPT) 69-71
CMV IgG (+/-) 72

8 1 73-74

APPENDIX 7 UK NATIONAL HCV REGISTER DATA COLLECTION FORMS

NATIONAL REGISTER HCV

NATIONAL REGISTER OF HCV INFECTIONS WITH A KNOWN DATE OF ACQUISITION

REGISTRATION FORM

The national register contains information on HCV infections with known dates of acquisition, and provides a facility for the future monitoring and long term assessment of HCV infection within the UK.

- No patient names are held in the HCV National Register. It is therefore very important that you retain the register number in your records and that you are able to trace the patient from either the register number or the identifier/reference number that you supply on the registration form (Question 1).
- Ethical approval for the register has been obtained from both the Public Health Laboratory Service and the Multi-Centre Research Ethics Committees.

Enquiries regarding either the HCV National Register or completion of the registration form should be directed to either:

Dr Helen Harris (Register Co-ordinator)
Telephone: 020 8200 6868

Ms Lisa Beck (HCV Research Assistant)
Telephone: 020 8200 6868

Register number: .....

A COLLABORATIVE PROJECT BETWEEN THE HEALTH PROTECTION AGENCY AND THE NATIONAL BLOOD AUTHORITY

Section 1: PATIENT DETAILS *(please insert details or tick boxes as appropriate)*

1. **Identifier by which you can recognise the patient in current and future correspondence (e.g. hospital number):**

   .....

2. **Date of birth (dd/mm/yy):** ...../...../.....

3. **Sex:** Male ☐ Female ☐

4. **Ethnic group:** White ☐ Black-Caribbean ☐ Black-African ☐ Black, other ☐ Indian ☐ Pakistani ☐
   Bangladeshi ☐ Chinese ☐ Asian, other ☐ Other, please specify: .....

5. **Country of birth:** .....

6. **If you are not the patient's GP, please give the name and address of their GP below:**

   Name: .....

   Address: .....

   .....

7. **Has the patient *ever* injected drugs (even if only once)?** Yes ☐ No ☐ Not known ☐

8. **Does the patient have any other known risk factors for HCV infection?** Yes ☐ No ☐ Not known ☐

   If **yes**, please give details: .....

9. **To your knowledge, does the patient have any other significant chronic viral infection?** Yes ☐ No ☐

   If **yes**, please specify: .....

10. **Does the patient suffer from any other significant medical conditions?** Yes ☐ No ☐

    If **yes**, please specify: .....

11. **Are you still responsible for the HCV-related care of the patient?**

    Yes ☐ ⇨ ***PLEASE COMPLETE THE REST OF THE FORM***

    No ☐ ⇨ ***PLEASE GIVE THE NAME AND ADDRESS OF THE CLINICIAN NOW RESPONSIBLE FOR THE HCV-RELATED CARE OF THIS PATIENT (AND THEN RETURN THE FORM TO US). PLEASE ALSO ENSURE THAT YOU INSERT YOUR DETAILS AT THE END OF THIS FORM SO THAT WE CAN CONTACT YOU IF WE NEED MORE INFORMATION ABOUT THIS PATIENT. THANK YOU VERY MUCH FOR YOUR HELP.***

    Name: .....

    Address: .....

    .....

Section 2: CURRENT CLINICAL STATUS

The next questions ask about the patient's current clinical status. In this context, clinical status is intended to reflect the patient's signs and/or symptoms of liver disease, *not* their test results.

1. **Has the patient died (please tick box)?** Yes ☐ No ☐

   If **yes**, please give date of death (dd/mm/yy): ...../...../..... and cause of death: .....

   If **no**, does the patient have: No clinical signs or symptoms of liver disease ☐ ⇨ ***PLEASE GO TO SECTION 3***

   Clinical signs or symptoms of liver disease (HCV-related) ☐

   Clinical signs or symptoms of liver disease (not HCV-related) ☐ ⇨ Details/cause: .....

2. **Please record any signs or symptoms of liver disease:**

   Spider naevi ☐ Hepatomegaly ☐ Splenomegaly ☐ Ascites ☐ Varices ☐ Bleeding varices ☐ Liver tumor ☐

   Other (please give details): .....

Section 3: TEST RESULTS

1. **Date of last consultation for HCV (dd/mm/yy):** ...../...../.....

2. **Has the patient been tested for hepatitis B infection, and if so, what were the results?**

HBsAg: Positive ☐ Negative ☐ Not tested ☐ Not sure ☐

anti-HBc: Positive ☐ Negative ☐ Not tested ☐ Not sure ☐

3. **Has the patient *ever* had a positive HCV PCR test?** Yes ☐ No ☐ Not tested ☐ Not known ☐

If **yes**, please give date of first known positive test (dd/mm/yy): ...../...../.....

4. **Date of latest HCV PCR test results (dd/mm/yy):** ...../...../..... Not done ☐ Not known ☐

Results (please tick box): Positive ☐ Negative ☐ Indeterminant ☐ Not known ☐

5. **Please insert the HCV genotype or serotype if known:** ..... Not known ☐

6. **Date of latest liver function tests (dd/mm/yy):** ...../...../..... Not done ☐ Not known ☐

Results (please tick box or enclose copy of report form): Normal ☐ Abnormal ☐ Not known ☐

If **abnormal**, please give results and test ranges: ALT ..... Range ..... AST ..... Range .....

Bilirubin ..... Range ..... Albumin ..... Range .....

INR .....

7. **Date of latest liver biopsy (dd/mm/yy):** ...../...../..... Not done ☐ Not known ☐

Results (please tick box): Normal ☐ Abnormal ☐ Not known ☐

If **abnormal**, please give results (enclose copy of report form, if possible):

Minimal change ☐ Chronic hepatitis ☐ Cirrhosis ☐ Hepatocellular carcinoma ☐

Fibrosis score (if known): ..... Scoring system: .....

Section 4: ANTIVIRAL DRUG TREATMENT

1. **Has the patient had any antiviral treatment for HCV?** Yes ☐ → *PLEASE CONTINUE WITH THIS SECTION*

No ☐ → *PLEASE GO TO QUESTION 3*

If **yes**, please insert details of treatment in the table below:

Course	Date started (dd/mm/yy)	Date finished (dd/mm/yy)	Interferon preparation (eg. Intron A®, Wellferon®)	Interferon dosage (mU)	Interferon schedule (eg. daily, twice weekly)	Other antiviral (eg. Ribavirin)	Dosage of other antiviral (please give units)	Schedule for other antiviral (eg. daily, twice weekly)
A								
B								
C								

2. **What was the response to treatment? (please tick only one of the 8 boxes below)**

Not relevant (still on treatment) ☐

Treatment stopped early (eg. due to side effects) ☐

No response (never became PCR negative) ☐

Response - Late relapse (PCR negative >12/12 after treatment but became positive at a later date) ☐

- Long term response (remains PCR negative 12/12 after treatment completed) ☐

- Sustained response (remains PCR negative 6/12 after treatment completed) ☐

- Immediate/initial response (PCR negative <6/12 after treatment completed) ☐

- Transient response (PCR negative during treatment but became positive after treatment) ☐

Section 4 continued overleaf

Section 4: ANTIVIRAL DRUG TREATMENT *continued*

3. **Has the patient taken part in any antiviral drug trials?** Yes ☐ No ☐

If **yes**, please give details: Name of trial: .....

Patient registration/code number: ..... Date of entry: ...../...../.....

4. **Has the patient received any other treatments for HCV? (eg. herbal treatments etc)** Yes ☐ No ☐

If **yes**, please give details: .....

.....

Section 5: CURRENT MANAGEMENT

1. **During the last 12 months what care has the patient received for HCV-related illness?**

None ☐ Outpatient only ☐ Inpatient (assessment only eg. liver biopsy) ☐ Inpatient (medical care) ☐

If the patient received **inpatient (medical care)** in the last 12 months for HCV-related illness, please give date and reason for each admission:

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

2. **What was the patient's alcohol intake at first diagnosis (in units of alcohol/week, if possible)?** ..... Not known ☐

3. **What is the patient's current alcohol intake (in units of alcohol/week, if possible)?** ..... Not known ☐

COMMENTS

If you have any comments that you would like to make, please do so in the space below:

Please print your details below so that we can contact you if we need more information about this patient:

Your name: .....

Date (dd/mm/yy): ...../...../..... Telephone number: .....

