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“High risk pregnancy subsequent to maternal infectious disease”

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Napoli, November 2007 *Annalisa AGANGI*

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INTRODUCTION.
INFECTIOUS DISEASE DURING PREGNANCY

The health and well-being of both mother and child largely depend on the environmental factors, which in other words is the environment that can influence the fertility of women or the outcome of pregnancy or of the foetus. Today, increased knowledge about basic virology and immunology, better diagnostic techniques and the development of vaccines and efficient antiviral open new perspectives for women in childbearing age, in pregnancy and for the foetus.

Infections in pregnancy are one of the major causes of foetal morbidity and mortality. Infections are quite easily diagnoses and can, in many cases, be prevented; hence, risk assessment to the developing foetus is primarily based on the positive diagnosis of maternal infection. The foetus may be affected only if the causative agent is transmitted to it. This generally occurs in less than half of the cases of primary maternal infection, and to a much lesser degree in maternal reinfection. It was therefore important to develop methods for the direct diagnosis of foetal infection and/or to prevent vertical transmission.

Maternal infections may spread to the embryo and foetus in one of two ways: ascending infections, from the upper vagina via the uterine cervix, infecting the amniotic fluid, or by hematogenous spread as a result of maternal viremia or bacteremia. Ascending infections are more common in bacterial infections, while the hematogenous spread is more common in viral infections.

The possible adverse effects on the embryo and foetus include, besides distinct congenital anomalies, neonatal and perinatal effects that may lead to embryonic and foetal death, spontaneous abortions, premature labour and disturbances of foetal growth. In addition, the infection may have long term sequels, as for Congenital Toxoplasmosis, or, it may condition the life of the child and the adult it will be, as for HIV infection.

In the 25 years since the first report, more than 65 million persons have been infected with HIV, and more than 25 million have died of AIDS. Worldwide, more than 40% of new infections among adults are in young people (15-24years of age) and 60% are women. This crisis demanded a unique and truly global response. There is good evidence that available behavioural prevention strategies are effective, yet key prevention services currently reach less than 10% of person at risk. In the meantime, there is also a need for research on new approaches to prevention (vaccines) and treatment. A comprehensive response to HIV should include intensified effort while simultaneously expanding access to treatment and care, this is especially true for women in childbearing age.

While Toxoplasmosis infection in women is often benign, Congenital Toxoplasmosis can lead to severe consequences for the foetus and the newborn, such as visual or neurological impairment or death. To deal with this problem, European countries have adopted contrasting public health policies, ranging from focusing on primary prevention in the general population to intensive screening programs. One hypothesis to explain the lack of consensus is the fact that the epidemiology of Congenital Toxoplasmosis varies across countries and time. Another is that the evidence regarding the advantages and disadvantages of possible interventions is not clear.

In Italy, both screening for HIV and Toxoplasmosis are strongly recom-

mended during pregnancy.

Behind the development of screening programs, as a special type of public health intervention, there is a precious rational and a decision-making process. The main factors can be summarised as:

1. *The severity of the public health issue*. The usual starting point, for a public health program to be justified, is the severity of the target health problem or its consequences.
2. *The interventions*. They can be aimed at preventing causes of a disease (primary prevention) or at detecting early dysfunction (secondary prevention) or, one consequences are unavoidable, to solve the problem or to avoid worsening of consequences (treatment, rehabilitations and corrective actions).
3. *The risk/effectiveness ratio of the intervention.* An intervention should be recommended only if its positive effects outweigh its negative effects.
4. *The efficacy of the intervention.* The efficiency of an intervention will consider: a) the minimal resources requirement needed to maximize the positive effect and minimize the negative effects; b) the costs of implement the intervention; and c) whether the expected effects justify these costs.

The first part of chapter 1 deals with HIV infection background trying to explain why this infection is considered a relevant public health problem specially during pregnancy and what have been done since now to reduce foetal risk related to the infection. In particular, in the second part, it has been reported the description of the population of HIV infected women that attend the University Hospital of Naples. The second chapter, describes the ongoing European Collaborative Study on HIV and in particular examines the rate of repeated pregnancy in HIV infected women in the European setting, trying to recognise predictive factors for maternity desire. Moreover in this chapter, the most recently results from the European Collaborative Study are discussed. The third chapter deals with Toxoplasma infection, in particular the efficacy of the screening program and the effect of targeted ultrasound are discussed. Chapter 4 and chapter 5 report some rare and challenging conditions that have been observed during the study period.

CHAPTER 1.
HIV INFECTION IN CAMPANIA REGION

1.1. Scientific background: HIV and pregnancy

According to the Joint United Nations Program on AIDS (UNAIDS) about 19,2 million of women are HIV (Human Immunodeficiency Virus) infected in the word and 800.000 children become infected every year mainly through maternal-to-foetal transmission of the virus (1). There is particular concern for the increasing numbers of infected people reported from Eastern Europe, where the current infrastructure may be unable to cope with a rapidly evolving epidemic. HIV infection and transmission thus remain an important issue in Europe.

HIV is an enveloped retrovirus whose infection results in profound deficiencies in cell-mediated and humoral immunity, secondary to both quantitative and qualitative defects, leading to a progressive dysfunction of the immune system with depletion of CD4 cells.

Diagnosis of HIV infection as part of routine prenatal care of pregnant women is very important because preventive therapies are now available. Prenatal diagnosis in the foetus is difficult because of the risk for bleeding and contamination of the sample with maternal blood or the possibility for accidental iatrogenic infection of the foetus.

HIV infection in infants and children has a different presentation from adults. Growth delay is an early and frequent finding of untreated perinatal infection, children are more likely than adults to have serious bacterial infections. The initial symptoms may be subtle, premature birth has been reported in 19%. Common clinical features seen during the course of HIV infection in children are lymphadenopathy, fevers, malaise, loss of energy, as well as recurrent and chronic otitis and sinusitis. Also commonly encountered are failure to thrive, sometimes associated with chronic diarrhoea, failure to grow, the presence and persistence of mucocutaneous candidiasis and many non-specific cutaneous manifestations (2).

Mother-to-child, or vertical, transmission of HIV-1 can take place before, during or after birth, with most transmission occurring around the time of delivery. The risk is associated with maternal HIV disease status, foetal exposure to infected maternal body fluids and breastfeeding, but the exact mechanism is not understood. Knowledge about the timing of mother-to-child transmission of HIV is sought in order to determine the period of greatest risk for the infant, as this would suggest approaches to help to prevent HIV infection and to suggest the optimum time of their initiation. If infection occurs mainly during early gestation, interruption of transmission may be difficult, whereas intervention may be more feasible if transmission occurs mainly during late gestation or at the time of delivery. If transmission at the time of delivery occurs through exposure of the foetus to infected maternal secretions, Caesarean section delivery or reduction of viral load in the birth canal would be beneficial. On the other hand, if transmission at the time of delivery occurs through maternal-foetal transfusion during labour, prophylactic antiretroviral treatment of the infant would be appropriate. Knowledge about postnatal transmission through breastfeeding would inform guidelines regarding infant feeding practices (3-6). Current data suggest that the majority of children are infected during immediate

peripartum period. The risk of vertical transmission without any intervention is about 25% (7). High viral load and low CD4 count have been suggested as risk factors for HIV vertical transmission. However there is not a viral load under which the transmission never occurs even if it is considered very rare when the woman has a undetectable viral load (HIV-RNA <1000 copies/ml) near the time of delivery (8,9). On the other hand, the risk of vertical transmission is increased in case of prolonged rupture of membranes (>4h) because of the exposure to maternal fluids (10), episiotomy, corioamnionitis and concomitant sexually transmitted diseases (11).

An important goal in the care for HIV-infected women is the prevention of foetal/neonatal infection. Identifying pregnant women who are HIV infected is essential, not only for the potential initiation of therapy but also for the coordination of optimal prenatal care. In theory mother-to-child transmission may be avoided by antiretroviral prophylaxis during pregnancy, elective caesarean section (12,13) and refraining breastfeeding. In Western Europe, where these interventions are available, the rate of vertical transmission has decreased from about 15 to 2% or less (7). The European Collaborative Study of 1254 HIV-infected mothers and their children estimated that caesarean section resulted in a 50% reduction of the transmission rate (14). The success of the AIDS Clinical Trials Group 076 (PACTG 076) protocol has had a major impact on the prevention of perinatal transmission of HIV-1 and has resulted in new guidelines. In that landmark study pregnant HIV-infected women received oral zidovudine, starting at 14 to 34 weeks of gestation, and intravenous zidovudine during labor and delivery, while the infants were treated with 6 weeks of oral zidovudine post partum. This resulted in a 67% reduction in the perinatal transmission rate from 25% to 8.3% (P=0.00006) (15).

Since the mid-1990s, potent and effective antiretroviral therapy (HAART) to delay progression of disease in HIV-infected adults has become the standard of care. Such regimens are now usually applied, as a consequence HIV-infected women may now make a positive choice to become pregnant and those who do become pregnant are less likely to have this pregnancy terminated, because their own disease is well managed and interventions to reduce the risk of vertical transmission are available (16). Pregnancy should not preclude the use of optimal therapeutic regimens. However, recommendations regarding the choice of antiretroviral drugs for treatment of infected pregnant women are subject to unique considerations. They include:

a. Possible changes in dosing requirements resulting from physiologic changes associated with pregnancy,
b. potential effects of antiretroviral drugs on the pregnant woman, and the potential short- and long-term effects of the antiretroviral drug on the foetus and newborn, which may not be known for certain antiretroviral drugs.

Although the protective effect of antiretroviral therapy for vertical transmission has been confirmed in many clinical trials (17-22) and in European (22,23) or American (24) observational cohorts, data are conflicting as to whether receipt of combination antiretroviral therapy during pregnancy is associated with adverse pregnancy outcomes such as preterm delivery or other pregnancy complications.

1.2. Epidemiological and clinical features of pregnant women with HIV: a 21-year perspective from a highly specialized regional center in Southern Italy

The HIV epidemic has seen many important changes over the past 20 years, especially the significant declines in AIDS incidence and deaths (16).

In Italy governmental HIV surveillance, based on reports of AIDS cases has shown, as elsewhere in Europe, a decline in AIDS cases since 1996 (25). Progressive changes in the characteristics of the HIV-infected population in Europe have also been reported with a gradual shift from injecting drug use (IDUs) sharing to heterosexual acquisition of infection. In the same time, the proportion of women among newly diagnosed HIV infections in Europe has increased, from 10% in 2001 to 29% in 2005 (26).

In this context we have judged mandatory to monitoring the demographic and biological characteristics of HIV infected pregnant women in our region (the Campania) and the extent and type of antiretroviral treatment and other interventions to reduce mother-to-child transmission given before, during or after pregnancy, to estimating the rate of vertical transmission in Campania. All this aspects have indirectly the mean goal to act as a public health resource on a regional basis.

Since our Department of Prenatal Care has been recognised as a referring centre for HIV/AIDS and other sexual transmitted diseases, we were providing with a unique opportunity to describe the changing characteristics of women with HIV in a region of the South West of Italy (Campania) over the past 21 years.

1.3. Our experience: Materials and Methods

- **Type of study**: This is a prospective cohort study;
- **Inclusion criteria**: The study population consisted of all HIV-infected pregnant women attending the Antenatal Clinic of the Department of Obstetrics and Gynecology of the University of Naples Federico II;
- **Data collection for pregnant women**: All HIV-infected women attended to our Department were enrolled and follow with their infected and uninfected children. The management of the pregnancies was based on standard protocols that were progressively updated according to advances in clinical care and treatment guidelines. Informed consent was obtained for the use of personal data and no woman declined to enter the study. We recorded socio-demographic data (date of birth, marital status, ethnic group, work), and information about clinical status (current HIV staging according to the 1993 CDC classification symptoms and date of onset), maternal antiretroviral therapy (ART) with details about the date of starting and adverse effects, virological and immunological status(quantitative HIV-RNA PCR, total lymphocytes, CD4 and CD8 cell counts), route of transmission, HIV status of the partner, and concomitant infectious diseases (TORCH, Lue). Data on pregnancy and neonatal outcomes were also recorded by our multidisclipinary team constituted by obstetricians and pediatricians. The data collected were entered into a Microsoft Access Database which holds all information on child and maternal follow-up.
- **Results**: By December 2006, 230 deliveries in 159 women had been monitored. The following were the main results we obtained:

  1. Deliveries in HIV-infected women increased from 0.16% (4/2,499) of all deliveries in 1985 to 0.73% (15/2042) in 2006;
  2. The socio-demographic profile of the women changed greatly over the study period. There was a shift from injecting drug use to heterosexual contact as the main transmission route, and an increased proportion of foreign women;
  3. The proportion of infected pregnant women receiving antiretroviral treatment increased from 27% (17/63) before 1996 to 81% (63/78) in 2006, with a corresponding decrease in the mother-to-child transmission rate from 36% (16/44) to 0.6% (1/157). Similar changes in the overall scenario of HIV and pregnancy are occurring in various parts of the world.

This work concluded with the publication of a paper on an international journal as reported above.

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Epidemiological and Clinical Features of Pregnant Women with HIV: A 21-Year Perspective from a Highly Specialized Regional Center in Southern Italy

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Recto running head: *HIV Trend in Pregnant Women in Southern Italy • Martinelli et al.*

Abstract

Purpose: To analyze the changes over two decades in HIV-infected pregnant women followed at a highly specialized regional center for antenatal care in southern Italy. **Method:** Since 1985, all HIV-infected pregnant women attending our center have been monitored using progressively updated protocols. **Results:** By December 2006, 230 deliveries in 159 women had been monitored. Deliveries in HIV-infected women increased from 0.16% (4/2,499) of all deliveries in 1985 to 0.73% (15/2,042) in 2006. The sociodemographic profile of the women changed greatly over the study period, and there was a shift from injecting drug use to heterosexual contact as the main transmission route and an increased proportion of foreign women. Subsequent to improvements in clinical care, the proportion of infected pregnant women receiving antiretroviral treatment increased from 27% (17/63) before 1996 to 81% (63/78) in 2006, with a corresponding decrease in the mother-to-child transmission rate from 36% (16/44) to 0.6% (1/157). **Conclusion:** The increasing number of HIV-infected pregnant women can be attributed to nonselective antenatal HIV screening, the spread of HIV infection through heterosexual contacts, and the desire of HIV-infected women to have children. In this context, highly specialized reference centers can play an important role in providing HIV-infected pregnant women with optimal care and in reducing mother-to-child transmission rates to very low levels. **Key words:** *AIDS, HIV, epidemiology, Italy, pregnancy*

The HIV epidemic has seen many important changes over the past 20 years, perhaps the most striking being a significant decrease in the incidence of AIDS and AIDS-related deaths.[1] In Italy, 56,076 cases of AIDS were reported between 1982 and 2005. The number of cases progressively increased between 1982 and 1995, decreased between 1995 and 2001, were stable between 2001 and 2004, and decreased between 2004 and 2005.[2]

Also the characteristics of the HIV-infected population in Europe have changed in the last two decades. Since 1998, a gradual shift from injecting drug use (IDU) needle sharing to heterosexual acquisition of infection has been recorded in Europe (infections through heterosexual contacts increased from 43% in 1998 to 59% in 2003[3]). Also in Italy, the cases of AIDS due to heterosexual contacts increased from 12% before 1994 to 42% in 2004–2005[2]. Although the proportion of women among newly diagnosed cases of HIV infection in Europe increased from 10% in 2001 to 29% in 2005, the mother-to-child transmission (MTCT) rate declined from around 15%–18% to below 2%.[4,5]

In 1995, the Italian Ministry of Health recommended that HIV testing be included in screening for women during pregnancy.[6] Since 1985, all HIV-infected pregnant women in Campania (southwest Italy) have been followed at our Antenatal Clinic. Consequently, we had a unique opportunity to monitor the changing characteristics of women with HIV over the past 21 years.

METHOD

Population

The study population consisted of all HIV-infected pregnant women attending the Antenatal Clinic of the Department of Obstetrics and Gynecology of the University of Naples Federico II in the Campania region (which has one of the highest birth rates in Italy) in southwest Italy. Our Antenatal Clinic has been designated a highly specialized regional center for antenatal care. HIV-infected women were followed according to standard protocols that were progressively updated according to advances in clinical care and treatment guidelines. Informed consent was obtained for the use of personal data, and no woman declined to enter the study. We recorded sociodemographic data and information about clinical status, maternal antiretroviral therapy (ART), virological and immunological status, route of transmission, HIV status of the partner, and concomitant infectious diseases. CD4 cell counts were monitored since 1991. Stage of infection was classified according to the 1993 Centers for Disease Control and Prevention (CDC) classification.[7] Data on pregnancy and neonatal outcomes were also recorded by our multidisclipinary team of obstetricians and pediatricians. Preterm delivery was defined as delivery before 37 completed weeks of gestation; iatrogenic preterm deliveries resulting from the mother's condition were excluded. A child was classified as HIV-infected after the onset of an AIDS-defining disease or detection of the virus by DNA polymerase chain reaction (PCR) on two different occasions or the persistence of antibody beyond 18 months.[8]

Statistical Analysis

Microsoft Office Access 2003 was used to manage and store data. Associations between categorical variables were tested with the chi-square test, Fisher exact test, and the chi-square test for trend. All *p* values were two-sided, using .05 as threshold of significance. Calculations were performed using the EPI-Info software, version 3.1 (CDC, Atlanta, Georgia, USA).

[h1]RESULTS

[h2]Maternal Characteristics

From January 1985 to December 2006, 192 HIV-infected women were followed, for a total of 230 pregnancies. Four of these pregnancies were ongoing and seven were lost to follow-up. Seventy-one women had more than one pregnancy during the study (56 had two pregnancies and 15 had three). Overall, four pregnancies were twin pregnancies, 12 ended in spontaneous abortion, 5 in a voluntary pregnancy termination, and 1 in a therapeutic abortion (because of fetal malformation). After exclusion of abortions and patients lost to follow-up, deliveries to HIV-infected women as a proportion of total deliveries increased from 0.16% (4/2,499) in 1985 to 0.73% (15/2,042) in 2006 ($p < .001$; **Figure 1**).

The mean (±*SD*) maternal age of women at their first prenatal visit was 30 ± 5 years, with an increase from 25 years in 1991 to 32 years in 2006 ($p = .054$). The median gestational age at first antenatal visit was 16 weeks (range 4–42 weeks), with a shift toward an earlier first visit from late in the third trimester or at delivery (median gestational age, 31 weeks; range 10–41 weeks) in the pre-HAART era (1985–1996) to early in the second trimester (median 12 weeks; range 4–42 weeks; $p < .001$) in the HAART era (1997–2006).

The sociodemographic characteristics of the women are summarized in **Table 1**. It is interesting to note differences between the pre- and post-HAART eras. Indeed, after the advent of HAART, more women were in a stable relationship (married or cohabiting) than were single or divorced/separated/widowed (p =.06), and more women were infected through heterosexual contact than were infected through IDU ($p < .001$).

At enrollment in the study, 197 women (86%) provided information about the time of their first positive HIV test. In 134 cases (68%), HIV was diagnosed before conception; in the remaining cases, it was diagnosed during pregnancy (first trimester, 57%; second trimester, 20%; third trimester/delivery, 22%). Preconception diagnosis was more common among Italian women (95/142, 67%) than among other nationalities considered collectively (38/88, 43%; p = .06). There were no significant differences between ex- or current IDUs (28/50, 56%) and women infected heterosexually (97/152, 63%; p = .62).

In the pre-HAART versus the post-HAART era, HIV testing was carried out before conception in 73% (16/22) and 67% (118/175) of cases, respectively, and after conception in 27% (6/22) and 33% (57/175) of cases (p = .61). Before HIV screening was introduced for pregnant women in Italy in March 1995, 67% (8/12) of women were diagnosed before conception and 33% (4/12) after conception. After 1995, 68% (126/185) of women were diagnosed before conception and 32% (59/185) after conception (p = 1.00).

Figure 1 shows the temporal trends for proportions of deliveries from women with HIV and mode of acquisition of HIV infection. The proportion of heterosexually acquired infection increased significantly from 28% (3/11) in 1985–1990 to 42% (16/33) in 1991–1996, to 77% (54/70) in 1997–2001, and to 68% (79/116) in 2002–2006 (p for trend = .008).

[h2]Maternal Management

In 1996, when zidovudine (ZDV) was approved for the prophylaxis of vertical transmission of HIV in Italy, we adopted the three-part (prenatal, intrapartum, and postnatal) AIDS Clinical Trial Group (ACTG) 076 regimen[9] to prevent vertical transmission of HIV. Before 1996, ZDV monotherapy was used in 12/44 (27%) pregnancies, depending on the mother's condition. Among the 157 pregnancies followed subsequently (excluding cases of spontaneous abortion, voluntary interruption, therapeutic abortion, and ongoing pregnancies), 141 (89%) received ART: 31 women were already undergoing ART at conception, and in 110 women treatment was started during pregnancy, at a median gestational age of 18 weeks (range 3–37). The 16 untreated women in the HAART era presented very late in pregnancy (at delivery or in the 10 preceding days) and there was not sufficient time to administer prophylaxis in some cases, whereas the women's health status had not been completely evaluated by an infectious disease specialist before labor started in other cases. Among the 141 women treated during pregnancy, 30 (20%) received ZDV monotherapy, 31 (22%) a two-drug therapy, and 80 (58%) a HAART regimen. The proportion of women receiving HAART increased from 27% (17/63) in 1997–2001 to 81% (63/78) in 2001–2004 ($p < .001$). (One woman received a two-drug regimen in the first half of pregnancy and then HAART.) The most common HAART regimens were ZDV + lamivudine (3TC) + nevirapine (NVP) (33/80; 41%) and ZDV + 3TC + nelfinavir (NFV) (19/80; 24%). Administration of HAART was not associated with nationality ($p = .28$) or mode of acquisition (heterosexual vs.IDU; $p = .49$). Few side effects (mild anemia fever, myalgia and rash) were recorded.

[h2]Pregnancy Outcome and Complications

Most women delivered by elective cesarean section after 37 weeks of gestation; vaginal delivery decreased from 32% (14/44) in the pre-HAART era to 6% (9/157) in the HAART era ($p < .001$; **Table 2**). ZDV intrapartum prophylaxis was applied in 98% (154/157) of the pregnancies in the HAART era: three did not receive intravenous ZDV because of very rapid labor after arriving at hospital, but they received the antenatal treatment and their babies the neonatal therapy.

Obstetrical complications were few: two cases of pre-eclampsia (one woman was on HAART from the first trimester, and the other from the second trimester), and one case of abruptio placentae (ZDV monotherapy since second trimester). Two women had major postpartum complications (rate of major postpartum complications was 1.2 per 100 deliveries): one postcesarean hysterectomy secondary to uterine atony after an elective cesarean section, and one had a wound dehiscence of a previous emergency cesarean section. There were three cases of fetal malformation: one cardiac malformation (mother not treated), one bilateral renal hypoplasia (mother treated with d4T+3TC+NFV before conception), and one gastroschisis (mother treated with AZT+3TC+NVP before conception). The congenital malformation rate was 1.3% (2/153) among ART-exposed infants and 2% (1/48) among those without ART exposure ($p = .56$, Fisher exact test). Complete follow-up regarding HIV infection status was available for 193 children (8 were lost to follow-up). Two cases were stillborn at 27 and 36 weeks: there were no maternal complications. The first mother received no ART because she delivered in 1992. The second mother started HAART (ZDV, 3TC, lopinavir/ritonavir) before conception. There were two neonatal deaths (at 3 days, attributable to a cardiac malformation [38 weeks gestation] and at 7 days because of neonatal sepsis [32 weeks gestation]). The overall mother-to-child transmission (MTCT) rate was 8% (17/201, 95% CI 5%–12%), but

only one infant acquired HIV infection vertically after 1996. In this case, the parents interrupted neonatal prophylaxis with ZDV at discharge. The MTCT rate prior to application of the ACTG 076 protocol and introduction of HAART was 36% (16/44, 95% CI 22%–51%) compared to 0.6% (1/157; 95% CI 0%–3.5%) in the HAART era.

Forty-seven women were HCV coinfected. Among their newborns (n = 48; 36 since 1996), there were no dual transmissions, nine transmissions of HCV infection (18.8%, 95% CI 8.9–32.6), and two cases of HIV transmission (4.2%; 95% CI 0.5–14.3). ART was not administered in either case.

Postnatal information on maternal health status was available for 111 mothers (80%): 105 (95%) were alive at a median of 4 years postpartum, and 6 (5%) died at an average of 4.5 years (range 1– 8) after delivery. Of these six deaths, only one was HIV-related (one woman who delivered in 1991 and died from AIDS in 1993); the remaining four women died from non-HIV related causes.

[h1]DISCUSSION

The experience of our highly specialized center reflects diverse aspects of the HIV epidemic and the advances that have been made in the care of pregnant HIV-infected women over the last 20 years. The increase by almost 4.5-fold in the HIV-infected caseload in our antenatal clinic since 1985 is probably a composite effect of various factors, namely, the desire for maternity among HIV-infected women, the general shift in the epidemic toward heterosexually acquired infections, the increasing number of infected women of foreign provenance in our region, and changes in the management of infected pregnant women (i.e.,

an increased use of ART, particularly of HAART), and support for childbearing provided to HIV-infected women by specialized reference centers.

Similar changes in the overall scenario of HIV and pregnancy are occurring in various parts of the world. Because pregnancy seems to have little influence on the progression of infection and given the actual low rate of vertical transmission, many people with HIV now contemplate having offspring.[9] A national study in the United States revealed that 69% of HIV-infected women and 59% of HIV-infected men expect to have at least one child.[10] A prospective cohort study in Europe found a substantially increased likelihood of subsequent live births after 1995 and shorter birth intervals among women on HAART and among Black women.[11]

We found that after the introduction of HAART, the vast majority of pregnant HIV-infected women in our population had a stable relationship (89%, 144/161). This condition probably strongly favors the desire for parenthood. Moreover, the high 5-year postdelivery survival rate among the subgroup of women with postnatal data is also reassuring with regard to future family life and confirms the good prognosis associated with HAART demonstrated in other cohorts.[12] However, our survival data should be interpreted with some caution because we lack follow-up information for about 20% of the women.

The increase in deliveries among our HIV-infected women may also reflect the introduction of screening programs for all pregnant women in Italy. In Italy, HIV screening started in 1995 and was recommended for all women only during the first trimester.[6] In 1998, HIV testing was recommended and was available free-of-charge for all women at a preconception visit, in addition to the first visit in pregnancy and the last visit before delivery.[13] The Italian policy of

first trimester screening includes, in addition to HIV testing, testing for antibodies against rubella, toxoplasma, and the Venereal Disease Research Laboratories (VDRL) and Treponema Pallidum Hemagglutination (TPHA) tests.

Our results indicate a shift in the characteristics of HIV-infected women from an easily recognizable high-risk population consisting largely of IDUs to a more heterogeneous population that includes an increasing number of women from countries with a high frequency of HIV infection. Our finding that 3/11 pregnant women in 1985–1990 compared to 88% in 2002–2006 were infected through heterosexual contact, with a concomitant decline in IDU-related acquisition, is similar to results obtained in other European countries.[14,15] In Latium, Italy, heterosexual contact surpassed IDU as the primary mode of acquisition among women in 1992, and in 2000 nearly 60% of HIV-positive women had acquired infection sexually.[16] In Sardinia, the overall rate of heterosexual transmission of HIV was 61%, and the rate was even higher rate among women (78% vs. 47% among men).[17] This epidemiological scenario illustrates the importance of recommending universal voluntary antenatal HIV testing for all pregnant women and not just for particular groups.

Notwithstanding national recommendations, a high proportion of women in south Italy are still diagnosed with HIV in the second or third trimester of pregnancy (14% in our population and about 40% in southeastern Italy).[18] There are three possible reasons for the high rate of late diagnosis: (a) women in the general population do not consider the possibility of being HIV infected; (b) not all women are screened; and (c) some women, especially those from abroad, may not have easy, timely access to antenatal services. Given the potentially negative effects of delayed application of anti-MTCT measures, these observations suggest that more effort should be made to inform prenatal care providers about the importance of HIV

screening. They also suggest that foreign women should be guaranteed easy access to antenatal care services.

The transmission rate of 36% in our early cases (pre-1997), which is higher than the 15% reported in another European context[15] but similar to the 26.6% rate observed in the United States without ZDV prophylaxis,[19] was obtained in a setting in which intervention measures were undefined, many of the infected women were drug users with concomitant morbidity, most had late antenatal care, and it was not possible to treat the majority of them effectively. Starting from 1997, infected pregnant women presented and were managed earlier during pregnancy and were treated more effectively. As a result, the rate of MTCT has decreased in our center to a very low level (0.6%), which is comparable to those of other European cohorts.[4,5]

It is the policy of our center to offer elective cesarean section irrespective of maternal plasma viral load. This policy is based on the low rate of complications in our population and on epidemiological findings suggesting that elective cesarean section is effective in reducing the risk of MTCT regardless of whether or not the mother is receiving ART.[4] We recorded one case of emergency peripartum hysterectomy, which corresponds to a rate (0.4%) similar to that observed in the general population (cesarean section and vaginal deliveries) of 0.8/1000 births.[20] The very low rate of preterm deliveries (7%) in our study might be related to the small number of complicated pregnancies that we observed.

In terms of specific subgroups, there were no differences in time of diagnosis between women with and without an IDU history. However, the proportion of Italian women who knew their

HIV diagnosis before conception was higher than that of non-Italian women. This finding may reflect reduced access of foreign women to HIV testing in their countries of origin.

Last, we also evaluated the HCV transmission rate in HIV/HCV-coinfected women. The HCV transmission rate of 19% that we observed is in line with a previous study showing an HCV transmission rate of 15% in coinfected mothers, which is higher than the 3.7% transmission rate among women with HCV infection only.[21]

[h1]CONCLUSION

In summary, in the fast evolving epidemiological scenario of HIV infection, specialized reference centers, by providing adequate and timely care and applying an updated standard protocol for obstetrical management and ART, may reduce the rates of vertical HIV transmission and obstetrical complications and may also reduce untoward effects of treatment. Despite the current low MTCT rates and the significantly earlier presentation for antenatal care in recent years, preconception testing and early presentation for prenatal care is still not optimal in south Italy. To ensure effective management of HIV-infected mothers, it is essential to obtain a timely diagnosis of HIV through adequate preconception counselling and HIV testing.

[h1]ACKNOWLEDGMENTS

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Table 1. Sociodemographic maternal characteristics pre-HAART and after the introduction of HAART

Characteristics	Pre-HAART (1985–1996) *n* (%)	HAART (1997–2006) *n* (%)
Gravidity (*n* = 214)	31 (14)	183 (86)
1	10 (32)	46 (25)
2	8 (26)	46 (25)
3 or more	13 (42)	102 (56)
Previous terminations (*n* = 214)	31 (14)	183 (86)
0	24 (77)	104 (57)
1	3 (10)	40 (22)
2	3 (10)	16 (9)
3 or more	1 (3)	23 (13)
Previous spontaneous abortions (*n* = 214)	31 (14)	183 (86)
0	27 (87)	153 (84)
1	3 (10)	21 (11)
2	1 (3)	6 (3)
3	0	3 (2)
Country of origin (*n* = 229)	41 (18)	186 (82)
Italy	35 (85)	107 (57)
Africa	5 (12)	48 (26)
East Europe	1 (2)	16 (9)
West Europe (excluding Italy)	0	7 (4)
South America	0	10 (5)
Marital status (*n* = 196)	35 (18)	161 (82)
Single	4 (13)	11 (7)

Married	22 (63)	90 (56)
Cohabitating	4 (11)	54 (34)
Divorced/separated/widowed	5 (14)	6 (4)
Route of transmission (n = 223)	41 (18)	182 (82)
Heterosexual contact	18 (44)	135 (74)
Injecting drug use[a]	23 (56)	26 (14)
Blood transfusion	0	2 (1)
Not known	0	19 (10)

[a]Present and past injecting drug users.