THANK YOU VERY MUCH FOR YOUR HELP

ALL THE INFORMATION YOU PROVIDE WILL BE TREATED IN CONFIDENCE

PLEASE RETURN THIS FORM TO:

Dr H E Harris (Register Co-ordinator)
Health Protection Agency
Immunisation Division, Communicable Disease Surveillance Centre
61 Colindale Avenue, London NW9 5EQ
Tel: 020 8200 6868 [redacted]

VAc01

NATIONAL REGISTER HCV

NATIONAL REGISTER OF HCV INFECTIONS WITH A KNOWN DATE OF ACQUISITION

FIRST FOLLOW-UP FORM

The national register contains information on HCV infections with known dates of acquisition, and provides a facility for the future monitoring and long term assessment of HCV infection within the UK.

- No patient names are held in the HCV National Register. It is therefore very important that you retain the register number in your records and that you are able to trace the patient from either the register number or the identifier/reference number that you supply on the form (Question 1).
- Ethical approval for the register has been obtained from both the Public Health Laboratory Service and the Multi-Centre Research Ethics Committees. There is no formal requirement to gain patient consent.

Enquiries regarding either the HCV National Register or completion of the form should be directed to:

Dr Helen Harris (Register Co-ordinator)
Telephone: 020 8200 6868 ext
Pager:
E-mail:

DATE OF REGISTRATION: ..../..../....

Register number: .....

Date of birth: ..../..../....

Soundex/initials: .....

Your reference: .....

A COLLABORATIVE PROJECT BETWEEN THE PUBLIC HEALTH LABORATORY SERVICE AND THE NATIONAL BLOOD AUTHORITY

Section 1: PATIENT DETAILS *(please insert details or tick boxes as appropriate)*

1. **Identifier by which you can recognise the patient in current and future correspondence (e.g. hospital number):**

   .....

2. **Are you still responsible for the HCV-related care of the patient?**

   Yes ☐ ➔ *Please continue*

   No ☐ ➔ *Please give the name and address of the clinician now responsible for the HCV-related care of this patient (and then return the form to us). Please also ensure that you insert your details at the end of this form so that we can contact you if we need more information about this patient.*
   THANK YOU VERY MUCH FOR YOUR HELP.

   Name: .....

   Address: .....

   .....

3. **Has this patient been seen, treated, or tested for HCV-related illness <u>since</u> they were registered (see front of form for date of registration)?**

   Yes ☐ ➔ *Please complete the rest of the form*

   No ☐ ➔ *Please go straight to Section 6*

Section 2: CURRENT CLINICAL STATUS

The next questions ask about the patient's current **clinical status**. In this context, **clinical status** is intended to reflect the patient's signs and/or symptoms of liver disease, *<u>not</u>* their test results. For example, a patient who has abnormal liver function, or whose biopsy indicates liver disease, should be classified as having "**no clinical evidence of liver disease**" if they have no physical signs or symptoms of liver disease.

1. **Has the patient died (please tick box)?** Yes ☐ No ☐

   If **yes**, please give date of death (dd/mm/yy): ..../..../.... and cause of death: .....

   If **no**, does the patient have: No clinical evidence of liver disease ☐ ➔ *Please go to section 3*

   Clinical evidence of liver disease (HCV-related) ☐

   Clinical evidence of liver disease (not HCV-related) ☐ ➔ Details/cause: .....

2. **Please record any signs and symptoms of liver disease:**

   Spider naevi ☐ Hepatomegaly ☐ Splenomegaly ☐ Ascites ☐ Varices ☐ Bleeding varices ☐ Liver tumor ☐

   Other (please give details): .....

Section 3: TEST RESULTS

1. **Date of last consultation for HCV <u>since</u> registration (dd/mm/yy):** ..../..../....

2. **Date of latest HCV PCR test results <u>since</u> registration (dd/mm/yy):** ..../..../.... Not done ☐ Not known ☐

   Results (please tick box): Positive ☐ Negative ☐ Not known ☐

3. **Date of latest liver function tests <u>since</u> registration (dd/mm/yy):** ..../..../.... Not done ☐ Not known ☐

   Results (please tick box or enclose copy of report form): Normal ☐ Abnormal ☐ Not known ☐

   If **abnormal**, please give results and test ranges: ALT ..... Range ..... AST ..... Range .....

   Bilirubin ..... Range ..... Albumin ..... Range .....

4. **Date of latest liver biopsy since registration (dd/mm/yy):** ...../...../..... Not done ☐ Not known ☐

Results (please tick box): Normal ☐ Abnormal ☐ Not known ☐

If **abnormal**, please give results (enclose copy of report form, if possible):

Minimal change ☐ Chronic hepatitis ☐ Cirrhosis ☐ Hepatocellular carcinoma ☐

Fibrosis score (if known): ..... Scoring system: .....

Section 4: ANTIVIRAL DRUG TREATMENT

1. **Has the patient had any antiviral treatment for HCV since registration?** Yes ☐ → *Please continue with this section*

No ☐ → *Please go to question 3*

If **yes**, please insert details of treatment in the table below:

COURSE	Date started (dd/mm/yy)	Date finished (dd/mm/yy)	Interferon preparation (eg. Intron A®, Wellferon®)	Interferon dosage (mU)	Interferon schedule (eg. daily, twice weekly)	Other antiviral (eg. Ribavirin)	Dosage of other antiviral (please give units)	Schedule for other antiviral (eg. daily, twice weekly)
A								
B								
C								

2. **What was the response to treatment? (please tick only one of the 8 boxes below)**

Not relevant (still on treatment) ☐

Treatment stopped early (eg. due to side effects) ☐

No response (never became PCR negative) ☐

Response - Late relapse (PCR negative >12/12 after treatment but became positive at a later date) ☐

- Long term response (remains PCR negative 12/12 after treatment completed) ☐

- Sustained response (remains PCR negative 6/12 after treatment completed) ☐

- Immediate/initial response (PCR negative <6/12 after treatment completed) ☐

- Transient response (PCR negative during treatment but became positive after treatment) ☐

3. **Has the patient taken part in any antiviral drug trials since they were registered?** Yes ☐ No ☐

If **yes**, please give details: Name of trial: .....

Patient registration/code number: ..... Date of entry: ...../...../.....

Section 5: CURRENT MANAGEMENT

1. **What care has the patient received for HCV-related illness since registration?**

None ☐ Outpatient only ☐ Inpatient (assessment only eg. liver biopsy) ☐ Inpatient (medical care) ☐

If the patient has received **inpatient (medical care)** for HCV-related illness since they were registered, please give date and reason for each admission:

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

Date (dd/mm/yy): ...../...../..... Reason: .....

2. **What is the patient's current alcohol intake (in units of alcohol/week, if possible)?** ..... Not known ☐

Section 5 continued overleaf

Section 5: CURRENT MANAGEMENT *continued*

3. How many cigarettes does the patient currently smoke each day?

None ☐ 0-9 ☐ 10-19 ☐ 20 or more ☐ Not known ☐

Section 6: SUPPLEMENTARY INFORMATION TO SUPPORT OTHER RESEARCH PROJECTS

1. If known, please advise the HCV genotype? ..... Not known ☐

2. Please advise the results of the following tests taken at or around the time of the latest liver biopsy (regardless of whether this biopsy was undertaken before or after the date of patient registration).

   If the patient hasn't had a liver biopsy, please place a tick in this box ☐ and go straight to question 3.

TEST	Date of test	Result (please give units)	Test range (where appropriate)
Platelets			
Bilirubin			
INR			
Albumin			
ALT			

3. If we needed a blood specimen, would you be willing to send us one when you next routinely take blood from this patient? Yes ☐ No ☐

COMMENTS

If you have any comments that you would like to make, please do so in the space below:

Please print your details below so that we can contact you if we need more information about this patient:

Your name: .....

Date (dd/mm/yy): ...../...../..... Telephone number: .....

THANK YOU VERY MUCH FOR YOUR HELP

ALL THE INFORMATION YOU PROVIDE WILL BE TREATED IN CONFIDENCE

PLEASE RETURN THIS FORM TO:

Dr H E Harris (Register Co-ordinator)
Immunisation Division, PHLS Communicable Disease Surveillance Centre
61 Colindale Avenue, London, NW9 5EQ
Tel: 020 8200 6868
[redacted]*phs.uk*

APPENDIX 8 SURVEY ON THE CLINICAL MANAGEMENT OF CHILDREN COINFECTD WITH HIV AND HCV

CLINICAL MANAGEMENT OF CHILDREN DIAGNOSED WITH HIV AND HEPATITIS C VIRUS COINFECTION

We are currently carrying out research on children coinfected with HIV and Hepatitis C virus and are keen to find out how centres manage and treat these children in light of their coinfection, given the lack of guidelines for management of this group. We would be very grateful if you could take the time to complete our questionnaire and provide us with as much information as you can.

Respondent's Name .....Centre.....
Position .....
Address.....
.....
Email .....

Background information

1. How many **HIV/HCV coinfected** children do you currently manage at your centre/clinic?.....

2.How many were Vertically coinfected .....
Parenterally coinfected .....
Unknown mode of acquisition .....

3.What is the age range of the HIV/HCV coinfected children currently in your care ?.....

4.Under what circumstances do you test children for possible HIV/HCV coinfection? (please circle/delete as appropriate)

Children followed from birth
Test all children born to HIV/HCV coinfected mothers for both HIV and HCV .....Y/N
Children presenting with HIV or HCV infection in childhood
Test all children with diagnosed HIV infection for HCV .....Y/N
Test all children with diagnosed HCV infection for HIV .....Y/N
Please specify any other conditions which would lead you to test for HIV or HCV infection:
.....
.....

5.What is your usual practice regarding HCV genotyping? (please circle/delete as appropriate)
Always perform.....Y/N
Sometimes perform .....Y/N
under what circumstances do you perform HCV genotyping?
.....
.....
Never perform .....Y/N

Policy for management of HIV/HCV coinfected children

6.Does your centre have a written policy for all or any aspects of the management of HIV/HCV coinfected children?.....Y/N
If yes, please email/send a copy of this policy. If no, please go to question 9.

7.When was the policy drawn up?.....

8.Have there been any recent changes to this policy?.....Y/N
If yes, what changes?
.....

9.At what level are decisions about the clinical management of HIV/HCV coinfected children made? (please circle/delete as appropriate)

National / regional / hospital-based / departmental / individual clinician

Management of HIV/HCV coinfected children

10. Is the schedule for the management of HIV/HCV coinfected children at your centre affected by whether the child is on HIV or HCV treatment?.....Y/N
If yes, in what way? e.g. treated children seen more often, seen less often, more frequent follow-up in initial period of starting treatment

11. Is the schedule for the management of HIV/HCV coinfected children at your centre affected by the age of the child, the duration of infection or the mode of acquisition of infection.....Y/N

If yes, in what way? e.g. younger children followed-up more regularly, vertically coinfected children followed-up more regularly than parenterally coinfected children

12. In comparison to HIV-only or HCV-only infected children, are HIV/HCV coinfected children followed up

more frequently / less frequently / at the same frequency?

13.Are any specific HCV-related laboratory tests requested for diagnosed HIV/HCV coinfected children that would **not** be requested for **HIV-only** infected children.....Y/N

If yes, which ones and how frequently are they usually carried out?
HCV RNA PCR annually / every 6 months / every 3 months / other (please specify)
..... ALT
annually / every 6 months / every 3 months / other (please specify)
..... AST
annually / every 6 months / every 3 months / other (please specify)
.....
Liver biopsy annually / every 6 months / every 3 months / other (please specify)
.....
Liver ultrasound annually / every 6 months / every 3 months / other (please specify)
.....
Other (please specify).....
annually / every 6 months / every 3 months / other (please specify)
.....

Treatment of HIV-only infected children

14.Which paediatric guidelines do you follow when treating **HIV-only** infected children?

CDC / PENTA / National guidelines / other (please specify)
.....

15.Do you have a preferred 1st line therapy for **HIV-only** infected infants/children? .....Y/N

If yes, please specify

in children younger than 1 year of age.....

in children older than 1 year of age.....

Treatment of HCV-only infected children

16. Have you ever treated **HCV-only** infected children? .....Y/N
If no, please go to Q 18.
If yes, have you ever initiated treatment before 3 years of age? .....Y/N
If yes, under what circumstances?
.....
.....

17. Have you always treated **HCV-only** infected children in the context of a treatment trial?..Y/N
If yes, please provide further details of the trial(s) and treatment(s)
.....
.....

Treatment of HIV/HCV coinfected children

18.Do you monitor **HIV/HCV coinfected** children receiving HIV treatment for evidence of hepatotoxicity? .Y/N
If no, please go to Q 21. If yes, please answer the following questions:

Please indicate below how you monitor possible hepatotoxicity?
ALT levels Y/N How often? .....
AST levels Y/N How often? .....
Other (please specify) .....How often?.....

19.Is there a level of hepatotoxicity at which you would **normally** switch HIV therapy?..... Y/N
If yes, please provide further details e.g. how many elevated liver enzyme tests and at what threshold do you consider the liver enzymes to be elevated?
.....
.....

20.Do you monitor drug-related hepatotoxicity more intensively in coinfected children than in HIV-only infected children? (e.g. more frequent testing).....Y/N
If yes, in what way?
.....
.....

21. Do you check for lipodystrophy more frequently in HIV/HCV coinfected children than HIV-only infected children? .....Y/N

22.Do you have a preferred 1st line HIV therapy for **HIV/HCV coinfected** children? .....Y/N
If yes, please specify
in children younger than 1 year of age.....
in children older than 1 year of age.....

23.Have you ever treated children for HIV and HCV infections at the same time? .....Y/N
If yes, are there specific HIV drug/drug combinations that you **avoid**? (please specify and give reasons)
.....
.....
.....

If no, would you ever consider treating children for HIV and HCV infection at the same time in specific circumstances? .....Y/N

(please give details)

.....

.....

24. **If you would not treat HIV and HCV concurrently,** how would you respond to the following situations?

In a child with known HIV/HCV coinfection from birth where HIV treatment was necessary before HCV could be treated would you

stop HIV therapy if HCV treatment became necessary?.....Y/N

OR

delay starting HIV therapy until HCV could be treated first?.....Y/N

Other response to situation (please specify)

.....

.....

In a child diagnosed with HIV/HCV coinfection later in childhood would you

treat HCV infection before starting HIV treatment?.....Y/N

OR

start HIV treatment and stop if HCV treatment became necessary?.....Y/N

Other response to situation (please specify)

.....

.....

Thank you for taking the time to fill out this questionnaire, your help is greatly appreciated and I will feedback the results when they are all collected. If there is any other information or comments on the management of HIV/HCV coinfected children you would like to add then please feel free to do so below.

Any other information or comments of the management of HIV/HCV coinfected children.

.....

.....

.....

.....

.....

.....

.....

.....

APPENDIX 9 PUBLICATIONS ARISING RFOM THIS RESEARCH

England, K., Thorne, C., and Newell, M.L. (2006). Vertically acquired paediatric coinfection with HIV and hepatitis C virus. *Lancet Infect.Dis.*; 6: 83-90.

Vertically acquired paediatric coinfection with HIV and hepatitis C virus

Kirsty England, Claire Thorne, Marie-Louise Newell

Both HIV and hepatitis C virus (HCV) can be transmitted from mother to child during pregnancy and delivery. Vertical transmission of HIV and HCV separately is most likely from HIV/HCV-coinfected mothers; however, transmission of both infections is less frequent. The effect of HCV coinfection on HIV-related disease remains unclear; whereas most studies indicate no effect, recent results suggest HCV in adults accelerates HIV progression. Little is known about how HIV coinfection affects HCV progression in children and the information available is based on small numbers of patients. Paediatric HIV treatment is extremely successful and it is vital to determine if HCV coinfection alters the effectiveness of this treatment. The hepatotoxicity of many HIV therapies and the possible negative impact of HCV on this treatment, alongside the interactions and contraindications of many HIV and HCV therapies, further limits the choice of paediatric treatments for coinfected children. Future research must therefore focus on vertically acquired HIV/HCV coinfection to inform treatment trials addressing coinfection management.

Lancet Infect Dis 2006; 6: 83–90

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Introduction

HIV and hepatitis C virus (HCV) are both blood-borne viruses and share transmission routes in adults, specifically injecting drug use and blood transfusion, making coinfection relatively common, although sexual transmission of HCV is much less frequent than for HIV.[1–3] With antiretroviral treatment for HIV infection improving and prolonging life in infected individuals,[4,5] the prevalence and effect of secondary infections and comorbidities such as HCV has increased in adult populations.[6] Symptoms related to HCV infection were not previously observed in coinfected patients because progression to liver disease or associated cancers can take as long as 20 years after HCV infection. However, with successful HIV treatment HCV-related mortality has become a leading cause of death in HIV-infected individuals.[6,7] The extent to which this situation is true in coinfected children is unclear.