Table 2. HIV-related parameters and pregnancy outcomes pre-HAART and after the introduction of HAART

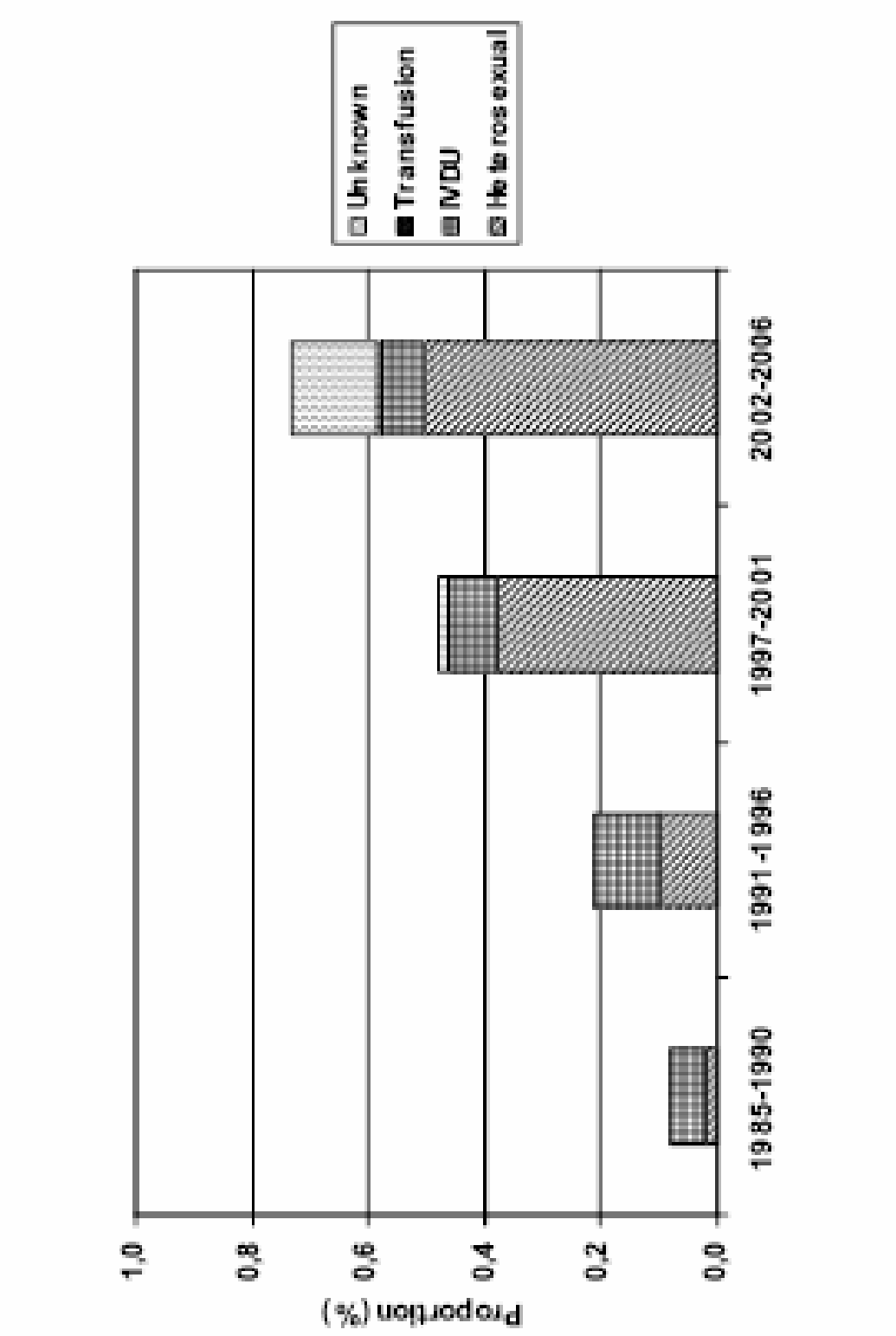
Parameters and outcomes	Pre-HAART (1985–1996) *n* (%)	HAART (1997–2006) *n* (%)
CD4 count/mm^3 at delivery (*n* = 124)	**16 (13)**	**108 (87)**
≥500 cells	6 (37)	41 (38)
200–499	7 (44)	59 (55)
<200	3 (19)	8 (7)
CDC clinical stage[7] at delivery (*n* = 192)	**41 (21)**	**151 (79)**
A	36 (88)	122 (81)
B	0	14 (9)
C	5 (12)	15 (10)
Pregnancy outcome (*n* = 230)		
Delivery	44	157
Vaginal delivery	14 (32)	9 (6)
Emergency cesarean section	6 (14)	28 (19)
Elective cesarean section	24 (54)	120 (76)
Spontaneous abortion	NR	12 (6)
Voluntary interruption	NR	5 (3)
Therapeutic abortion	NR	1 (1)
Lost	NR	7 (4)
Ongoing	NR	4 (2)

Gestational age at delivery (*n* = 176)	**29 (16)**	**147 (84)**
Median (range)	39 (36–42)	38 (28–42)
Preterm delivery	1 (3)	11 (7)[a]
Birthweight (*n* = 166)	**29 (17)**	**137 (83)**
Mean ± *SD*	2868 ± 435	2869 ± 516
<2500 g	5 (18)	24 (18)
≥2500 g	24 (82)	113 (82)

Note: NR = not recorded; data not collected before 1997.

[a]Three cases were excluded because we performed an iatrogenic preterm delivery because of the mother's condition: two cases of severe pre-eclampsia and one of severe diabetic nephropathy.

Figure 1. Deliveries to HIV-infected women as a proportion of total deliveries 1985–2006 and distribution of maternal mode of acquisition. IVDU = intravenous drug use.



CHAPTER 2.
HIV INFECTION IN EUROPE

2.1. The European point of view in HIV infection in pregnancy: the European Collaborative Study

One of the reason of the success in the prevention of vertical transmission of HIV infection is the collaboration of most of the centres dedicated to the treatment of HIV infection. The Department of Obstetrics and Gynaecology of the University of Naples Federico II collaborates with the long-standing and ongoing European Collaborative Study (ECS).

Since 1985, the ECS has substantially contributed to research internationally on HIV infection in pregnancy and childhood, influencing policy and management. The ECS network includes more than 20 referral centres for HIV infected pregnant women and their children with central epidemiological expertise, and it currently expanding into Eastern Europe.

The ECS is a multicenter European prospective study, it consists of a coordinating centre at the Institute of Child Health in London and a network of export clinicians from 9 European countries.

The aim of this project is to investigate the consequences of HIV infection in pregnancy and after delivery in women and their children, in the area of antiretroviral therapy and other interventions for treatment and prophylaxis. This includes:

1. to monitor demographic and biological characteristics of HIV- infected pregnant women in Europe, and the extent and type of anti-retroviral treatment and other interventions to reduce mother-to-child transmission given before, during and after pregnancy,
2. to document the rate of MTCT across Europe in relation to interventions to reduce transmission,
3. to determine benefits and potential risks of these intervention,
4. to assess the health and social circumstances of infected and uninfected children, in the era of antiretroviral therapy to delay disease progression,
5. to act as a public health resource on a European basis.

2.2. General Methodology and data collection

The ECS is a prospective birth cohort study with approximately 7000 mother-child pairs. HIV-infected pregnant women are enrolled and followed up during pregnancy, with their children (both infected and uninfected) followed up prospectively from birth. Only women identified as HIV infected before, during pregnancy or at delivery are eligible for inclusion. The ECS uses only routinely collected data for its core study, including socio-demographic details and clinical and laboratory test results; thus no extra patient visits or tests are required as a result of enrolment in the study.

ECS centres systematically identify HIV infected pregnant women according to local practice, thus in Italy we perform HIV screening test during the preconceptional visit, during the first and third trimester of pregnancy. Clinical and laboratory information is collected from enrolled women 1-3 times during pregnancy; the amount of follow-up information depending on the trimester at which the women were enrolled. Initial information collected includes mother's full obstetric history, marital status, ethnic group, likely route of HIV acquisition (injecting drug use, needle sharing, high risk sexual partner), HIV history and current HIV status including treatment details. During pregnancy and at delivery, virological and immunological data on both qualitative and quantitative HIV-DNA PCR and HIV-RNA PCR, total lymphocytes, CD4 and CD8 cell counts are reported. Required delivery information includes gestational age in completed weeks, birthweight in grams, the administration of prophylactic ART during labour or delivery including type of therapy and dose mode of delivery and any perinatal problems after delivery. Each child is followed according to local centre policy.

The collaboration consist, firstly, in returning follow-up questionnaires to the co-ordinating centre in London on a regular basis. There is a high level of knowledge integration between the participants of the ECS, as the creations of knowledge and dissemination of results is a primary objective of the collaboration. The ECS has a policy of publishing all its work under the ECS authorship. Anyway all ECS clinicians are involved in the writing of ECS papers and they all approve manuscript before submission to journals.

2.3. Maternity desire in HIV infected women in Europe

Inside the ECS research group, there is an exchange of experience because researches from ECS centres are welcome to the co-ordinating centre for carried out specific projects.

On this basis, I made ad hoc visit to the coordinating centre to investigate how the subsequent childbearing of HIV-infected mothers enrolled in the ECS has changed over time and identify factors predictive of further childbearing.

The specific background was that the maternity desire recorded in other international study is growing and the feeling of the expertise that the number of subsequent pregnancy is increasing over the years. One national study of HIV-infected individuals in the USA found that about 28% of participants wanted children in the future (1), and cohorts of HIV-infected women from Sub-Saharan Africa, Europe and North America show that many HIV-infected women choose to have children after learning of their infection (2,3).

There are few medical concerns that should affect HIV-infected women's reproductive choices. Pregnancy seems to have little influence on the progression of HIV disease, because the transient decline I maternal immunocompetence observed during gestation spears to resolve after delivery. For many HIV-Infected women, these medical concerns can be outweighed by psychological factors influencing decision about fertility and contraception. Existing data indicated that diagnosis with HIV modifies but does not remove the wish to have children for many women and men (2,3). For some, having a child might be perceived to help combat the dehumanising effects of living with HIV and related anxieties about the future (4,5). Importantly, the desire to have a child might be altered bt the availability of highly active antiretroviral therapy, because infected individuals receiving treatment can come to view HIV as a chronic condition (6).

The psychological influences on fertility desires are paralleled by social norms, which view reproduction as an integral part of women's life. Family and partner influences can be fundamental in shaping women's decisions to have a child. The central role of childbearing in women's social identities might be particularly marked in parts of Africa, where a woman's failure to have children can be accompanied by stigmatisation and discrimination rivalling that of HIV infection itself (7).

Although some individual and societal factors might encourage reproductive desire, in other situations concerns about the possibility of an infected child, and/or a child orphaned because of AIDS, might discourage childbearing (4,5).

To carry on this project all HIV infected women enrolled between end 1986 and November 2003 were considered. From the total population of 3911 HIV-infected women, two groups were distinguished: 3693 with only one and 218 with subsequent live births.

This analysis has allowed the publication of the paper reported below.

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Increasing likelihood of further live births in HIV-infected women in recent years

European Collaborative Study[a,1]

Objective To examine how the subsequent childbearing of HIV-infected mothers enrolled in the European Collaborative Study (ECS) has changed over time and identify factors predictive of further childbearing.

Design Prospective cohort study.

Setting Centres in nine European countries included in the ECS, enrolled between end 1986 and November 2003.

Population HIV-infected women (3911): 3693 with only one and 218 with subsequent live births.

Methods Univariable and multivariable logistic regression analyses to obtain odds ratios (OR) and 95% confidence intervals (CI). Kaplan–Meier (KM) analyses to estimate cumulative proportions of women having a subsequent live birth.

Main outcome measures Subsequent live birth.

Results In multivariable analysis adjusting for time period, ethnicity, maternal age and parity, black women were more likely [adjusted odds ratio (AOR) 2.45; 95% CI, 1.75–3.43], and women >30 years less likely (AOR 0.54, 0.37–0.80), to have a subsequent live birth. Time to subsequent live birth significantly shortened over time, with an estimated 2% of women having a subsequent live birth within 24 months of enrolment pre-1989 versus 14% in 2000–2003 ($P < 0.001$). Estimated time to subsequent live birth was shorter for black than for white women ($P < 0.001$).

Conclusions The likelihood of subsequent live births substantially increased after 1995 and birth intervals were shorter in women on HAART and among black women. Numbers are currently too small to address the issue of advantages and disadvantages in the management of subsequent deliveries.

INTRODUCTION

The vast majority of HIV-infected women are in their reproductive years, with an estimated 78% of women newly diagnosed with HIV infection in West Europe in 2002 aged between 13 and 39 years, 73% in Central Europe and 85% in Eastern Europe,[1] but little is known about the effect of HIV on decisions regarding childbearing, or on the ability to become pregnant.

In a study among HIV-infected women predating the HAART era, an HIV diagnosis nearly halved the live birth rate, from 10.2 per 100 women-years prediagnosis to 5.7 afterwards.[2] In a large European study, the age-adjusted pregnancy incidence before HIV diagnosis remained stable in the first four years postdiagnosis but reduced significantly four or more years after diagnosis.[3] Although pregnancy rates did not change significantly in relation to the availability of prophylactic zidovudine to prevent mother-to-child transmission (MTCT),[4] the proportion of pregnancies continuing to a live birth significantly increased since 1995.[3] It has recently been suggested that HIV-infected women in Europe are now more likely to want to become pregnant,[5] probably in response to the substantial reductions in the MTCT rate to 1–2% now achievable[6–8] and the availability of highly active antiretroviral therapy (HAART) to delay HIV disease progression in both the infected mother and her infected child.[9–12]

To date, the growing group of HIV-infected women in Europe who have had more than one live birth following their HIV diagnosis has received little attention. These women are not only of interest with regard to child-bearing decisions, but also because there are some important questions regarding their optimal management. The main objectives of this analysis were to examine how the subsequent childbearing of HIV-infected mothers enrolled in the European Collaborative Study (ECS) has changed over time and to identify socio-demographic and HIV-related factors predictive of subsequent live births. To examine this

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[1] ECS collaborators may be found in the Appendix.

question, we compared the characteristics of women with and without at least one subsequent live birth enrolled in the ECS.

METHODS

The ECS is a cohort study, in which HIV-infected women are identified during pregnancy and their infants prospectively followed according to standard clinical and laboratory protocols.[13] The ECS was set up in 1986 and includes centres in nine European countries.[13] In ECS centres, all pregnant women are offered antenatal HIV testing, and those found to be infected invited to participate in the study; pregnant women already known to be HIV infected as the result of earlier testing are also invited to take part. Informed consent is obtained before enrolment, according to local guidelines, and local ethics approval has been granted. Infants born to infected women subsequent to their index pregnancy within the ECS are also enrolled in the study, with prospective follow up as above.

Information collected at enrolment and during pregnancy includes current antiretroviral treatment (ART), maternal immunological and virological status, maternal illicit drug use (IDU) history and other socio-demographic characteristics. Information on maternal CD4 cell count was routinely collected from 1992 onwards and on RNA viral load from 1998. Laboratory tests including HIV RNA PCR, serology and CD4 cell count measurements, were carried out locally, with assays used recorded. Maternal CD4 cell count and HIV RNA copy number nearest the time of delivery were used in the analyses here.

In this analysis, children with a positive virological or serological marker of infection and/or children aged more than 18 months with persistence of antibody were included as infected. If a child was HIV antibody-negative and no virus or antigen had ever been detected, they were classified as uninfected.

Women who had only the delivery at enrolment (index pregnancy) were included in the 'one live birth' group and women who had at least one additional live birth after the index pregnancy were classed in the 'subsequent live births' group. Among the group of women with only one live birth, 72 delivered twins and 3 three infants, but no multiple pregnancies were observed in the group with subsequent live births. IDU-related mode of acquisition included women who had a history of IDU themselves, or who had sexual partners who had a history of IDU.

Univariable comparisons were assessed with the χ^2 test for categorical variables and a two-tailed unpaired *t* test for continuous variables. Univariable and multivariable logistic regression analyses were used to obtain unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI). Kaplan–Meier (KM) analyses were performed to estimate the cumulative proportion of women having a subsequent live birth,[14] and the log-rank test used to test statistical significance between strata. Data entry was carried out using MS Access 2000 and analyses using SAS statistical software (v8.02, SAS Institute, Cary, North Carolina, USA) and STATA (version 6; College Station, Texas, USA).