Both HIV and HCV can be vertically transmitted from an infected mother to her child, but in Europe the number of coinfected children is small and the pooling of expertise in this area is vital if management recommendations are to be made. The prevalence of antenatal HIV infection in western Europe is 0·2–0·4%, resulting in approximately 3800–7600 children born to HIV-infected mothers in western Europe each year. In this population, most infections are acquired sexually and occur in women of sub-Saharan African origin who are rarely coinfected with HCV. In the remaining women, who have acquired HIV infection through drug use, coinfection with HCV is much more common. It can be estimated that 150–300 children are born to mothers coinfected with HIV and HCV in western Europe each year. Because of these small numbers the guidelines for management in this group of children remain unclear and as coinfected numbers increase it becomes more important to understand how maternal coinfection affects risk of vertical infection with HIV, HCV, or both, and the subsequent disease progression.[7] In addition, advances in paediatric HIV treatment have meant that therapy in children of all ages is both readily available and extremely effective and the possibility that coinfection with HCV might hinder this effectiveness is of vital importance. We review information regarding vertical transmission of HIV and/or HCV from coinfected mothers, disease progression, and treatment of both HIV and HCV in coinfected children.

Mother-to-child transmission of HIV from coinfected mothers

Transmission of HIV infection from a mother to child may occur before (across the placenta), during (via exposure to contaminated maternal secretions), and after delivery (through breastfeeding).[8,9] The most important factors associated with an increased risk of mother-to-child transmission of HIV are maternal plasma HIV RNA viral load, vaginal delivery, and mode and duration of breastfeeding.[10–13] Elective caesarean section delivery before onset of labour and rupture of membranes, prophylactic antiretroviral therapy during pregnancy and/or neonatally, and breastfeeding avoidance independently substantially reduce mother-to-child transmission rates,[10] which range from 15% to 40% without intervention. In settings where all interventions can be safely implemented, the advent of highly active antiretroviral therapy (HAART) has reduced HIV vertical transmission rates to below 2%.[14]

Vertical transmission of HIV occurs more often from women coinfected with HIV and HCV than from those infected with HIV only.[15–20] For example, HIV transmission occurred from 16% (53/326) of mothers infected with HIV only, compared with 26% (42/161) of HIV/HCV-coinfected women (table), suggesting that HCV/HIV coinfection nearly doubles the HIV mother-to-child transmission rate (odds ratio [OR] 1·82, p=0·001).[16] However, the transmission rates reported and sample sizes used in these studies vary considerably and do not always compare coinfected mothers with

Study	Methods/sample	Total HIV VTR	HIV VTR from HIV-only infected	HIV VTR from coinfected	Total HCV VTR	HCV VTR from HCV only infected	HCV VTR from coinfected	VTR of coinfection	Significance of results
Giovannini et al[18]	Prospective follow-up of 49 HIV-infected mothers in Italy, 25 coinfected with HCV	14/49 (28·6%)	2/24 (8·3%)	12/25 (48%)	..	..	..	..	..
Paccagnini et al[19]	Prospective follow-up of 70 HCV-infected mothers in Italy, 53 coinfected with HIV	..	..	16/53 (30·2%)	14/70 (20%)	2/17 (12%)	12/53 (23%)	..	Higher HCV VTR from coinfected (p<0·05)
Hershow et al[16]	Prospective follow-up of 487 HIV-infected mothers, 161 confected with HCV	95/487 (19·5%)	53/326 (16%)	42/161 (26%)	..	..	..	..	Higher HIV VTR from coinfected (OR 1·8, p=0·01)
Tovo et al[17]	Prospective follow-up of 245 children born to HCV-infected mothers in Italy, 165 mothers coinfected with HIV	..	..	22/165 (13·3%)	28/245 (11·4%)	3/80 (3·7%)	25/165 (15·1%)	6/165 (3·6%)	Higher HCV VTR from coinfected (p<0·001)
Papaevangelou et al[15]	Prospective study of 100 HIV-infected women in New York, 54 coinfected with HCV and who had 63 children	..	..	16/63 (25%)	..	..	9/62 (16·4%)	6/63 (9·5%)	..
Thomas et al[21]	Prospective follow-up of 155 HIV/HCV-coinfected mothers (Women and Infants Transmission Study)	..	..	41/155 (26·5%)	..	..	13/155 (8·4%)	7/155 (4·5%)	..
Nigro et al[23]	Prospective follow-up of 23 HIV-infected children born to 22 HIV/HCV-coinfected mothers	..	..	..	..	..	2/22 (9%)	2/22 (9%)	..
Zanetti et al	Prospective follow-up of 188 children born to 291 HCV-infected mothers in Italy, 40 HIV coinfected				17/291 (5·8%)	8/251 (3·2%)	9/40 (22·5%)		Higher HCV VTR from coinfected (p<0·001)
Gibb et al[22]	Follow-up of 441 HCV-infected women in UK and Ireland, 22 coinfected with HIV	..	..	..	30/441 (6·7%)	21/328 (6·4%)	4/22 (18·6%)	..	Higher HCV VTR from coinfected (OR 3·8, p=0·05)
EPHN[20]	Prospective follow-up of 1474 HCV-infected women in Europe, 503 coinfected with HIV	..	..	55/491 (11·2%)	136/1474 (9·2%)	60/916 (6·6%)	70/503 (13·9%)	..	Higher HCV VTR from coinfected OR 2·3 (95% CI 1·6–3·4)
Ferrero et al[25]	Prospective follow-up of 170 HCV-infected pregnant women and their 188 children in Italy	..	..	..	5/188 (2·7%)	3/151 (2%)	2/37 (5·4%)	..	..

OR=odds ratio, VTR=vertical transmission rate, ..=not reported

Table: **Mother-to-child transmission of HIV, HCV, and HIV/HCV coinfection**

HIV-only infected mothers. Reported HIV transmission rates from HIV/HCV-coinfected mothers range from 13·3% to 30% in settings where initiatives to prevent mother-to-child transmission of HIV are available and where few HIV-infected women breastfeed.[17–20] The lowest HIV transmission rates, from the most recent studies, likely reflect the use of zidovudine in pregnancy to reduce mother-to-child transmission.[26] There have been few studies to assess the effect of HIV/HCV coinfection on HIV transmission rates in the past 5 years. Despite one recent study finding the HIV mother-to-child transmission rate to be 6·2% (10/161),[27] lower than the 11·2% (55/491) of coinfected women from the same study investigated between 1992 and 2000,[24] the impact of HAART on the prevention of HIV mother-to-child transmission, which became widely used after 2000, in a maternally HIV/HCV-coinfected population remains unclear.

Mother-to-child transmission of HCV from coinfected mothers

The effect of HIV/HCV coinfection on vertical transmission of HCV has received more recent attention than that of HIV and the evidence to support an increase in the transmission rate from coinfected mothers in comparison with mothers only infected with HCV is more complete. The HCV mother-to-child transmission rate in HCV-only infected women ranges from 4% to 10%,[20,22–24,28–30] higher in women with a high HCV viral load.[22,31] There is no evidence to support a protective effect of elective caesarean section on HCV vertical transmission,[23,24] although HCV transmission can occur intrapartum.[32] Similarly, no substantial increase in transmission rates has been observed among breastfeeding women.[24] There is substantial evidence to support an increase in HCV vertical transmission from mothers coinfected with HIV compared with mothers infected with HCV only. One study including more that 100 children from HCV/HIV-coinfected mothers found HCV transmission rates to be significantly higher with a 2·3 times increase (95% CI 1·6–3·4) (table).[24] Furthermore, acquisition of infection of HCV is more likely in infants also becoming infected with HIV,[18,19] which has been taken to imply that transmission of HIV in utero may facilitate transmission of HCV. However, it is more plausible that women transmitting HIV have both high HIV RNA and HCV RNA viral loads and are thus prone to transmit both viruses. Treatment during pregnancy to prevent HIV transmission by lowering HIV RNA viral load may also lower the risk of HCV transmission and may thus be vital to the prevention of mother-to-child transmission of both these viruses.