RESULTS

By November 2003, 3911 HIV-infected pregnant women had been enrolled, 3693 (94%) of whom had one live birth and 218 (6%) had at least one subsequent live birth. There were a total of 473 births in the group of women with subsequent live births: of the 218 women who had a second baby, 31 went on to have a third, of whom 5 went on to have a fourth, and 1 a fifth baby. The time between the index birth and subsequent infants was 32 months overall (min 7.7; max 170.6 months), varying from 42.15 ± 29.26, 33.71 ± 21.60 and 28.89 ± 10.30 months between the first and second, the second and the third, the third and the fourth subsequent live births, respectively. The main socio-demographic characteristics and mode of acquisition of HIV infection of the mothers at enrolment are presented in Table 1.

Black women (mostly born in sub-Saharan Africa) were significantly more likely to have a subsequent live birth than women of other ethnicities (Table 1). This was reflected in the association between mode of acquisition of infection and subsequent childbearing, with a significantly higher proportion of women who acquired HIV infection in high prevalence areas having a subsequent live birth. Most women were married or cohabiting at the time of enrolment, and marital status did not significantly affect the likelihood of subsequent childbearing. Previous obstetric history, both prior pregnancies resulting in deliveries and previous abortions, showed no influence on the number of subsequent live births. Women with subsequent live births were significantly younger at their first delivery compared with women without any further live births. With regard to characteristics specific to HIV infection, women having a subsequent delivery had a lower mean RNA level at enrolment, although the proportions with viral loads $\leq$500 copies/mL were not significantly different between the two groups (Table 1), and they did not differ in terms of health status as indicated by the CD4 count and maternal clinical stage at enrolment. With regard to trends in subsequent child bearing over time, 6.8% (56/819) of women enrolling in 1985–1989 had a further delivery, decreasing to 5.3% (60/1133) in 1990–1994, rising again to 7.6% (80/1058) in 1995–1999. In the most recent time period, only 2.5% (22/882) of women enrolling had gone on to have a subsequent delivery, but this reflects their shorter time since the index delivery.

To further explore factors shown to be univariably associated with the likelihood of a woman to have a subsequent live birth, a multivariable logistic regression analysis was carried out (Table 2). Adjusting for time period, maternal ethnicity, age and parity, both ethnicity and maternal age remained significantly associated with the likelihood of a

Table 1. Maternal socio-demographic characteristics at enrolment: women with only the index pregnancy ($n = 3693$) compared with 218 women with subsequent live births.

Variables	Only index live birth, *n* (%)	Subsequent live births, *n* (%)	χ^2 (*P*)
Ethnicity	$n = 3529$	$n = 215$	20.83 (<0.001)
White	2728 (77)	139 (65)	
Black	656 (19)	67 (31)	
Other	145 (4)	9 (4)	
Mode of acquisition	$n = 3693$	$n = 218$	18.87 (<0.001)
IDU related	2270 (61)	110 (50)	
Origin area of high prevalence	595 (16)	59 (27)	
Other heterosexual	556 (15)	33 (15)	
Not stated	272 (7)	16 (7)	
Marital status	$n = 1914$	$n = 142$	1.95 (0.38)
Single	455 (24)	30 (21)	
Married/cohabiting	1337 (70)	99 (70)	
Divorced/separated/widowed	122 (6)	13 (9)	
Parity	$n = 3402$	$n = 207$	6.46 (0.09)
0	1916 (56)	132 (64)	
1	911 (27)	48 (23)	
2	361 (11)	13 (6)	
≥3	214 (6)	14 (7)	
Previous miscarriage	$n = 3251$	$n = 204$	0.11 (0.75)
Yes	639 (20)	42 (21)	
Previous terminations	$n = 3302$	$n = 204$	1.13 (0.30)
Yes	1256 (38)	70 (34)	
Timing of HIV test	$n = 3557$	$n = 211$	2.74 (0.25)
Prepregnancy	1925 (54)	102 (48)	
Antenatal	1333 (37)	88 (42)	
At delivery	299 (8)	21 (10)	
Maternal age	$n = 3339$	$n = 212$	(<0.002)
Mean	28.28	26.72	
SD	5.08	4.8	
HIV RNA level	$n = 997$	$n = 66$	(0.05)
Mean [SD]	24,169 [108,389]	13,914.79 [41,885]	
Range	20–1,980,000	40–290,000	
≤500 copies/mL	434 (44)	31 (45)	
CD4 count at enrolment	$n = 2027$	$n = 89$	1.12 (0.57)
Median (range) (cells/mm^3)	420 (1–2350)	390 (5–1840)	
<200 cells/mm^3	271 (14)	11 (12)	
200–499 cells/mm^3	978 (48)	48 (54)	
≥500 cells/mm^3	778 (38)	30 (34)	

woman to have a subsequent live birth (Table 2). Black women were nearly two and a half times more likely to have a subsequent live birth than white women, while women over 30 years at enrolment were about half as likely to have a further delivery compared with those aged less than 25 years.

In a subanalysis involving 1960 women, adjusting for maternal CD4 cell count, the increased risk of subsequent live births among black women remained (OR, 3.49; 95% CI, 2.16–5.63; $P < 0.001$), but the difference in terms of maternal age was not statistically significant. Maternal immune status was not a significant predictor of subsequent live births: compared with women with CD4 cell counts above 500 per mm^3 the risk was similar in women with CD4 counts below 200 (AOR, 1.11; 95% CI, 0.68–1.79; $P = 0.58$) and of 200–499 cells/mm^3 (AOR 0.90; 0.43–1.84; $P = 0.61$).

To investigate whether the abovementioned trend in terms of subsequent live births was a real change in childbearing in the study population or a reflection of other factors such as the increased immigration of women from sub-Saharan countries with higher fertility, we investigated

Table 2. Multivariable analysis of likelihood of a subsequent live birth.

	Any subsequent live birth		Subsequent live birth within three years of index delivery	
	OR (95% CI)	AOR (95% CI)	OR (95% CI)	AOR (95% CI)
Time period				
1985–1994	1.00	1.00	1.00	1.00
1995–2003	0.95 (0.71–1.25)	0.87 (0.63–1.19)	1.35 (0.92–1.99)	1.24 (0.81–1.90)
Ethnicity				
White	1.00	1.00	1.00	1.00
Black	2.10 (1.54–2.85)	2.45 (1.75–3.43)	2.13 (1.41–3.23)	2.15 (1.38–3.36)
Asian, oriental, other	1.30 (0.65–2.62)	1.45 (0.71–2.93)	1.38 (0.55–3.45)	1.40 (0.55–3.56)
Maternal age at delivery				
<25 years	1.00	1.00	1.00	1.00
25–30 years	0.65 (0.47–0.90)	0.65 (0.46–0.91)	0.63 (0.40–1.00)	0.60 (0.38–0.97)
>30 years	0.51 (0.36–0.73)	0.54 (0.37–0.80)	0.66 (0.41–1.05)	0.62 (0.38–1.03)
Parity				
0	1.00	1.00	1.00	1.00
1	0.80 (0.57–1.12)	0.81 (0.57–1.15)	1.0 (0.63–1.54)	0.98 (0.62–1.54)
≥2	0.73 (0.48–1.12)	0.73 (0.47–1.15)	0.76 (0.42–1.37)	0.71 (0.38–1.31)

predictors of subsequent live births within three years of enrolment. From a multivariable logistic regression analysis, involving 3471 women, adjusting for time period, maternal ethnicity, age and parity, ethnicity was still a significant predictor of subsequent live births (Table 2). Black women were twice as likely to have more than one baby than white women. In a subanalysis, adjusting for maternal CD4 cell count, black women were nearly three times more likely to have subsequent live births than white women (AOR, 2.80; 95% CI, 1.55–5.10; $P < 0.001$).

In KM analyses of time to subsequent live birth, stratified by various explanatory variables, black women had a significantly shorter time to subsequent live birth than white women, with an estimated 9% and 14% having a subsequent live birth within 24 months and within 36 months of the index delivery, respectively, compared with 4% and 6% for white women ($P < 0.001$) (Fig. 1). There was no significant association between maternal age at enrolment and time to subsequent live birth. Time to subsequent live birth decreased significantly over time: an estimated 2% and 3% of women having a subsequent live birth by 24 months after the index delivery in the periods ≤1989 and 1990–1994, respectively, compared with an estimated 10% in 1995–1999 and 14% in 2000–2003 ($P < 0.001$) (Fig. 2). As the increasing enrolment of black women in the more recent years of the study[13] could have biased this trend, we carried out KM analyses separately for black and white women. In both groups, differences over time remained significant ($P < 0.001$), with an estimated 2% of black women and 3% of white women having had a subsequent live birth within 24 months after the index delivery in 1990–1994 compared with 16% and 15%, respectively, in 2000–2003.

In Table 3, the use of antenatal ART, neonatal prophylaxis, mode of delivery and pregnancy outcome in terms of prematurity and infant HIV infection status for the two groups (index pregnancy only group and subsequent live birth group) are presented. There was no difference in the prematurity rate between the index pregnancy only group and the first pregnancy of the subsequent childbearing group. The infection status of the index child did not affect the likelihood of having a subsequent child ($\chi^2 = 0.70$; $P = 0.40$ for index and first child of women with subsequent births). Comparisons of the use of interventions for PMTCT between the index pregnancy only and the subsequent pregnancy groups are complicated by the longitudinal nature of the data. In a subanalysis, limited to the period since 1997, which could be considered the established HAART era, there were

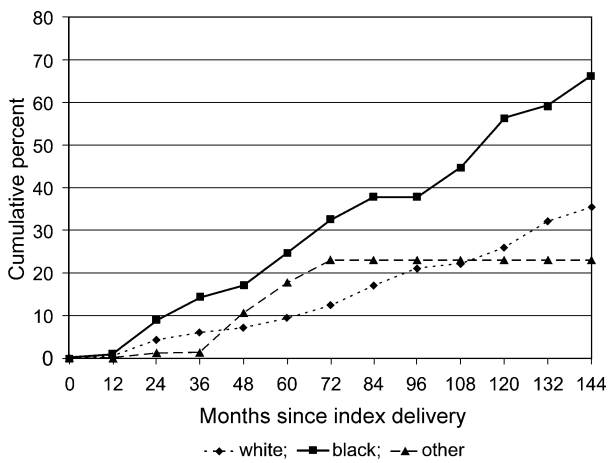

Fig. 1. KM analysis of time to subsequent pregnancy by ethnicity ($n = 3911$).

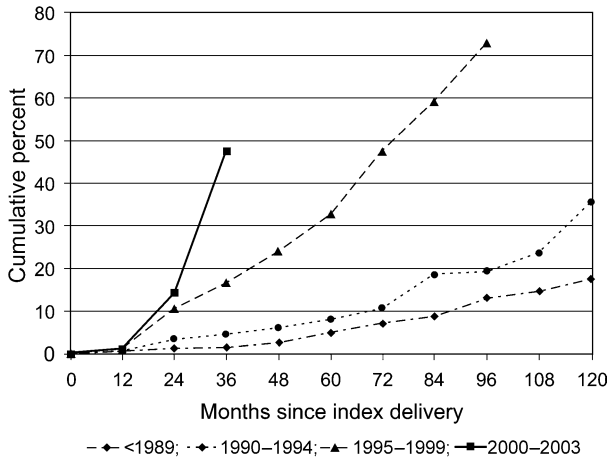

Fig. 2. KM analysis of time to subsequent pregnancy by time period ($n = 3911$).

no significant differences between the index pregnancy group and the first pregnancy of the subsequent pregnancy group in terms of receipt of HAART ($P = 0.13$) (Table 4).

Comparison of the obstetric and therapeutic management of the first and subsequent pregnancies reflected changes over time in policy and practice. In a subanalysis, limited to deliveries since 1997, the use of HAART increased significantly with increasing pregnancy number ($\chi^2_{trend} = 7.14$, $P = 0.008$) (Table 4). Overall, there was a high concordance between first and second pregnancies with regard to antenatal ART: for example, of the 127 women not receiving ART in their first pregnancy, 77 (61%) had none in their subsequent pregnancy (reflecting the timing of delivery, prior to 1994). Within the group of women receiving HAART in their first pregnancy ($n = 28$), 22 (79%) were also on HAART in their second delivery, with two receiving dual therapy and four none. The elective CS rate was higher among second deliveries compared with first deliveries ($\chi^2 = 7.16$; $P = 0.007$), but not between the second and the third deliveries ($\chi^2 = 0.09$; $P = 0.76$), reflecting the very high rate of emergency CS delivery (24%) recorded for the latter group, which was associated with prematurity (Table 3). Concordance in the obstetric management of deliveries within women was high: 91 (67%) of 135 women had repeated vaginal/instrumental deliveries and 57 (89%) of 64 had a repeated elective CS in the second delivery. Of the women with three subsequent live births, 12 (32%) of 37 had a repeated vaginal/instrumental delivery and 5 (73%) of 11 women had a repeated elective CS compared with their second live birth. Although the emergency CS rate was low among second deliveries to women having a vaginal delivery or elective CS in their first delivery, it was high among women with a previous emergency CS (4/16, 25%) suggesting an underlying risk factor.

Because the study covers a long time period, what may have been appropriate care at the time of the index pregnancy may no longer be considered the optimum in subsequent pregnancies. We assessed the use of prophylactic ART in relation to which interventions to reduce vertical transmission were known to be effective at the time of the delivery. Prophylactic ART was not used by 4/145 (3%) black, 14/166 (8%) white IDU and 6/132 (5%) white non-IDU women, when this was the standard of care of the time. The difference between white non-IDU women and black women was not statistically

Table 3. Use of PMTCT interventions in women with only the index pregnancy and for each pregnancy of women with subsequent live births.

	Only index pregnancy (3693 mothers and 3771 infants*), *n* (%)	**Subsequent pregnancy subgroup (218 mothers with 473 total pregnancies since enrolment)**		
		First child ($n = 218$), *n* (%)	Second child ($n = 218$), *n* (%)	Third or more ($n = 37$), *n* (%)
Antenatal ART	$n = 3691$	$n = 218$	$n = 218$	$n = 37$
No therapy	2117 (57.5)	128 (59)	85 (39)	12 (32)
Monotherapy	598 (16)	46 (21)	37 (17)	5 (14)
Combination therapy	198 (5.5)	14 (6)	16 (7)	4 (11)
HAART	778 (21)	30 (14)	79 (37)	16 (43)
Mode of delivery	$n = 3719$	$n = 215$	$n = 212$	$n = 37$
Vaginal or instrumental	1895 (51)	135 (63)	98 (46)	15 (41)
Elective caesarean	1360 (37)	64 (30)	96 (45)	13 (35)
Emergency caesarean	464 (12)	16 (7)	18 (9)	9 (24)
Neonatal prophylaxis	$n = 1413$	$n = 55$	$n = 115$	$n = 24$
Yes	1159 (82)	46 (84)	93 (81)	20 (83)
Child HIV infection status	$n = 3002$	$n = 212$	$n = 186$	$n = 34$
Infected	324 (11)	19 (9)	(6.5)	4 (12)
Preterm delivery	$n = 3771$	$n = 218$	$n = 218$	$n = 37$
<37 weeks	701 (19)	38 (17)	47 (22)	13 (35)

* Twins are included.

Table 4. Use of ART in pregnancy in the HAART era (post-1997). Values are presented as *n* (%).

	Only index pregnancy group ($n = 1243$)	Subsequent pregnancy group		
		First pregnancy ($n = 51$)	Second pregnancy ($n = 119$)	Third pregnancy ($n = 24$)
None	143 (12)	7 (14)	16 (13)	1 (4)
Mono or dual therapy	356 (28)	19 (37)	28 (24)	7 (29)
HAART	744 (60)	25 (49)	75 (63)	16 (67)

significant (Fisher's Exact Test, $P = 1.00$), but that between white non-IDUs and IDUs was ($\chi^2 = 5.07$; $P = 0.024$), which reflects the lack of antenatal care in the IDU population.

DISCUSSION

Our results show that in Europe the number of HIV-infected women who have subsequent live births has increased in recent years. Reproductive decision making is a difficult issue and in the early years of the HIV epidemic physicians may have been asked to counsel women about factors that were incompletely understood such as maternal and fetal risks related to pregnancy in HIV-infected women. Furthermore, aspects such as socio-cultural background, past reproductive history, maternal disease progression status and concerns about the risk of MTCT complicated the decision-making process. However, in the past eight years or so, advances have been made not only in the treatment of HIV-infected women to preserve her own health and that of their infected children,[9–12] but also in the prevention of MTCT. So whereas in the early studies HIV-infected women may have been advised to postpone childbearing[15] this is no longer the case. The composite effect of these advances is likely to underlie the increased number of subsequent live births in HIV-infected women observed in our cohort.[16,17]

The main factors associated with the likelihood of having a subsequent live birth were maternal ethnicity and time of enrolment. Black women were more likely to have subsequent live births even after adjusting for other variables. This reflects the generally higher fertility in women born in sub-Sahara Africa, even when living in Europe. The temporal trend highlighted in the KM analysis confirms the observations from physicians in Europe that HIV-infected women are now more likely to become pregnant than in the past.[5] The time-related trend was seen in both white and black women and suggests that all women are likely to be influenced by the availability of ART and prophylaxis.

In a longitudinal analysis of data from the U.S. Adult Spectrum of HIV Disease Project, women on HAART were more likely to become pregnant in the first instance, and to have subsequent pregnancies, than women on other ART regimens,[18] while Massad *et al.*[19] found termination of pregnancy to be significantly less common in the HAART era than in previous years in the Women's Interagency HIV Study.

Stephenson *et al.*[2] showed that having a previous live birth did not appear to influence the likelihood of termination of pregnancy. In accordance with these results, we found little differences in parity and abortion/miscarriage rates between the women with only one and those with more children enrolled in the ECS. However, we do not have data on miscarriage or termination of pregnancy after the enrolment in the ECS. Several studies have shown that pregnancy in HIV-infected women is more common in younger and healthier women[3,18–21] and this appears to be true for additional pregnancies as well, as shown here and elsewhere.[16,22]

HAART taken in pregnancy is highly effective in reducing risk of vertical transmission,[6–8] although questions remain regarding the short and long term safety of these new combinations on children.[23] There is an increasing number of women with long durations of exposure to HAART during pregnancy, as seen here with the increase of HAART in subsequent pregnancies. Elective CS delivery has a significant independent effect in reducing vertical transmission.[24–27] Our results show that the mode of delivery in a previous pregnancy affects that in a subsequent pregnancy. As the rate of repeated emergency CS reported here shows, there is a higher risk of emergency CS in some women probably independent of both their HIV infection and their antiretroviral therapy.

Limitations of our study are that the number of subsequent live births is an under-estimation of the number of pregnancies occurring in HIV-infected women after the diagnosis of infection as we were only able to investigate subsequent live births, and that we cannot distinguish the group of women who become pregnant by choice from those who experienced unwanted pregnancies. Also, our subgroup of women with subsequent live births is still too small to evaluate adequately fetal outcome in women with subsequent live births, or to assess the impact of repeat exposure to antiretroviral prophylaxis on maternal health and MTCT risk.

In conclusion, we confirm the increasing number of subsequent live births in recent years is more evident among black women and this should be taken in account in the planning of ART for women of childbearing age. If the increase in subsequent live births is confirmed by others, the number of children born to HIV-infected women will increase faster than the number of HIV-infected mothers. Attention should be focussed on improving health care for childbearing women without adding potential risks for planned or unplanned pregnancies.

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2.5. The collaborative research on HIV in Europe

During these 20 years, a vast amount of experience relating to the challenges of long-term, collaborative research has been accumulated. The result of this unique collaboration is evident in the impressive publication record of ECS, with important contribution to research internationally on HIV infection in pregnancy and childhood and informing management and policy.

In the selected publications reported above, many and different aspects of HIV infection in pregnancy and childbearing are analysed.

In the first paper (Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe), we discuss about the potential risks of adverse pregnancy outcomes that may be associated with the spread of highly potent, complex combinations of drugs throughout pregnancy.

In the second paper (Mother-to-Child Transmission of HIV Infection in the Era of Highly Active Antiretroviral Therapy) we investigate the effects of elective Caesarean section delivery, duration of rupture of membranes, and prematurity. We also describe the small number of infants who acquired HIV infection, despite maternal exposure to prophylactic interventions.

The third paper (Time to Undetectable Viral Load after Highly Active Antiretroviral Therapy Initiation among HIV-Infected Pregnant Women) have addressed the question of which highly active antiretroviral therapy (HAART) regimens are more effective for optimal viral response in antiretroviral-naive, human immunodeficiency virus (HIV)–infected pregnant women.

The fourth paper (HIV-infected pregnant adolescents and youth: results from a European cohort study) deals with the sub-group of young, pregnant HIV-infected women aged<25 years enrolling in the study. This sub-group have diverse needs for services during and after pregnancy, including harm reduction services and psychosocial support, in addition to a universal need for prevention of mother-to-child transmission services.

Research Letters

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Successful therapy of hepatitis B with tenofovir in HIV-infected patients failing previous adefovir and lamivudine treatment

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Three HIV-infected patients with chronic hepatitis B (genotype A) were switched to adefovir therapy after unsuccessful lamivudine treatment. Surprisingly, adefovir therapy failed, although none of the virus isolates displayed mutations known to be associated with adefovir resistance (A181V, N236T). In two isolates we identified hepatitis B virus DNA polymerase mutation L217R, in one case we found multiple frameshifts in the same region. In all cases adefovir was replaced by tenofovir, resulting in a significant drop in the viral load.

Current therapies for hepatitis B virus (HBV) infections are based on the application of interferon, lamivudine or combinations of both drugs. For lamivudine, the development of resistant HBV strains has been observed in 25% of treated patients per year. Moreover, in HBV/HIV co-infected patients the development of lamivudine resistance is more frequent than in HBV mono-infected patients, making a therapy alternative to lamivudine indispensable [1–4]. Therefore, the new drug adefovir serves as an alternative therapy for the treatment of chronic HBV infection. Adefovir passed clinical studies successfully [5], and so far two mutations mediating resistance to adefovir have been described [6,7]. The frequency of the mutation A181V is approximately 2.5% per year with hitherto unkown relevance, whereas 1.7–2.5% per year of adefovir-treated patients carry the resistance mutation N236T [6,7]. Moreover, adefovir is also active against HIV, but HIV-DNA polymerase mutations selected during treatment with adefovir did not influence the sustained response in viral load for 6–12 months [8].

Studies by Perrillo *et al.* [9] and Peters *et al.* [10] demonstrated that 8–15% of patients infected with lamivudine-resistant HBV exhibit initial non-response to adefovir. Unfortunately, the reasons for adefovir non-response in those studies remained unclear. In line with those observations, we detected three out of 20 patients chronically infected with HBV and co-infected with HIV, who despite good compliance did not respond to adefovir dipivoxil with a decrease in HBV DNA.

Patient 1 was a 39-year-old white man with a long-term HIV infection [Centers for Disease Control and Prevention (CDC) stage B2] and chronic hepatitis B e antigen-positive hepatitis B. Antiretroviral therapy (ART) was started in 1996; current ART consisted of lamivudine/abacavir/efavirenz with an HIV viral load below 25 copies/ml and good immunological recovery (CD4 cell count 672 cells/μl; 30%). Liver function tests (LFT) fluctuated but never exceeded twice the upper limit of normal (alanine aminotransferase; ALT). On ultrasound examination the liver appeared normal. Because of a high HBV load while receiving lamivudine-containing ART, adefovir treatment was started and was continued for 6 months without any response in the HBV load (Fig. 1). Adefovir was replaced by tenofovir, resulting in a significant drop in the HBV load after 4 months and maintained virological control of HIV infection.

Patient 2 was a 34-year-old white man with chronic hepatitis B e antigen-positive hepatitis B and HIV infection diagnosed on the occasion of a *Pneumocystis jeroveci* pneumonia and cryptosporidial diarrhea (CDC stage C3). ART was started initially with zidovudine/lamivudine/indinavir/ritonavir and subsequently with zidovudine/lamivudine/efavirenz, with good virological and immunological response (HIV viral load less than 25 copies/ml, CD4 cell count 487 cells/μl, 21%). Initially on treatment with lamivudine, LFT were normal/minimally elevated and the HBV load was 1000 particles/ml, but increased 2 years after the initiation of lamivudine therapy in parallel with LFT (ALT up to four times the upper limit of normal). On ultrasound examination the liver was moderately enlarged. Adefovir was started, but no decrease in the HBV load or LFT was observed over 5 months (Fig. 1). ART was changed to tenofovir/lamivudine/efavorenz. Two months later the hepatitis B viral load and ALT decreased significantly.

The third patient was a 37-year-old white man with HIV infection. ART started with didanosine/stavudine/ritonavir when the patient became symptomatic from HIV infection (CDC stage B2), with good virological and immunological response. During ART the patient acquired acute hepatitis B infection and ART had to be interrupted. Later, ART with zidovudine/lamivudine/efavirenz and IFN-α (12 months) was started. Six months later zidovudine was substituted by stavudine because of neutropenia. During this ART, HBV-DNA became negative, whereas hepatitis B

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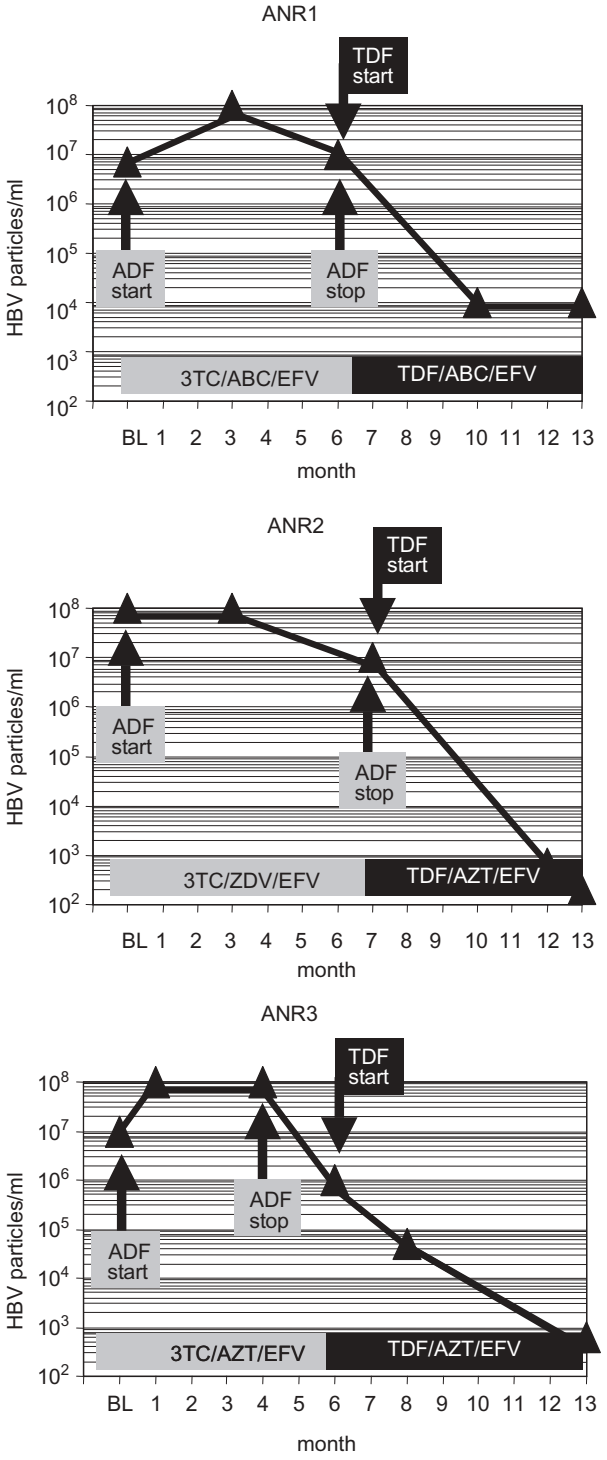
surface and e antigen remained positive. However, HBV-DNA rebounded, with the patient still being on a lamivudine-containing ART with an HIV load below 25 copies/ml. LFT (ALT) ranged between twice and five times the upper limit of normal. On ultrasound examination, the spleen and liver were enlarged, with increased echogenicity of the liver. Although adefovir therapy was started, no decrease in the hepatitis B viral load was observed after 3 months (Fig. 1). Under treatment with tenofovir the HBV load dropped by more than 3 log. ALT levels decreased in all three patients after tenofovir treatment was started (data not shown).



Adefovir was useless in all of the three patients. Sequencing analysis [11] revealed no amino acid exchange at position 236 nor at position 181, the position described to mediate resitance to adefovir [6,7]. All patients shared the same HBV genotype (genotype A) with the identical genotypical lamivudine resistance pattern (YVDD, L180M) as determined by line probe assay (Inno-LiPA HBV–DR, Innogenetics, Gent, Belgium) upon baseline. Sequencing analysis of the isolates revealed mutations at amino acid 217 (patients 1 and 3) or 215–226 (patient 2). Sequences obtained from sera collected immediatly before the initiation of adefovir therapy showed that patients 1 and 3 displayed the mutation before the treatment started, whereas patient 2 developed the domain exchange during ongoing adefovir therapy.

Two further patients not described here included in a tenofovir study exhibited the L217R mutation, and responded well to tenofovir. Four control HBV strains responding to adefovir sequenced in our laboratory exhibited wild-type sequence L217.

No stop codons but single amino acid exchanges were introduced in the overlapping hepatitis B surface open-reading-frame by the polymerase gene mutations. Based on GenBank data, the hepatitis B surface amino acid exchanges did not have a significant impact on the outcome of the hepatitis.

The observation that differences in genotypic pattern in HCV and HIV correlate with subsequent virological response to treatment [12,13] in combination with our data suggest similar phenomena for HBV, of which its polymerase shares high homology with the HIV polymerase. On the basis of our observations we (carefully) conclude that: (1) the polymerase domain amino acids

Fig. 1. Course of hepatitis B viral load in three chronically HIV/hepatitis B virus-infected patients (adefovir-non-responders) during antiretroviral therapy; antiretroviral therapy was initially combined with adefovir and was switched to combination with tenofovir after no significant decrease of hepatitis B viral load was observed as a result of adefovir. All patients share the genotype A and identical lamivudine resistance patterns (L180M/YVDD). Sequencing analysis of the isolates revealed mutations at amino acid 217 (patients 1 and 3) or 215–226 (patient 2) (see main text). ABC, Abacavir; ADF, adefovir; ANR, adefovir non-responders; BL, baseline; D4T, stavudine; EFV, efavirenz; IDV, indinavir; TDF, tenofovir;

215–226 might mediate adefovir resistance; (2) the combination of the lamivudine resistance pattern L180M/YVDD plus HBV genotype A may predetermine resistance to adefovir; and most important (3) in analogy to earlier observations [2–4], therapy changes to tenofovir should be considered for adefovir non-responders. Although an in-vitro assay confirming the hypothesis that the amino acid region 215–226 might be responsible for resistance is missing, this assumption is confirmed by the recent patent application of Bartholomeusz *et al.* [14].

In summary, at this stage, we recommend tenofovir for the treatment of adefovir-non-responders, probably also in HBV-mono-infected patients.

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Predicted genotypic resistance to the novel entry inhibitor, BMS-378806, among HIV-1 isolates of subtypes A to G

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BMS-378806 targets virus entry by inhibiting the binding of HIV-1 gp120 to the CD4 receptor. Env sequences ($n = 1226$) of subtypes A–G were analysed to determine the frequency of mutations associated with resistance to BMS-378806. In line with reported sensitivity data, background genotypic resistance to BMS-378806 among non-B HIV-1 viruses was found to be higher than in subtype B. These data suggest that BMS-378806 may have reduced efficacy against non-B viruses.