Vertical transmission of HIV/HCV coinfection

Few studies have focused on vertical transmission of HIV/HCV coinfection. Reported transmission rates range from 3·6% to 9·5%,[18–20,21] with the lower transmission rates from the two studies with more than

150 coinfected mothers (table).[19,40] These findings would suggest that, whereas single transmission of HCV and HIV from coinfected mothers is more likely than from singly infected mothers, the mother-to-child transmission of both viruses simultaneously occurs less frequently than single-virus transmission from a coinfected mother (figure 1, panel 1).

HIV disease progression in coinfected children

In the absence of effective antiretroviral therapy or other supportive prophylactic management, a quarter of vertically HIV-only infected children will have progressed to serious disease or death by the age of 1 year, with a 40% cumulative incidence of HIV-related disease or death by 6 years of age.[13] HIV-related disease progression is measured by levels of immunological and virological manifestations, specifically CD4 cell count and HIV RNA viral load, and the presence of non-specific clinical symptoms such as hepatomegaly, splenomegaly, lymphadenopathy, and HIV-related manifestations or opportunistic infections.[14] Since recommendations for the use of more active combination antiretroviral therapies were introduced in 1997/98 and paediatric antiretroviral therapy became more widely available for children at an earlier stage in their disease progression before serious clinical or immunological deterioration, children have been shown to be substantially less likely to progress to serious HIV-related diseases or death. Most HIV-infected children now treated with combination antiretroviral therapy remain symptom free for at least 10 years.[15]

The effect of paediatric HIV/HCV coinfection on progression of HIV disease remains unclear, since only two studies have investigated this area in children. Two coinfected children in one study were without signs of HIV progression when last seen at 10 and 10·6 years of age.[21] The authors therefore suggest that coinfection may lead to slower progression of HIV disease but the sample size and details do not warrant this conclusion. Papaevangelou and colleagues[18] found no significant differences in HIV disease progression between nine coinfected children and three infected with HIV only. Although the plasma HIV RNA viral load at 6 months of age in coinfected children was higher than in infants with HIV infection only, the difference was not statistically significant.

Evidence of the impact of HIV/HCV coinfection on HIV disease progression in adult populations is a little more extensive, albeit still limited. Whereas in four studies including 116–1685 coinfected people there was no evidence of an effect of HCV coinfection on progression to HIV disease,[16–19] in two studies with 650–1157 coinfected patients, HIV progression was substantially accelerated.[40,41] Specifically, in a study by Carlos and co-workers[40] on 902 HIV infected patients, 72% (650/902) of whom were also infected with HCV, CD4 cell count 2 years after the initiation of therapy

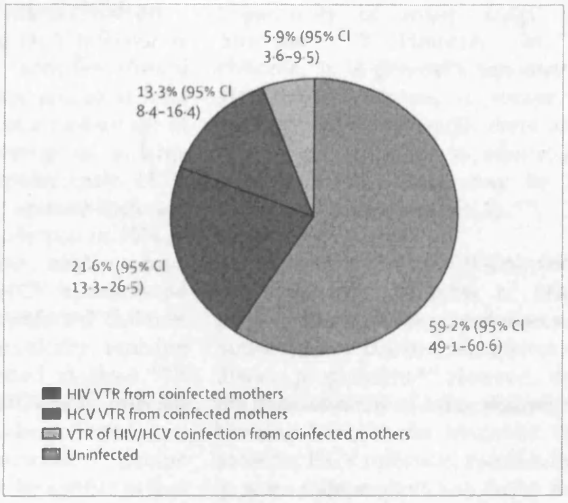

Figure 1: **Estimated vertical transmission rate (VTR) of HIV and/or HCV from HIV/HCV-coinfected mothers**
Estimated vertical transmission rates are mean rates from three studies providing information on all types of vertical transmission.[18–20] Mean rates from these three studies are in line with mean rates from all studies.

was significantly lower in coinfected individuals compared with HIV-only infected individuals ($p<0·001$). In response to treatment, the mean increase in CD4 cell count over a 2-year period was significantly less than in HCV-coinfected individuals (11% *vs* 19%, $p<0·05$) and the mean decrease in plasma HIV RNA over the same period was significantly lower in coinfected patients than in HIV-only infected individuals (5% *vs* 54%, $p<0·05$), although this association was not significant in multivariable analysis adjusting for sex, age, CD4 cell count, use of HAART, and adherence to therapy.[40] Differences in the effect of HIV/HCV coinfection on HIV disease progression between studies may be partly explained by suggestions that coinfected individuals with HCV genotype 1 are at an increased risk of progression to AIDS-related mortality compared with those with HCV non-genotype 1, although this relation is not yet supported by strong evidence.[47]

Panel 1: **Mother-to-child transmission of HIV, HCV, and HIV/HCV coinfection**

- HIV transmission rates from HIV/HCV-coinfected mothers range from 13·3% to 30% and are substantially higher than those from HIV-only infected mothers.
- HCV transmission rates from HIV/HCV-coinfected mothers range from 5·4% to 23% and are substantially higher than those from HCV-only infected mothers.
- Vertical transmission of HIV/HCV coinfection occurs less frequently than transmission of HIV or HCV alone. Coinfection transmission rates range from 3·6% to 9·5%.

HCV disease progression in coinfected children

The progression of vertically acquired HCV infection is slow in the first 10–15 years of life, with few clinical symptoms. Only 22% of children experienced at least one minor clinical manifestation before a median age of 4·2 years during prospective follow-up in a large European cohort.[43] In this study, hepatomegaly, HCV RNA viraemia, and increased alanine aminotransferase levels were indicative of active HCV infection in 30% of HCV-infected children.[43] Only four studies have separated results on progression of HCV in coinfected infants, with between two and 26 coinfected children included. Three studies report chronically evolving hepatitis in at least 50% of coinfected children,[18,19,21] which is similar to that found in HCV-only infected children.[43] One study reports the number of coinfected children with persistently abnormal alanine aminotransferase levels (>40 IU/L) to be similar to that in HCV-only infected children, but the number of coinfected children with a high proportion of positive HCV RNA PCR results is reported to be greater than in HCV-only infected children, albeit not significantly so.[43] No formal comparison was made between progression in coinfected children compared with HCV-only infected children, and insufficient details were given to draw firm conclusions about HCV disease progression in the context of paediatric HIV/HCV coinfection.

The effect of HCV/HIV coinfection on liver-related morbidity and mortality in adult populations has been more extensively studied than in children with higher numbers of coinfected adults than children. In HCV-infected adults it is widely acknowledged that coinfection with HIV accelerates the course of HCV-associated liver disease progression, particularly in patients who are more immunodeficient. HCV/HIV coinfection is thus associated with increased liver fibrosis progression, increased rate of liver decompensation, cirrhosis, hepatocellular carcinoma, and liver-related mortality, particularly due to the hepatoxicity of many drugs used to treat HIV infection.[2,4,5,44–48] However, in HCV-only infected children, it is generally assumed that progression of HCV-related disease is slower than in HCV-only infected adults (although there are few studies where timing of infection in adults is known with any precision); the same may be true of HCV/HIV-coinfected children (panel 2).[49,50]

Panel 2: **Disease progression of HIV and HCV in vertically coinfected children**

- Paediatric HIV disease progression in the context of HCV coinfection remains unclear. Until recently it was accepted that disease progression in HIV-only infected individuals was not different from that in coinfected individuals, although current evidence of lower CD4 cell counts and higher HIV viral loads in coinfected adult patients contradicts this. The effect of HCV coinfection on HIV disease progression in children remains unknown.
- Limited evidence does not support an effect of HIV/HCV coinfection on paediatric HCV disease progression but studies in adult populations suggest that the use of HIV treatment might accelerate HCV disease progression in coinfected patients.