The increasing prevalence of HIV-1 isolates that are resistant to reverse transcriptase and protease inhibitors has resulted in a search for alternative therapeutic targets. A new class of drugs termed entry inhibitors have become the subject of increasing interest, particularly since the licensing of the first drug in this class, enfuvirtide (T20) (Roche/Trimeris Inc., Durham, NC, USA) [1]. BMS-378806, a small molecule inhibitor of HIV-1 [2], is a further candidate within the entry inhibitor class that targets virus entry by inhibiting the binding of HIV-1 gp120 to the CD4 receptor. The affinity of BMS-378806 for the gp120 molecule is similar to that of soluble CD4 cells, with binding occurring close to the CD4 cell-binding pocket [2,3]. BMS-378806 exhibits potent antiviral activity against subtype B HIV-1 viruses, including both R5 and X4 viruses [4]. Analysis of a limited number of viruses of non-B subtypes suggest that the drug may be less efficacious in these viruses; the EC_{50} value for subtype C (EC_{50} 243 nM) and the other subtypes (EC_{50} > 1000 nM) is reported to be higher compared with subtype B viruses (EC_{50} 61.5 nM) [4].

HIV-1 viruses resistant to BMS-378806 have been selected *in vitro*, and mutations within the virus envelope conferring resistance to BMS-378806 have been

Table 1. Frequency of mutations conferring resistance to BMS-378806.

		HIV-1 subtype											
		B ($n = 790$)	A ($n = 82$)		C ($n = 217$)		D ($n = 62$)		CRF01_AE ($n = 48$)		F ($n = 14$)		G ($n = 13$)	
Observed *in vitro*	Fold resistance[a]	Freq (%)	Freq (%)	*P*	Freq (%)	*P*	Freq (%)	*P*	Freq (%)	*P*	Freq (%)	*P*	Freq (%)	*P*
M475I	40	0.63	1.23	NS	0		3.23	NS	**83.33**	< 0.0001	0		0	
R350K, M475I	15	0.25	0		0		0		**77.08**	< 0.0001	0		0	
D185N, R350K, M475I	ND	0.25	0		0		0		**12.5**	< 0.0001	0		0	
I595F	8	1.9	0		0.92	NS	0		0		0		0	
M434I	4	0.76	**36.59**	< 0.0001	**17.05**	< 0.0001	**4.84**	0.0225	2.08	NS	0		0	
M434I, I595F	498	0	0		**0.92**	0.0463	0		0		0		0	
M434I, K655E	ND	0.13	0		0		0		0		0		0	
M434V	ND	0.25	0		0.46	NS	0		0		0		0	
V68A, M434I	77	0	0		0		0		0		0		0	
V68A, S440R	ND	0.13	0		0		0		0		0		0	
M426L, K655E	ND	0	0		0		0		0		0		0	
S440R	ND	65.4	**6.1**	< 0.0001	**2.76**	< 0.0001	**4.84**	< 0.0001	**12.5**	< 0.0001	0		0	
Not observed alone *in vitro*														
M426L	116	5.82	2.44	NS	4.61	NS	8.06	NS	0		0		15.38	NS
V68A	< 4	0.38	0		0.46	NS	0		0		0		0	
D185N	ND	6.2	**54.88**	< 0.0001	**53.46**	< 0.0001	**16.13**	0.0073	**16.67**	0.0122	**42.86**	0.0002	**53.85**	< 0.0001
R350K	0.6	9.75	8.64	NS	**5.07**	0.0299	16.13	NS	**87.5**	< 0.0001	**71.43**	< 0.0001	15.38	NS
K655E	ND	0.63	0		**6.91**	< 0.0001	3.23	NS	0		0		0	
M434T	ND	0.25	1.22	NS	1.38	NS	0		0		0		0	

[a]Lin *et al.* [4].Values in bold indicate $P < 0.05$, *P* values indicate significance of mutation frequency compared with subtype B.

identified [4]. These mutations result in the reduced binding of g120 to BMS-378806, with many mutations occurring near the CD4-binding pocket on gp120 [1]. Reported mutations include M475I, M434I/V, M426L, D350K, D185N, K655E, I595F, V68A and S440R. Some mutations have only been observed in combination with other mutations, therefore the biological significance of some single mutations is not known. Nevertheless, their existence may predispose viruses to acquire a second mutation, resulting in resistance to BMS-378806.

In this study we have analysed a total of 1226 Env sequences of subtypes A–D, F–G and CRF01_AE, obtained from the Los Alamos database in order to determine the frequency of naturally occurring resistance mutations to BMS-378806. The occurrence of resistance mutations, either individually or in combination, was expressed as a percentage for each subtype (Table 1). In line with sensitivity data [4], the level of background resistance to BMS-378806 among HIV-1 subtypes other than B, including subtype C, which is now responsible for the majority of new infections worldwide, was found to be considerably higher than that observed in subtype B. The results of this analysis suggest some degree of genotypic resistance to BMS-378806 in all subtypes, including subtype B in which the majority of efficacy tests have been performed [4]. With the exception of the S440R amino acid change that is common in subtype B (65.4%) and for which the fold increase in resistance to BMS-378806 is not known, and M426L (in 5.82% of sequences, shown to increase resistance by more than 100-fold), all other mutations reported to confer resistance occur naturally at a lower frequency in subtype B (less than 1%).

The M434I mutation, which is associated with a four-fold increase in resistance, was more common in subtype A (36.59%, $P < 0.0001$) and C (17.05%, $P < 0.0001$). This is reflected in the median EC_{50} reported for these subtypes: 243 nM in subtype C and 1132 nM in subtype A compared with subtype B (61.5 nM) [4]. Furthermore, within subtype C, two ($P = 0.0463$) of the 217 sequences analysed also contained the double mutation M434I, I595F, which is reported to result in a 498-fold increase in resistance to BMS-378806. The naturally occurring existence of this double mutant may indicate the absence of a significant fitness disadvantage in such viruses. Both subtypes A and C also contained the D185N mutation at a high frequency (53.46%, $P < 0.0001$ and 54.88%, $P < 0.0001$, respectively).

Subtype D showed relatively low background genotypic resistance to BMS-378806, an observation that is not in accord with the relatively high EC_{50} of 2183 nM [4]. Mutations observed included M475I (3.32%, $P = 0.0863$, resulting in 40-fold increased resistance), M434I (4.84%, $P = 0.0225$) and S440R (4.84%, $P < 0.0001$). A number of other mutations were observed singly in sequence data but have not been reported *in vitro*. These include M426L (8.06%), which has been associated with a 116-fold increase in resistance.

Sequences of CRF01_AE suggested extremely high resistance to BMS-378806, again confirming the efficacy data available for this subtype (a range of > 2000 to $> 10\,000$ nM). A total of 83.33% ($P < 0.0001$) of sequences contained the M475I mutation that confers 40-fold resistance, with 77.08% ($P < 0.0001$) of these changes occurring in conjunction with R350K and 12.5% ($P < 0.0001$) also with D185N.

Sequences of subtypes F and G were unusual as none of the mutation combinations observed *in vitro* were present (although few sequences were analysed). This result is surprising in view of the low efficacy observed *in vitro* (estimated EC_{50} for these subtypes ranging from 214 to > 5000 nM) [4]. Subtype G showed evidence of the M426L mutation (15.38%, $P = 0.1795$). Both subtypes also contained a high incidence of the D185N mutation (42.86%, $P = 0.002$ and 53.85%, $P < 0.0001$, respectively) and the R350K mutation (71.43%, $P < 0.0001$ and 15.38%, $P = 0.6516$, respectively). It may be that the presence of these mutations in isolation is sufficient for reduced drug efficacy.

Isolates of subtypes other than B have previously been shown to be less sensitive to BMS-378806 [4]. Although it is not always possible to infer the phenotype from genotypic data, these results suggest that reduced efficacy in non-B viruses may be caused by high levels of genotypic resistance within the drug-naive population. These high levels of background genotypic resistance to BMS-378806 suggest that these resistance mutations do not confer a fitness disadvantage that is characteristic of viruses resistant to the reverse transcriptase and protease inhibitors and possibly also enfuvirtide [5,6].

BMS-378806 is a prototype drug likely to be followed by derivatives that may have broader specificity. Nevertheless, the high genotypic resistance to BMS-378806 may limit its usefulness in non-B populations, particularly in subtype C and CRF01_AE viruses. Future drugs based on BMS-378806 will need to be designed with these results in mind in order to expand the subtype range of this drug.

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Viral load and CD4 cell response to protease inhibitor-containing regimens in subtype B versus non-B treatment-naive HIV-1 patients

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We compared the response to protease inhibitor-containing highly active antiretroviral therapy in 175 HIV-1 treatment-naive patients harbouring subtype B versus non-B. No difference in the proportion of patients with viral loads below 400 copies/ml was observed at month 24. However, there was a significant difference in the median CD4 cell increase at month 24. Whether this is caused by viral or immune factors warrants further investigation.

Studies on highly active antiretroviral therapy (HAART) in individuals infected with non-B subtypes are needed to identify potential subtype-specific differences in the response to therapy. We compared the efficacy of a protease inhibitor-containing regimen (with two nucleoside analogues) in treatment-naive patients harbouring either B or non-B subtypes. Reverse transcriptase and protease sequencing was performed using the Viroseq version 2.0 method (Celera Diagnostics, USA) and the TruGene HIV-1 sequencing kit (Bayer Diagnostics, Berkeley, CA, USA), and viral subtyping was done using I-subtyping, the HIV-SEQ program from Stanford University and was confirmed by the subtyping tool from the National Center for Bioinformatics.

To evaluate the response to HAART, the proportion of patients achieving a plasma HIV viral load below 400 copies/ml and the absolute CD4 cell count after 6, 12 and 24 months were computed. Baseline demographic and laboratory characteristics were compared between B and non-B groups using chi-square and Fisher exact tests. Wilcoxon/Kruskall–Wallis tests were used for univariate analyses. A multiple linear regression was performed for the CD4 cell count evolution from baseline to month 24. As a result of the non-normality of CD4 cell count variation, this value was square root transformed. The following variables were included in the model: subtype, sex, baseline parameters [viral load, total lymphocyte count (absolute and percentage), CD4 cell count (absolute and percentage), Centers for Disease Control and Prevention stage and drug regimens at treatment initiation].

We identified 175 patients, of whom 56 (32%) were infected by subtype B and 119 (68%) by non-B subtype virus, including subtypes A (21), C (25), D (eight), G (eight), H (two), J (one), recombinant forms (AE seven, AG 22) and mosaics (22). Most B patients were Caucasians (90%) and homosexual (71%), whereas most non-B patients were Africans (89%) and heterosexual (100%). The median baseline viral load (4.9 versus 4.8 log) and CD4 cell count (264 versus 225 cells/mm^3) were comparable in both groups. There was no statistical difference in the use of any antiretroviral drug between both groups. There was no difference between the groups in median follow-up (1536 versus 1617 patient-days; $P = 0.42$). The proportion of patients who achieved undetectable viral load at month 24 was 52% for the B subtype and 64% for the non-B subtype (intent to treat analysis; missing equals failure). The CD4 cell increase at month 24 was significantly lower in the non-B group: +161 versus +235 cells/mm^3 ($P < 0.02$). Among the different non-B subtypes, differences were noted in the CD4 cell response, although none was significant: subtype A: +140; C: +158; circulating recombinant forms (CRF): +165; mosaics: +148; and others +185 cells/mm^3. As subtype A had the lowest CD4 cell increase, we compared the CD4 cell response between group B and group AA patients, i.e. 66 patients, harbouring group A, CRF-AG, CRF-AE and mosaics containing at least a part of subtype A. There was a statistically significant difference between B and AA patients in terms of CD4 cell increase at month 24: +235 versus +162 ($P < 0.03$). At treatment initiation, the viral load was lower in the AA group (4.7 log versus 4.9 log; $P < 0.02$) and the CD4 cell count was lower, although not statistically signifi-

cant (211 versus 264 cells/mm^3). In multiple linear regression, baseline viral load and the percentage of total lymphocytes were the only variables associated with the CD4 cell variation.

Most of the available data on the clinical outcome of HAART in non-B HIV-1 subtypes suggest that patients respond equally well regardless of the subtype. One report [1] has, however, suggested that the subtype may affect the clinical response in the long term but factors such as adherence and psychosocial conditions should be taken into account. Other studies did not confirm these findings [1–4]. Our study, performed in a single centre providing the same therapeutic attitude as well as similar psychosocial support to both B and non-B patients, argues against a role for viral subtypes in treatment responses in terms of viral load. However, the CD4 cell increase at month 24 was significantly lower in the non-B group. Larger CD4 cell increase after HAART initiation have been shown to be associated with higher baseline HIV-1 viral loads [5]. Baseline CD4 cell count also play a role in CD4 cell response, higher baseline CD4 cell count being associated with higher CD4 cell increases [6,7]. In our study, the median viral load at treatment initiation was equivalent in both groups, and baseline CD4 cell level was slightly lower, although not statistically significant, in the non-B patients.

Moreover, in multiple linear regression, baseline CD4 cell level was not predictive of CD4 cell response at month 24. We explored the hypothesis that the difference in CD4 cell response could be caused by some specific non-B subtypes. We could not find any statistically significant differences in CD4 cell response among the different non-B subtypes, but showed that patients harbouring subtype A were responsible for all the difference in CD4 cell increase at month 24, although the viral load response did not differ. The same baseline parameters as the global analysis were evaluated and did not influence the CD4 cell response.

Differences in CD4 cell response could be caused by viral or host characteristics, which should be further explored. A more rapid depletion of CD4 lymphocytes and higher levels of plasma HIV-1 RNA has been observed in human peripheral blood lymphocyte of severe combined immunodeficient mice infected with A and C-clade HIV-1 isolates in comparison with B-clade-infected mice [8]. However, in a study evaluating viral fitness *in vitro* in peripheral blood mononuclear cell culture [9], primary CD4 T cells and macrophages from different human donors, decreased fitness of subtype C compared with subtype B was observed. These apparently contradictory findings together with our observations underline the need for robust prospective trials on greater numbers of patients infected with non-B subtypes to establish whether such differences should be taken into account in the clinical setting.

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First-line efavirenz versus lopinavir–ritonavir-based highly active antiretroviral therapy for naïve patients

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Ninety-seven consecutive patients started anti-HIV therapy based on efavirenz (46) or lopinavir–ritonavir (51) in an observational study. Despite the significantly more compromised immunological-clinical baseline conditions of patients starting lopinavir–ritonavir, a mean

clinical-laboratory follow-up of 17 months showed a comparable laboratory response and therapy interruption or change rate, although the toxicity profile of the two compounds proved significantly different. Randomized studies comparing these two recommended first-line treatments are warranted, particularly from a pharmacoeconomic viewpoint.

The 2002–2004 international guidelines for antiretroviral management consider lopinavir–ritonavir highly active antiretroviral therapy (HAART) or efavirenz-based HAART to be the two alternative first-line choices for HIV-infected antiretroviral drug-naive patients [1,2]. However, no randomized comparative study has been performed until now, dealing with these two profoundly different therapeutic strategies (the first based on a boosterized protease inhibitor, and the second on a non-nucleoside reverse transcriptase inhibitor), with regard to both efficacy and tolerability issues [3–5].

The most important variables conditioning both the efficacy and safety parameters of lopinavir–ritonavir and efavirenz-based HAART were assessed in 97 consecutive antiretroviral-naive patients followed since the year 2002 in an open-label observational study conducted at our outpatient reference centre in Bologna, Italy. After giving their written informed consent, 51 consecutive patients treated with lopinavir–ritonavir plus two nucleoside analogues were compared with 46 patients who received efavirenz plus two nucleoside derivatives, selected by the physician in charge from all available molecules (including zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and tenofovir). At baseline, the two patient groups under consideration were statistically comparable with regard to all demographic and epidemiological features, as well as the mean plasma HIV viraemia (4.5 ± 1.4 versus 4.3 ± 1.6 $\log_{10}$ HIV-RNA copies/ml, for the lopinavir–ritonavir and efavirenz groups, respectively; Table 1). However, a previous or concurrent diagnosis of AIDS ($P < 0.0001$), or a significantly lower mean CD4 lymphocyte count ($P = 0.002$), were found among patients who received lopinavir–ritonavir as opposed to efavirenz-treated subjects. During the subsequent follow-up period, which until now ranged from 10 to 33 months (mean 16.8 ± 6.9 months), the laboratory workout showed a comparable virological response (with regard to the time and rate of both viral decay and the rate of viral suppression) in the presence of only one case of virological failure in the efavirenz group (explained by the appearance of a mutation at codons 103 and 181, at genotyping assay), versus no failed patient found in the lopinavir–ritonavir group (ns) (Table 1). Notwithstanding the significantly lower mean initial CD4 lymphocyte count, lopinavir–ritonavir-treated individuals achieved a more rapid and elevated immune recovery ($P = 0.011$ and $P = 0.005$, respectively), compared with those who received an efavirenz-based combination. Early (first month) interruptions as a result of a poor tolerability rate proved similar: eight patients were recorded in the lopinavir–ritonavir group, compared with six episodes among efavirenz-treated patients, although short-term untoward events predominanly involved the entire gastrointestinal tract for lopinavir–ritonavir, compared with the central nervous system for efavirenz-containing regimens ($P = 0.0011$). The overall need for regimen interruption or substitution attributable to toxicity, untoward events, insufficient patient adherence, or laboratory-clinical failure, as measured throughout the entire study period, tested quite low and was comparable between the two study groups (21.6% on the whole), as was the mean time to treatment interruption, but the observed mid-term toxicity rate proved

Table 1. A comparison of selected features of efavirenz versus lopinavir–ritonavir-treated patients in our open-label clinical-laboratory experience.

Patient features	Efavirenz ($n = 46$)	Lopinavir–ritonavir ($n = 51$)	P value
Male : female ratio	31 : 15	35 : 16	ns
Age in years (mean ± SD and range)	37.2 ± 11.4 (21–62)	38.3 ± 13.8 (33–68)	ns
Type of risk for HIV disease (IVDU, heterosexuals, MSM)	15/18/13	15/21/15	ns
Mean baseline viraemia ($\log_{10}$ HIV-RNA copies/ml)	4.3 ± 1.6	4.5 ± 1.4	ns
Mean baseline CD4 lymphocyte count (cells ± SD)	293.4 ± 96.5	196.8 ± 111.4	$P = 0.002$
No. with full-blown AIDS disease	10	35	$P < 0.0001$
No. who reached undetectable viral load (< 50 copies/ml) in study period	45	51	ns
Mean time to complete viral suppression (months ± SD)	6.8 ± 1.1	6.3 ± 1.5	ns
Mean time to peak CD4 lymphocyte count (months ± SD)	12.1 ± 6.5	9.4 ± 3.5	$P = 0.011$
Mean peak CD4 lymphocyte count (mean ± SD) reached in study period	556.5 ± 102.4	612.2 ± 88.7	$P = 0.005$
Early (first month) treatment interruption caused by poor tolerability	6	8	ns
Overall treatment interruptions throughout the entire study period	10	11	ns
Mean time to interruption (months ± SD)	8.9 ± 2.1	9.2 ± 3.1	ns
No. developing hyperlipidemia	24	5	$P = 0.0016$

IVDU, intravenous drug users; MSM, men who have sex with men.

significantly different from a qualitative point of view. Lopinavir–ritonavir-treated patients experienced dyslipidemia in more than 50% of overall cases (serum triglycerides > 200 mg/dl, or cholesterolemia > 220 mg/dl), versus nearly 10% of individuals belonging to the efavirenz group ($P = 0.0016$; Table 1).

Our experience conducted on 97 consecutive antiretroviral-naive patients treated with lopinavir–ritonavir versus efavirenz-based HAART, although limited by its open-label observational design, showed a comparable virological response, although an apparently more potent and rapid immunological recovery was attained in the lopinavir–ritonavir group, even taking into account the more severe initial immunodeficiency and AIDS rate in the latter patient group. On the other hand, although efavirenz-treated patients experienced more infrequent adverse events, and benefited from a low pill burden and an expected improved adherence (three capsules once a day at bedtime for efavirenz, versus three capsules twice a day for lopinavir–ritonavir) [3–5], the rate of early treatment interruptions tested similarly in the two study groups. The last update of international guidelines for antiretroviral treatment proposed either lopinavir–ritonavir or efavirenz-based HAART as equivalent first-line treatments for HIV disease in antiretroviral-naive patients [1,2], but in the absence of randomized comparative studies, the selection of lopinavir–ritonavir or efavirenz-based regimens should take into careful account the initial immunological and disease status of the patient, because lopinavir–ritonavir-based combinations seem to offer a better recovery in this last (more compromised) patient group, although efavirenz is better accepted and is more easy to administer. Further data are strongly warranted on long-term outcomes, the viral resistance burden, and tolerability issues on these two very different antiretroviral regimens proposed as first-line combinations for naive HIV-infected patients [1,2]. Given also the profoundly different expenditures associated with the administration of these two therapeutic strategies [6] (an efavirenz-based combination costs nearly 30% less than a lopinavir–ritonavir regimen in Italy), an appropriate pharmacoeconomic assessment of these two first-line selected regimens also seems highly desirable.

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Causes of death in HIV infection: the key determinant to define the clinical response to anti-HIV therapy

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Studies have shown an increased risk of new AIDS/death among injecting drug users (IDU) starting highly active antiretroviral therapy (HAART). Of 3872 patients starting HAART in the EuroSIDA study, 819 were IDU (21.2%). During 14 769 person-years of follow-up, 499 patients progressed to new AIDS/death. Compared with homosexual individuals, IDU had an increased incidence of new AIDS/death, but only for non-HIV deaths. There is an urgent need to define and standardize causes of death in observational studies.

Since the introduction of highly active antiretroviral therapy (HAART), morbidity and mortality has fallen to one-fifteenth of the level seen before HAART [1]. In an early study of 6645 patients from the EuroSIDA study, after adjustment there were no differences in the risk of death according to the exposure group [2]. The Antiretroviral Treatment Cohort Collaboration studied approximately 12 000 treatment-naive patients starting HAART, of whom 17% were injecting drug users (IDU). Recent findings demonstrated an increased risk of new AIDS/death or death among IDU after starting HAART [3,4], although the study was not able to differentiate between HIV-related and other causes of death. It is therefore unclear to what extent this finding is the result of a poor response to HAART in IDU compared with other patient groups.

EuroSIDA is a longitudinal pan-European (including sites in Israel and Argentina) observational study of 9802 patients with HIV, which has been described elsewhere [1]. The cause of death has been recorded since the beginning of the study (www.chip.dk). We

sought to describe differences in clinical progression after starting HAART according to exposure group and for different events (new AIDS/death, HIV-related death or non-HIV-related death). Patients were classified as dying from an HIV-related event when the cause of death was indicated as HIV related, resulting from an AIDS-defining event or caused by invasive bacterial infection. All other causes of death were classified as non-HIV deaths. A sensitivity analysis categorized deaths from invasive bacterial infections as non-HIV related.

Patients included in this analysis had started HAART (two or more nucleoside reverse transcriptase inhibitors plus at least one protease inhibitor, non-nucleoside reverse transcriptase inhibitor or abacavir), had a CD4 cell count and viral load measured in the 6 months before starting HAART and some prospective follow-up. Patients may have received non-HAART antiretroviral treatment before starting HAART. Those who were treatment naive at starting HAART were included in the Antiretroviral Treatment Cohort Collaboration. Patients were followed to their first event or their last follow-up, at latest December 2003. Poisson regression was used to determine the incidence rate ratio (IRR) of clinical progression after adjustment for confounding variables. These included age, previous AIDS diagnosis, previous antiretroviral treatment, HAART regimen started, hepatitis C status, date started HAART, and both CD4 cell count and viral load as time-updated variables.

A total of 3872 patients were included; 1767 were homosexual (45.6%), 819 were IDU (21.2%), 991 were heterosexual (25.6%) and 295 (7.6%) belonged to other exposure groups. A total of 1514 patients were treatment naive (39.1%), and the most common HAART regimen was a single-protease inhibitor-containing regimen ($n = 2758$, 71.2%). The median CD4 cell count at starting HAART was 220 [interquartile range (IQR) 104–341/mm^3] and viral load was 4.52 (IQR 3.70–5.15 $\log_{10}$ copies/ml). The median date of starting HAART was June 1997 (IQR 2/97–3/99).

During 14 769 person-years of follow-up 499 patients progressed to new AIDS/death; 296 patients died, and of these, 194 (66.5%) were non-HIV related. Compared with homosexual individuals (Fig. 1), after adjustment, there was a significantly increased incidence of new AIDS/deaths among IDU [IRR 1.36; 95% confidence interval (CI) 1.02–1.82, $P = 0.039$]. However, there was no increased incidence of AIDS (IRR 0.86, $P = 0.45$) or HIV-related death (IRR 1.01, $P = 0.99$). The increase was confined to non-HIV deaths, in which IDU had over a twofold increased incidence (IRR 2.53; 95% CI 1.64–3.92, $P < 0.0001$). The increased incidence in IDU was more pronounced when deaths from invasive bacterial infections were not included as HIV-related deaths (IRR 3.03; 95% CI 1.88–4.87, $P < 0.0001$). A significantly lower proportion of IDU were known to have died from HIV-related causes (24.0% versus 39.1% in all other exposure groups combined,

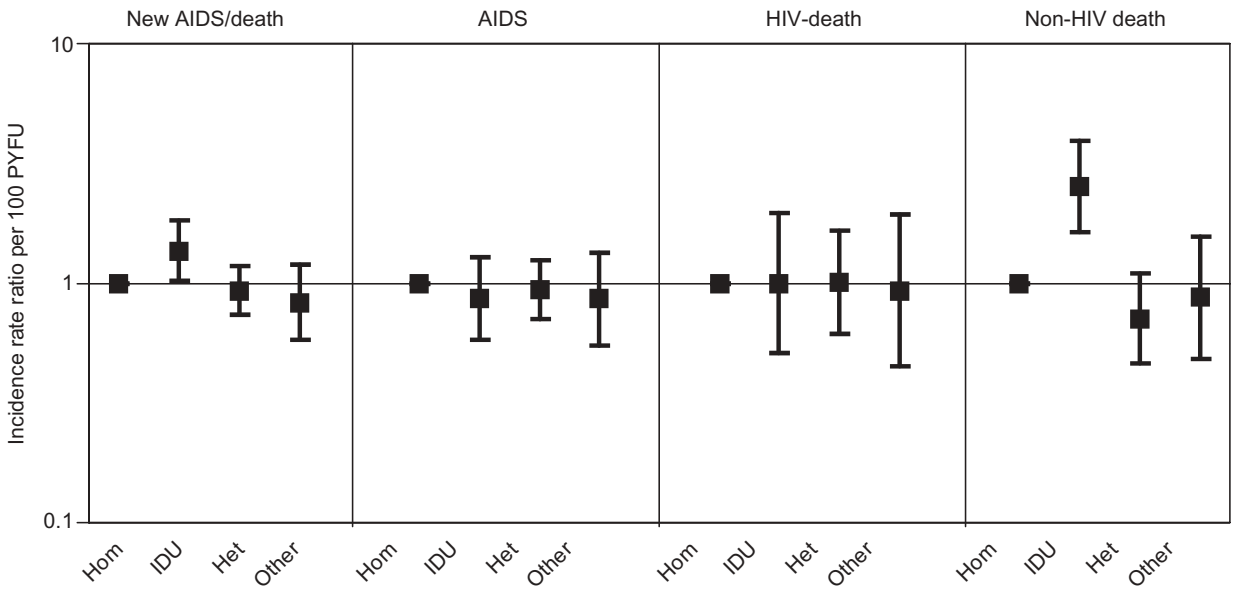

Fig. 1. Adjusted incidence rate ratios of new AIDS/death, AIDS, HIV or non-HIV death after starting highly active antiretroviral therapy. Adjusted for age, AIDS, previous antiretroviral treatment, highly active antiretroviral therapy (HAART) regimen started, hepatitis C status, date started HAART and both CD4 cell count and viral load as time-updated variables. Het, Heterosexual individuals; Hom, homosexual individuals; IDU, injecting drug users; PYFU, person-years of follow-up.

$P = 0.010$, chi-squared test), and a significantly higher proportion were reported to have died from liver-related events (20.8 versus 4.0% in all other exposure groups combined, $P < 0.0001$, chi-squared test). There were no significant differences between IDU and all other exposure groups combined in the proportion of patients who died from suicide or drug overdose, after a cardiovascular event, unknown cause or any other cause ($P > 0.3$ all comparisons, chi-squared test).

The EuroSIDA study has demonstrated an increased clinical progression among IDU after starting HAART. IDU are known to have an increased risk of chronic liver disease, bacterial infections, sepsis, drug overdoses and suicides [5], particularly among active IDU. The increased incidence was only found for non-HIV deaths, and was significant after adjustment for relevant confounding variables. In particular, the increased incidence could not be explained by co-infection with hepatitis C, although a higher proportion of IDU died from liver-related disease. We do not have data on current injecting drug use and were not able to adjust for this, but it is unlikely to explain completely the increased incidence. The introduction of HAART, more specific information on the cause of death and longer follow-up are likely explanations for the differences compared with earlier findings [2] in which we did not find an increased risk of death in IDU.

IDU had no increased incidence of AIDS or HIV-related death. This suggests, on average, that IDU who participated in EuroSIDA and started HAART had a comparable response to other exposure groups, were able to adhere to complex regimens, and benefited from a similar reduction in the risk of AIDS or HIV-related death. EuroSIDA patients may not be wholly representative of those with HIV across Europe, but do represent a heterogeneous population who have been treated according to local guidelines, which will differ between centres and over time.

In summary, these results show the importance of collecting data on the precise cause of death. Without this information results could have been misinterpreted as IDU having an inferior response to HAART compared with other exposure groups. As the proportion of non-HIV-related deaths increases in observational cohorts, there is a need to define and standardize causes of death more effectively, particularly as observational studies will increasingly be used for pharmacovigilance and the monitoring of serious adverse events.

Appendix

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Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe

European Collaborative study prepared by Claire Thorne, Deven Patel and Marie-Louise Newell

Highly active antiretroviral therapy (HAART) may be associated with adverse pregnancy outcomes. Among 4372 live births in the European Collaborative Study, the prematurity rate increased to 24.9% in 2000–2004. Antenatal HAART use initiated pre-pregnancy was strongly associated with prematurity (AOR 2.05, 95% CI 1.43, 2.95), particularly severe prematurity. The implication of increased prematurity is evidenced in high neonatal mortality in these groups (0.66% for infants at 34–36 weeks and 7.37% at < 34 weeks' gestation).

HIV-infected women taking highly active antiretroviral therapy (HAART) could face possible adverse events in pregnancy. Although we and other European observational studies have shown a substantially increased risk of premature delivery associated with antenatal combination antiretroviral therapy [1–3], which was not seen in a study in the USA [4], this was generally felt to be clinically manageable. There is no information documenting the consequences of premature delivery for the infants. Increasing numbers of infected women in Europe are taking highly potent, complex combinations of drugs throughout pregnancy, often initiated before or in early pregnancy. The impact of these changes in HIV treatment on pregnancy outcome is unclear, but we recently noted a worrying increase in severe pregnancy-related adverse events, including neonatal deaths, which are described here.

Since 1986 HIV-infected women identified before or during pregnancy and their infants are followed according to standard clinical and laboratory protocols, in the ongoing European Collaborative Study [2,3]. We define prematurity and severe prematurity as delivery before 37 and 34 weeks' gestation, respectively, neonatal death as death within the first 28 days of life, and perinatal mortality as stillbirths and deaths of live born infants within the first week of life. Data entry was carried out using MS Access 2000 and analyses using SAS statistical software (v8.02, SAS Institute, Cary, NC, USA).