Treatment of HIV/HCV-coinfected adults

The substantial benefits of HAART in decreasing progression to AIDS-related diseases and death probably outweigh any detrimental effects on the liver or HCV disease progression.[4,46] However, there is consensus that the hepatotoxicity of some antiretroviral drugs is a major limiting factor in the treatment options for coinfected patients; HCV infection, specifically with HCV genotype 3, is an independent risk factor for HAART-associated hepatotoxicity in adults.[1,4,51,52] On the other hand, HAART has been associated with a reduced likelihood of liver fibrosis and related disease progression in coinfected patients. Indeed, Quirishi and colleagues[4] identified HAART as an independent predictor of surviving liver disease (OR 0·11, 95% CI 0·02–0·56),[4] and the duration on HIV antiretroviral therapy has also been associated with less liver fibrosis. In summary, treatment to prevent HIV disease progression in coinfected patients should be a priority, although the drugs used must be chosen wisely to reduce the likelihood of hepatotoxicity.[53]

The disparity between studies in the effect of HAART on liver disease highlights the problem of comparing cohorts, simply because of the varying nature of both HIV and HCV disease.[4] However, it could also be explained by the type of drugs used, with protease inhibitors substantially less likely to result in liver fibrosis in coinfected patients than nucleotide reverse transcriptase inhibitors or non-nucleotide reverse transcriptase inhibitors.[55] On the other hand, there is evidence that HCV infection reduces the effect HAART has on increasing CD4 cell counts in coinfected patients.[41] Greub and colleagues[41] found the probability of failing to increase CD4 cell counts by at least 50 cells per μL 1 year after the start of HAART was 25% (95% CI 22·5–27·7) in coinfected patients and 16% (95% CI 14·4–17·8) in HIV-only infected patients (hazard ratio 0·79, 95% CI 0·72–0·87).[41] Given the hepatotoxic nature of many HIV treatments and the possible detrimental effects of HCV on HIV disease progression, it is advisable to screen all HIV-infected patients for HCV so that HIV infection can be managed appropriately.

The treatment of HCV in coinfected patients is also problematic since this group may not respond as well to anti-HCV therapies as HCV-only infected individuals.[56–59] This situation is exacerbated by the fact that anti-HCV therapy is often poorly tolerated and highly toxic, with up to half of patients developing symptoms such as fatigue, fever, myalgia, hair loss, and depression.[60] These side-

effects lead to poor compliance, especially in coinfected populations where the incidence of depressive symptoms and mitochondrial toxicity due to treatment is higher than in HCV-only infected patients, and among whom it is estimated only 20% will benefit from HCV therapy.[6,7,59] However, some HCV treatments are more effective than others;[60] the rate of sustained HCV virological response (SVR) in coinfected adults treated with pegylated interferon alfa-2b (or alfa-2a) plus ribavirin (SVR 27–44%) is significantly higher than in those treated with interferon alfa-2b (or alfa-2a) plus ribavirin (SVR 12–21%; $p<0.05$),[61–65] but this is also true in HCV-only infected adults.

Anti-HCV treatment is given only for a limited time (usually 12–48 weeks),[62] unlike HIV therapy which is for life. A recently published consensus statement[57] from the first European consensus conference on the treatment of chronic hepatitis B and C in HIV-coinfected patients states that, in adults, the initiation of HIV treatment in coinfected patients should be as in an HIV-only infected population. European, UK, and US guidelines recommend that HIV treatment is offered to all patients with CD4 cell counts less than 350 cells per μL.[6,66] However, in adults with CD4 cell counts just above the threshold for initiation of HIV treatment, HIV-related immune deficiency should be improved with HAART before starting any anti-HCV treatment, since interferon-based HCV therapy may cause decreases in CD4 cell count.[57] If HCV is detected before HAART is clinically indicated or at a point where the CD4 cell count is not yet indicative of immune deficiency, then appropriate HCV therapy—usually with pegylated interferon alfa and ribavirin—should begin immediately.[57]

Treatment of HIV/HCV-coinfected children

To date, there are no studies focusing specifically on the treatment of either HCV or HIV in children coinfected with both viruses. The recommendations regarding the treatment of coinfection are based on adult data or are extrapolated from recommendations for the treatment of paediatric HIV or HCV alone. The concomitant use of antiretroviral therapy and anti-HCV therapy is complicated by the interactions of many drugs, since most HAART agents have the potential to cause hepatotoxicity.[66] Treatment with interferon alfa-2b plus ribavirin is most commonly recommended for effective HCV treatment in children. Although recent trials show promising results for the use of pegylated interferon,[67] ribavirin can enhance the phosphorylation of didanosine, thus increasing the risk of associated toxicity and therefore the use of both these agents concurrently should be avoided. Similarly, ribavirin and zidovudine are both associated with anaemia and, where possible, should not be administered together.[66] Didanosine and zidovudine are both major components of many antiretroviral therapy regimens, especially those available to children, where the hepatotoxic nature of many HIV drugs is more problematic given the limited number of paediatric treatments available. It is therefore harder to find paediatric anti-HIV therapy that does not exhibit hepatotoxic properties. All non-nucleoside reverse transcriptase inhibitors are hepatotoxic, especially nevirapine, which can cause or intensify liver disease in HCV-infected children. Protease inhibitor-based combination antiretroviral therapy regimens including lopinavir, ritonavir, nelfinavir, and indinavir

Panel 3: Evidence on treatment of HIV/HCV-coinfected individuals

- All evidence relating to the treatment of coinfected individuals comes from adult populations or is extrapolated from guidelines for the treatment of paediatric HIV and HCV separately.
- The hepatotoxic nature of many HIV drugs, such as nevirapine and other non-nucleoside reverse transcriptase inhibitors, is the major limiting factor in the HIV treatment options of coinfected individuals; however, the benefits of HAART on the HIV disease progression of these patients probably outweighs these detrimental effects.
- Drug interactions make the treatment of coinfected children particularly problematic and the use of HIV and HCV treatments simultaneously should be carefully managed, in addition to the careful choice of HIV therapy to ensure progression to HCV disease is not accelerated.

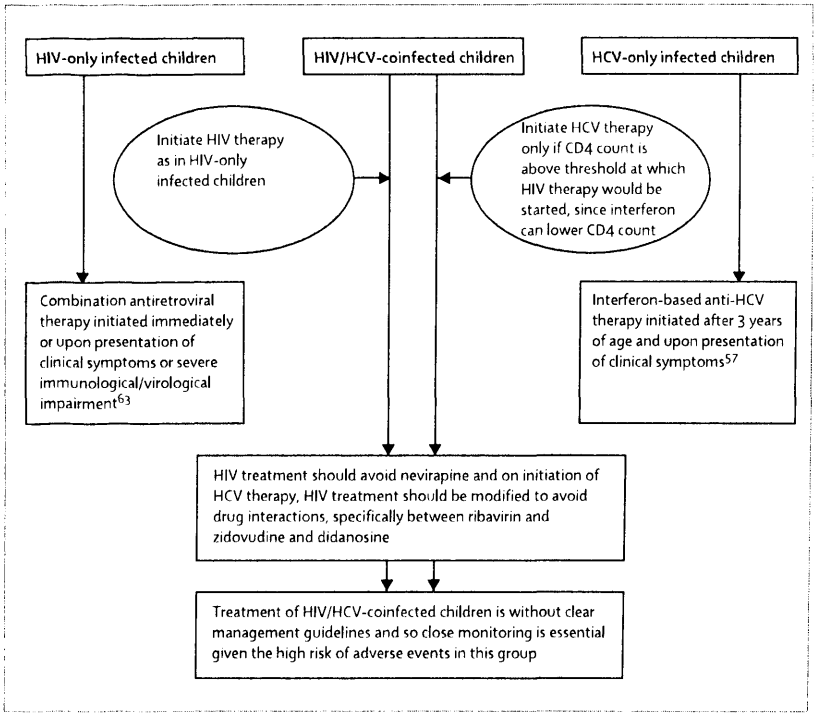

Figure 2: Recommendations for the treatment of HIV/HCV-coinfected children

Panel 4: Recommendations for treatment of coinfected children

- HIV therapy in HIV/HCV-coinfected children should be initiated as in HIV-only populations, although nevirapine should be avoided where possible.
- HCV treatment should only be initiated in children with a CD4 cell count greater than the level recommended for the initiation of HIV therapy, since interferon has been shown to reduce CD4 cell count.
- The modification of HIV therapy during HCV therapy may be necessary to avoid concurrent use of ribavirin and didanosine or zidovudine.
- The close monitoring of treatment and clinical status in coinfected children is vital given the high risk of adverse events in this group.

are also associated with exacerbation of chronic liver disease, albeit in rare cases.[77] Nucleoside reverse transcriptase inhibitors are more appropriate, although some have been shown to cause severe hepatotoxicity in rare cases.[78] At present there are no guidelines on how to resolve these issues in children and so the management of paediatric coinfection remains difficult and continues to be based on guidelines for the treatment of HIV and HCV separately.

Interferon-based HCV treatment is not recommended in children before 3 years of age but HIV treatment is often started immediately in infants with clinical symptoms and severe immunological and virological impairment.[68] It has been suggested that early initiation of HIV therapy in all HIV-infected children is more successful than a delayed approach, and in many cases HIV treatment will commence long before HCV therapy can be considered.[72] As HCV-infected children exhibit few clinical symptoms in the first 10–15 years of life and are therefore unlikely to require HCV treatment, HIV treatment may continue for many years without the need for concomitant HCV therapy. However, because it remains unclear whether HIV coinfection increases HCV disease progression in children, the possibility that HCV treatment in this group may be necessary earlier than in HCV-only infected children must be considered. As with adult populations, HIV treatment in HIV/HCV-coinfected children should be initiated as per guidelines for HIV-only infected children. However, close monitoring is essential to ensure that HIV treatment is not exacerbating HCV progression and the use of nevirapine should be avoided where possible. If and when HCV therapy becomes necessary, care should be taken to ensure that CD4 cell counts are above the recommended level for the initiation of HIV therapy before initiation of interferon, which is known to lower the CD4 cell count.[57] Additionally, it may be necessary to modify the HIV treatment to ensure drug interactions, specifically between ribavirin and zidovudine or didanosine, do not result in increased liver toxicity and fibrosis (panel 3, panel 4, and figure 2).

Discussion

The available evidence on paediatric HCV/HIV coinfection suggests that vertical transmission of both HIV and HCV separately occurs more often from mothers coinfected with HIV and HCV than from singly infected mothers. Transmission of HIV/HCV coinfection has been rarely studied but the rates reported imply that coinfection is transmitted less frequently than either HIV or HCV infection alone from coinfected mothers.

The bulk of the evidence does not support any change in HIV disease progression due to coinfection with HCV. However, a small number of recent, large studies have shown changes in HIV disease progression in adults, implying a detrimental effect of HCV on HIV disease resulting in more severe liver lesions and increased HCV RNA. There are few studies documenting HCV disease progression in light of vertically acquired HIV/HCV coinfection, and these studies have not compared HCV in coinfected infants with that of HCV-only infected infants; the effect of HIV on HCV progression in coinfected children remains unclear.

Evidence relating to the treatment of coinfected individuals predominantly comes from studies of adult populations and there are many conflicting findings. Some evidence suggests that the hepatotoxic nature of many HIV treatments results in an accelerated or increased level of liver-related morbidity in coinfected individuals and that HCV therapy is more poorly tolerated in coinfected patients and may cause CD4 cell count decline.

HCV treatment with interferon is not currently available for children under 3 years of age. It is unclear whether coinfection accelerates HCV disease progression and whether coinfected children may therefore need to receive HCV treatment at an earlier age than HCV-only infected children, most of whom remain symptom free for at least 15 years. Although HCV therapy is given for a relatively short period of time, the possible need to initiate earlier HCV therapy in HIV/HCV-coinfected children makes choosing appropriate HIV therapies important. HIV therapy in coinfected children should be initiated as would be the case in HIV-only infected children, although nevirapine should be avoided where possible and close monitoring of HCV progression is vital. Where the additional initiation of HCV treatment becomes necessary, HIV therapy may need to be modified to avoid concurrent use of ribavirin with didanosine or zidovudine.

This review of available research on paediatric HIV and HCV coinfection has highlighted a number of vital areas where evidence is insufficient to inform clinical practice and management. Most of the available

Search strategy and selection criteria

An English language literature search was carried out using the Pubmed database for studies examining HIV/HCV coinfection. The keywords used in the search were "HIV", "human immunodeficiency virus", "AIDS", "acquired immune deficiency syndrome", "HCV", "hepatitis C", "children", "infants", "transmission", "vertical transmission", and "mother-to-child-transmission". Reference lists of related articles were searched for relevant studies, as were the abstracts of recent conferences.

information comes from studies of mother-to-child transmission with a focus on HIV or HCV transmission in the light of maternal coinfection. What is now especially important given the management complexity is an emphasis on the transmission of coinfection and the resultant HIV and HCV disease. These studies would in turn inform treatment trials, vitally addressing appropriate management of this group, whose disease progression in adulthood has been shown to vary from those only infected with HIV or HCV and whose treatment is complicated by the limited number of available paediatric drugs and the difficulties of treating HIV and HCV simultaneously while avoiding drug interactions and the worsening of HIV or HCV disease.

Conflicts of interest

We declare that we have no conflicts of interest.

References

1 Bica I, McGovern B, Dhar R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; **32**: 492–97.

2 Bonacini M, Puoti M. Hepatitis C in patients with human immunodeficiency virus infection: diagnosis, natural history, meta-analysis of sexual and vertical transmission, and therapeutic issues. *Arch Intern Med* 2000; **160**: 3365–73.

3 Qurishi N, Kreuzberg C, Luchters G, et al. Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection. *Lancet* 2003; **362**: 1708–13.

4 Rosenthal E, Poiree M, Pradier C, et al. Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study). *AIDS* 2003; **17**: 1803–09.

5 Rockstroh JK, Spengler U. HIV and hepatitis C virus co-infection. *Lancet Infect Dis* 2004; **4**: 437–44.

6 Braitstein P, Palepu A, Dieterich D, Benhamou Y, Montaner JS. Special considerations in the initiation and management of antiretroviral therapy in individuals coinfected with HIV and hepatitis C. *AIDS* 2004; **18**: 2221–34.

7 Sulkowski MS, Mehta SH, Torbenson M, et al. Hepatic steatosis and antiretroviral drug use among adults coinfected with HIV and hepatitis C virus. *AIDS* 2005; **19**: 585–92.

8 Nduati R, John G, Mbori-Ngacha D, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA* 2000; **283**: 1167–74.

9 Newell ML. Mechanisms and timing of mother-to-child transmission of HIV-1. *AIDS* 1998; **12**: 831–37.

10 Thorne C, Newell ML. Epidemiology of HIV infection in the newborn. *Early Hum Dev* 2000; **58**: 1–16.

11 Wilfert CM, Stringer JS. Prevention of pediatric human immunodeficiency virus. *Semin Pediatr Infect Dis* 2004; **15**: 190–98.

12 Scarlatti G. Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. *AIDS Rev* 2004; **6**: 67–78.

13 Mofenson LM. Advances in the prevention of vertical transmission of human immunodeficiency virus. *Semin Pediatr Infect Dis* 2003; **14**: 295–308.

14 Newell ML. Prevention of mother-to-child transmission of HIV: challenges for the current decade. *Bull World Health Organ* 2001; **79**: 1138–44.

15 Giovannini M, Tagger A, Ribero ML, et al. Maternal-infant transmission of hepatitis C virus and HIV infections: a possible interaction. *Lancet* 1990; **335**: 1166.

16 Hershow RC, Riester KA, Lew J, et al. Increased vertical transmission of human immunodeficiency virus from hepatitis C virus-coinfected mothers. *J Infect Dis* 1997; **176**: 414–20.

17 Paccagnini S, Principi N, Massironi E, et al. Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis J* 1995; **14**: 195–99.

18 Papaevangelou V, Pollack H, Rochford G, et al. Increased transmission of vertical hepatitis C virus (HCV) infection to human immunodeficiency virus (HIV)-infected infants of HIV- and HCV-coinfected women. *J Infect Dis* 1998; **178**: 1047–52.

19 Thomas DL, Villano SA, Riester KA, et al. Perinatal transmission of hepatitis C virus from human immunodeficiency virus type 1-infected mothers. *J Infect Dis* 1998; **177**: 1480–88.

20 Tovo PA, Palomba E, Ferraris G, et al. Increased risk of maternal-infant hepatitis C virus transmission for women coinfected with human immunodeficiency virus type 1. *Clin Infect Dis* 1997; **25**: 1121–24.

21 Nigro G, D'Orio F, Catania S, et al. Mother to infant transmission of coinfection by human immunodeficiency virus and hepatitis C virus: prevalence and clincial manifestations. *Arch Virol* 1997; **142**: 453–57.

22 Zanetti AR, Tanzi E, Romano L, et al. A prospective study on mother-to-infant transmission of hepatitis C virus. *Intervirology* 1998; **41**: 208–12.

23 Gibb DM, Goodall RL, Dunn DT, et al. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. *Lancet* 2000; **356**: 904–07.

24 European Paediatric HCV Network, Pembrey L, Tovo PA, Newell ML. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. *Br J Obstet Gynaecol* 2001; **108**: 371–77.

25 Ferrero S, Lungaro P, Bruzzone BM, Gotta C, Bentivoglio G, Ragni N. Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990–2000). *Acta Obstet Gynecol Scand* 2003; **82**: 229–34.

26 Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N Engl J Med* 1994; **331**: 1173–80.

27 European Paediatric HCV Network, Tovo PA, Pembrey L, Newell ML. A significant gender, but not elective caesarean section effect on mother-to-child transmission of hepatitis C virus infection. *J Infect Dis* 2005; **192**: 1872–79.

28 Granovsky MO, Minkoff HL, Tess BH, et al. Hepatitis C virus infection in the mothers and infants cohort study. *Pediatrics* 1998; **102**: 355–59.

29 Resti M, Azzari C, Mannelli F, et al. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. *BMJ* 1998; **317**: 437–41.

30 Thomas SL, Newell ML, Peckham CS, Ades AE, Hall AJ. A review of hepatitis C virus (HCV) vertical transmission: risks of transmission to infants born to mothers with and without HCV viraemia or human immunodeficiency virus infection. *Int J Epidemiol* 1998; **27**: 108–17.

31 Ruiz-Extremera A, Salmeron J, Torres C, et al. Follow-up of transmission of hepatitis C to babies of human immunodeficiency virus-negative women: the role of breast-feeding in transmission. *Pediatr Infect Dis J* 2000; **19**: 511–16.

32 Mok J, Pembrey L, Tovo PA, Newell ML. When does mother to child transmission of hepatitis C virus occur? *Arch Dis Child Fetal Neonatal Ed* 2005; **90**: F156–60.

33 The French Pediatric HIV Infection Study Group and European Collaborative Study. Morbidity and mortality in European children vertically infected by HIV-1. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **14**: 442–50.

34 European Collaborative Study. Gender and race do not alter early-life determinants of clinical disease progression in HIV-1 vertically infected children. *AIDS* 2004; **18**: 509–16.

35 European Collaborative Study. Fluctuations in symptoms in human immunodeficiency virus-infected children: the first 10 years of life. *Pediatrics* 2001; **108**: 116–22.

36 Staples CT, Rimland D, Dudas D. Hepatitis C in the HIV (human immunodeficiency virus) Atlanta V.A. (Veterans Affairs Medical Center) Cohort Study (HAVACS): the effect of coinfection on survival. *Clin Infect Dis* 1999; **29**: 150–54.

37 Dorrucci M, Pezzotti P, Phillips AN, Lepri AC, Rezza G. Coinfection of hepatitis C virus with human immunodeficiency virus and progression to AIDS (Italian Seroconversion Study). *J Infect Dis* 1995; **172**: 1503–08.

38 Sulkowski MS, Moore RD, Mehta SH, Chaisson RE, Thomas DL. Hepatitis C and progression of HIV disease. *JAMA* 2002; **288**: 199–206.

39 Rockstroh J, Konopnicki D, Soriano V. Hepatitis B and hepatitis C in the EuroSIDA cohort: prevalence and effect on mortality, AIDS, progression and response to HAART. 11th Conference on Retroviruses and Opportunistic Infections: San Fransisco, CA, USA, Feb 8–11, 2004. Abstract 799.

40 Carlos MJ, Castilla J, Lopez M, Arranz R, Gonzalez-Lahoz J, Soriano V. Impact of chronic hepatitis C on HIV-1 disease progression. *HIV Clin Trials* 2004; **5**: 125–31.

41 Greub G, Ledergerber B, Battegay M, et al. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet* 2000; **356**: 1800–05.

42 Yoo TW, Donfield S, Lail A, Lynn HS, Daar ES. Effect of hepatitis C virus (HCV) genotype on HCV and HIV-1 disease. *J Infect Dis* 2005; **191**: 4–10.

43 European Paediatric HCV Network. Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. *Clin Infect Dis* 2005; **41**: 45–51.

44 Mohsen AH, Easterbrook P, Taylor CB, Norris S. Hepatitis C and HIV-1 coinfection. *Gut* 2002; **51**: 601–08.

45 Nelson KE, Thomas DL. Reciprocal interaction of human immunodeficiency virus and hepatitis C virus infections. *Clin Diagn Lab Immunol* 2001; **8**: 867–70.

46 Tedaldi EM, Baker RK, Moorman AC, et al. Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. *Clin Infect Dis* 2003; **36**: 363–67.

47 Fuster D, Planas R, Muga R, et al. Advanced liver fibrosis in HIV/HCV coinfected patients on antiretroviral therapy. *AIDS Res Hum Retroviruses* 2004; **20**: 1293–97.

48 Mathews G, Bhagani S. The epidemiology and natural history of HIV/HBV and HIV/HCV co-infections. *J HIV Ther* 2003; **8**: 77–84.

49 Guido M, Rugge M, Jara P, et al. Chronic hepatitis C in children: the pathological and clinical spectrum. *Gastroenterology* 1998; **115**: 1525–29.

50 Garcia-Monzon C, Jara P, Fernandez-Bermejo M, et al. Chronic hepatitis C in children: a clinical and immunohistochemical comparative study with adult patients. *Hepatology* 1998; **28**: 1696–701.

51 Borgia G, Reynaud L, Gentile I, Piazza M. HIV and hepatitis C virus: facts and controversies. *Infection* 2003; **31**: 232–40.

52 Alberti A, Clumeck N, Collins S, et al. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. *J Hepatol* 2005; **42**: 615–24.

53 Tural C, Fuster D, Tor J, et al. Time on antiretroviral therapy is a protective factor for liver fibrosis in HIV and hepatitis C virus (HCV) co-infected patients. *J Viral Hepat* 2003; **10**: 118–25.

54 Manns MP, Wedemeyer H. Treatment of hepatitis C in HIV-infected patients: significant progress but not the final step. *JAMA* 2004; **292**: 2909–13.

55 Macias J, Castellano V, Merchante N, et al. Effect of antiretroviral drugs on liver fibrosis in HIV-infected patients with chronic hepatitis C: harmful impact of nevirapine. *AIDS* 2004; **18**: 767–74.

56 Carrat F, Bani-Sadr F, Pol S, et al. Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: a randomized controlled trial. *JAMA* 2004; **292**: 2839–48.

57 Chung RT, Andersen J, Volberding P, et al. Peginterferon alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. *N Engl J Med* 2004; **351**: 451–59.

58 Laguno M, Murillas J, Blanco JL, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. *AIDS* 2004; **18**: F27–36.

59 Torriani FJ, Rodriguez-Torres M, Rockstroh JK, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 2004; **351**: 438–50.

60 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147–71.

61 Butt AA. Hepatitis C virus infection: the new global epidemic. *Expert Rev Anti Infect Ther* 2005; **3**: 241–49.

62 National Institute for Clinical Excellence. Interferon alfa (pegylated and non-pegylated) and ribavirin for the treatment of chronic hepatitis C. http://www.nice.org.uk/pdf/TA075guidance.pdf (accessed Dec 16, 2005).

63 British HIV Association (BHIVA). BHIVA guidelines. http://www.bhiva.org/guidelines/2005/BHIVA-guidelines/DRAFT-2005.pdf (accessed Dec 16, 2005).

64 US Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Developed by the Panel on Clinical Practices for Treatment of HIV Infection convened by the Department of Health and Human Services, USA. October 6, 2005. http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf (accessed Jan 4, 2006).

65 European AIDS Clinical Society. European guidelines for the clinical management and treatment of HIV infected adults in Europe 2001. http://www.eacs.ws/download/European_Treatment_Guidelines.pdf (accessed Dec 16, 2005).

66 Nelson M, Mathews G, Brook MG, Main J, the BHIVA Coinfection Committee on behalf of the British HIV Association. BHIVA guidelines on HIV and chronic hepatitis: coinfection with HCV and hepatitis C virus infection (2005). *HIV Med* 2005; **6** (suppl 2): 96–106.

67 Wirth S, Pieper-Boustani H, Lang T, et al. Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. *Hepatology* 2005; **41**: 1013–18.

68 Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in paediatric HIV infection. March 2005. http://aidsinfo.nih.gov/ContentFiles/PediatricGuidelines.pdf (accessed Jan 4, 2006).

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