There were 4372 live births to women enrolled between 1986 and the end of April 2004, of whom 3131 (74%) were white, 914 (22%) were black and 171 (4%) were of other ethnicity. The median maternal age at the time of delivery was 28.3 years (range 10–47). The overall crude prematurity rate was 18.9% (828/4372), with a significant recent increase from 16.4% (136/832) in 1985–1989, 15.7% (187/1192) in 1990–1994, and 18.0% (205/1142) in 1995–1999 to 24.9% (300/1206) in 2000–2004 ($\chi^2 = 30.1$, $P < 0.002$). Similar trends were observed in the prevalence of low birthweight and very low birthweight. The rate of elective caesarean section in the European Collaborative Study was high (65.4%, 783/1198, in 2000–2004), reflecting its use in the prevention of mother-to-child transmission [5]. The median gestational age among infants delivered by elective caesarean section was 38 weeks, although some centres had a policy of scheduling elective caesarean section at 36 weeks [5]. The increase in prematurity among women delivering by emergency caesarean section or vaginally (thus with a 'spontaneous' delivery with rupture of the membranes or initiation of labour) was from 16.1% (108/670) in 1985–1989, 15.0% (147/982) in 1990–1994, to 20.0% (118/591) in 1995–1999 and 37.1% (154/415) in 2000–2004 ($\chi^2 = 66.4$, $P < 0.002$).

The prematurity rate among women taking monotherapy (predominantly zidovudine) in pregnancy was 16.8% (118/704), 13.4% (34/254) for dual therapy, and 25.5% (274/1075) for HAART ($\chi^2 = 26.6$, $P < 0.002$). In univariable and multivariable logistic regression analysis, HAART in pregnancy, particularly when initiated before pregnancy, was strongly predictive of prematurity (Table 1). Taking severe prematurity as the outcome gave adjusted odds ratios (AOR) of a similar size for most explanatory variables, but a stronger association with HAART [AOR 2.50, 95% confidence interval (CI) 1.19–5.24, $P = 0.016$ when initiated during pregnancy, and AOR 4.41, 95% CI 2.06–9.41, $P < 0.002$ when inititated pre-pregnancy]. Although adjusting for unaccounted centre-associated variation through a variable for random effect at the centre level improved the model's goodness of fit, the AOR for the variables of interest here remained at a similar magni-

Table 1. Risk factors for delivery before 37 weeks' gestation in 2279 mother–child pairs.

	N	OR	*P* value	AOR (95% CI)	*P* value
Maternal age (years)					
< 25	501	1.00		1.00	
26–34	1463	1.15	0.32	1.05 (0.78–1.41)	0.75
⩾ 35	315	1.82	< 0.002	1.45 (0.98–2.13)	0.06
Maternal ethnic group					
White	1572	1.00		1.00	
Black	628	0.93	0.54	0.73 (0.55–0.96)	0.02
Other	79	1.22	0.48	1.01 (0.56–1.81)	0.97
Maternal IDU in pregnancy					
No	2073	1.00		1.00	
Yes	206	1.75	< 0.002	2.15 (1.48–3.12)	< 0.002
Maternal CD4 cell count					
⩾ 500 cells/mm^3	847	1.00		1.00	
200–499	1117	1.13	0.33	1.00 (0.77–1.29)	1.00
< 200	315	2.02	< 0.002	1.95 (1.40–2.72)	< 0.002
Mode of delivery					
Vaginal	975	1.00		1.00	
Emergency caesarean section	300	5.87	< 0.002	5.23 (3.80–7.20)	< 0.002
Elective caesarean section	1004	1.62	< 0.002	1.37 (1.02–1.82)	0.03
Antenatal antiretroviral use					
None	944	1.07	0.62	1.01 (0.71–1.41)	0.95
Mono/dual therapy		1.00		1.00	
HAART started antenatally	446	2.03	< 0.002	1.88 (1.34–2.65)	< 0.002
HAART started prepregnancy	321	2.19	< 0.002	2.05 (1.43–2.95)	< 0.002

AOR, Adjusted odds ratio; CI, confidence interval; HAART, highly active antiretroviral therapy; IDU, injection drug use; OR, odds ratio.

tude. A sub-analysis limited to 1275 women with emergency caesarean section or vaginal deliveries identified the same risk factors for premature delivery as above, although the HAART-associated risk was more pronounced (HAART started antenatally: AOR 3.25, 95% CI 1.99–5.31, and pre-pregnancy: AOR 4.00, 95% CI 2.26–7.08).

To assess the clinical consequences of this increase in prematurity and low birthweight we investigated neonatal and perinatal deaths. There were 28 neonatal deaths (eight in the most recent period); with a median gestation of 31 weeks (range 22–40) and median birthweight of 1213 g (range 500–3940). The neonatal mortality rate was 73.7 per 1000 (16/217) for infants born before 34 weeks, 65.5 per 1000 (4/611) for those born at 34–36 weeks, and 2.26 per 1000 (8/3544) for term infants ($\chi^2_{\text{trend}} = 110.0$, $P < 0.002$). The few deaths preclude multivariable analysis, but univariably there was a strong relationship between gestational age and neonatal mortality: 71% of the neonates who died (20/28) were premature versus 18% of surviving infants (808/4344, $P < 0.002$). Other univariable associations (ethnicity, mode of delivery, maternal age) disappeared when controlling for gestational age. The overall neonatal mortality rate fluctuated at approximately six to seven per 1000 between 1985 and 2003, compared with three to four per 1000 in the general population in Europe. The perinatal mortality rate was 18 per 1000 (95% CI 12.2–25.4; 31/1725) in 1998–2003, reaching 21.7 per 1000 (95% CI 5.95–54.7) in 2003, substantially higher than the five to nine per 1000 in the general European population.

Our findings of a substantially increased risk of severely curtailed pregnancy duration among women taking HAART antenatally, particularly when initiated pre-pregnancy, coupled with the very high neonatal mortality rate associated with delivery at these early gestations, are very concerning. Although not denying its prevention of mother-to-child transmission benefit, we would suggest that these data are taken into consideration when making therapeutic decisions for HIV-infected women of childbearing ages whose clinical, immunological and virological status does not indicate a need for the early initiation of HAART.

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Changes in HIV prevalence among young Thai men as defined by hepatitis C co-infection as a marker for mode of transmission

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To obtain a better understanding of the evolving HIV-1 epidemic in Thailand, we utilized antibody to hepatitis C virus (HCV) to indicate the mode of HIV-1 transmission. Although the proportion of men with HCV co-infection increased between 1995 and 2000, the prevalence was similar, whereas the prevalence of men not co-infected decreased (1.93–0.46%). This suggests that HIV-1 infection associated with parenteral transmission has been stable despite a dramatic reduction in the sexual transmission of HIV-1.

When the Royal Thai Army Medical Department noticed a high prevalence of HIV-1 infection among northern recruits in 1989, it established a screening and prevention programme for HIV infection in conjunction with biennial military induction. This programme has provided critical surveillance data for the country, has been instrumental in the development of policy, and informative for monitoring trends in HIV risk behaviour [1]. HIV-1 in Thailand was initially described as two epidemics; one of subtype B among injection drug users (IDU) occurring predominantly in Bangkok, and a second with the circulating recombinant form, 01_AE (formerly subtype E), among female sex workers and their clients in the north. More recently, concurrent with the reduction in high-risk sexual behaviour, it has been demonstrated that the proportion of HIV infections attributable to injection drug use has increased among young Thai men from

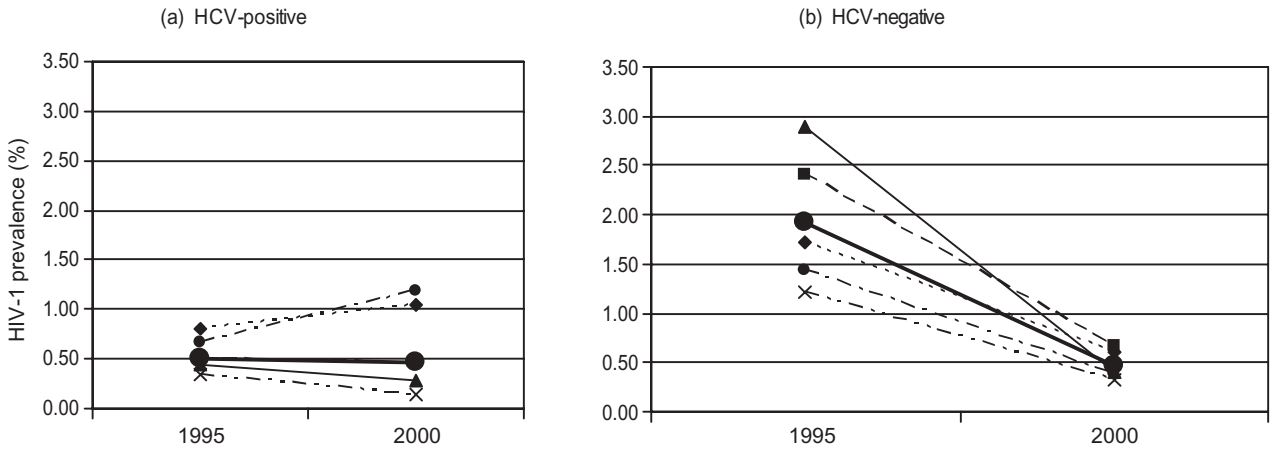

Fig. 1. Changes in prevalence of HIV-1 infection among young Thai men by the presence and absence of hepatitis C virus co-infection. - - - -◆- - - - Bangkok; – – –■– – – central; ——▲—— north; — -×— - northeast; – –●– – south; ——●—— total.

the north in the period from 1991 to 1998 [2]. As hepatitis C virus (HCV) is often associated with parenterally acquired HIV infection as a result of a common mode of transmission, it may be useful to use co-infection as an indicator of risk behaviour associated with injection drug use when this information is either unavailable or unreliable and there is a high prevalence of HCV infection among IDU [3]. In a study of IDU in Bangkok, 96% of those with HIV infection were co-infected with HCV [4]. In a community vaccine preparatory cohort study on the eastern seaboard of Thailand, more than 90% of those participants who reported previous injection drug use were HCV infected [3]. Therefore, HCV serology in this setting may facilitate the evaluation of differing trends in the mode of HIV transmission. We previously reported the high proportion of HIV/HCV co-infection among young Thai men in 2000 [5]. This current analysis builds on this information to characterize the epidemic in terms of the mode of transmission at two timepoints separated by 5 years.

Sera were tested for HIV-1 by two different sequential HIV enzyme immunoassays (EIA) [6], and by a third-generation hepatitis C EIA (UBI Biomedical, Beijing, China). Samples were considered HIV-1 positive if both assays were reactive and confirmed by western blot (HIV 2.2 Blot, GeneLab, Singapore), and HCV positive if the HCV EIA was repeatedly reactive. Confirmatory testing for HCV was not performed because of the low rate of false positives previously obtained on a subset of samples with low HCV EIA reactivity that were tested by recombinant immunoblot assay (RIBA HCV 3.0; Chiron, Emeryville, CA, USA; P. Chanbancherd, unpublished data) and the high proportion of HCV antibody reactivity in the HIV-infected population tested. Subjects with HIV infection were referred for counselling, care, and management at military hospitals or Thai Ministry of Public Health facilities. All samples tested for HCV were unlinked to any personal identifiers.

Among recruits whose residence location was known, HIV prevalence decreased from 2.4% (1243 out of 51 131 screened) in 1995 to 1.1% (711 out of 62 296 screened) in 2000 ($P < 0.0001$ by chi-square statistic). Within this overall decline in HIV prevalence, there are two trends that differ considerably based on the presence or absence of HCV co-infection. The overall proportion of HCV co-infection increased from 20.8% (249 out of 1196) in 1995 to 49.5% (282 out of 570) in 2000, using available sera from HIV-infected men. The regions with the highest proportion and largest increases in HCV co-infection in 2000 were the south (75.0% compared with 31.8% in 1995) and Bangkok (63.0% compared with 22.1%). The overall prevalence of HIV-infected recruits with HCV co-infection did not change significantly (from 0.5 to 0.4%, Fig. 1a), whereas the prevalence of HIV-infected recruits without HCV infection decreased nearly fourfold, from 1.9 to 0.5% over this time period (Fig. 1b). There was an increase in the prevalence of HCV-infected men in Bangkok and southern Thailand in 2000 compared with 1995 ($P = 0.005$ by chi-square statistic).

These distinct trends in the Thai HIV epidemic, when assessed using a marker to provide information about different routes of transmission, reaffirms the known success in reducing sexually transmitted HIV-1. In contrast, the data strongly suggest that IDU-related transmission has not decreased in the 5-year period. These data are consistent with a previous study of risk factors for HIV-1 infection in a study of young men tested during military recruitment in northern Thailand [2], in which the percentage of HIV-infected men who indicated previous injecting drug use increased from

15% in 1995 to 25% in 1998. The proportion of men with HCV co-infection in this study increased from 13.9% in 1995 to 41.0% in 2000 in the same region. Notably, the highest prevalence of HIV/HCV co-infection was observed among men from southern Thailand. The epidemiology of HIV in this region may more closely resemble that of bordering Malaysia, where the majority of HIV transmissions are thought to occur among IDU [7,8]. This study highlights the importance of dissecting HIV-1 national epidemics despite objective evidence of successful prevention efforts. Successful public health interventions in Thailand have reduced the impact of commercial sex work and mother-to-child transmission on the epidemic; however, this analysis, which utilizes a surrogate marker for parenteral transmission, reinforces the recent assessment by the Joint UN Programme on HIV/AIDS (UNAIDS) that IDU will continue to fuel the HIV-1 epidemic in Thailand [9].

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The mother-to-child HIV transmission epidemic in Europe: evolving in the East and established in the West

European Collaborative Study*

Objectives: To carry out an epidemiological analysis of the emerging epidemic in an Eastern European country and to compare the approach to prevention of mother-to-child transmission (MTCT) with that in Western Europe.

Design: Prospective cohort study established in 1985 in Western Europe and extended to Ukraine in 2000.

Methods: Data on 5967 HIV-infected pregnant women and their infants (1251 from Ukraine and 4716 from Western/Central Europe) was analysed. Factors associated with transmission were identified with logistic regression.

Results: HIV-infection among pregnant women enrolled in Western European centres has shifted from being largely injecting drug use (IDU)-related to heterosexually-acquired; in Ukraine IDU also gradually declined with women increasingly identified without specific risk factors. In Ukraine in 2000–2004 most (80%) women received single dose nevirapine (sdNVP) and/or short-course zidovudine prophylaxis [MTCT rate 4.2%; 95% confidence interval (CI), 1.8–8.0 for sdNVP with short-course zidovudine]; 2% ($n = 27$) received antenatal HAART and 33% ($n = 418$) delivered by elective caesarean section (CS); in Western European centres 72% of women received HAART (MTCT rate 1.0%; 95% CI, 0.4–1.9) and 66% delivered by elective CS during the same period.

Conclusions: Our findings indicate distinct differences in the epidemics in pregnant women across Europe. The evolution of the MTCT epidemic in Ukraine does not appear to be following the same pattern as that in Western Europe in the 1980s and 1990s. Although uptake of preventive MTCT prophylaxis has been rapid in both Western Europe and Ukraine, substantial challenges remain in the more resource-constrained setting in Eastern Europe.

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Keywords: epidemiology, vertical transmission, prevention of perinatal transmission, Europe, antiretroviral agents, pregnancy, Ukraine, mother-to-child transmission

Introduction

An estimated 580 000 HIV-infected people live in Western Europe and 1.3 million in Eastern Europe and Central Asia [1]. The HIV epidemic in Western Europe was established in the early 1980s, mostly among homosexual men and injecting drug users (IDUs). Today, heterosexual transmission prevails, accounting for 58% of new infections in 2003, largely associated with origin from sub-Saharan Africa [2,3]. It was not until the mid- to late-1990s that the HIV epidemic fully emerged in Eastern Europe, where two countries (the Russian

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Federation and Ukraine) bear the brunt of the epidemic [2,4,5], and although initially focused among IDUs, new HIV infections among non-IDU heterosexuals are now rapidly increasing [2]. HIV seroprevalence in Central Europe is substantially lower than that elsewhere in Europe, with most HIV diagnoses concentrated in Romania and Poland [5].

Use of antenatal HAART, elective caesarean section (CS) delivery and no breastfeeding is highly effective in reducing mother-to-child transmission (MTCT) of HIV [6–8]. Prevention of MTCT (PMTCT) programmes have resulted in only around 250 vertically-acquired HIV infections in Western Europe annually, despite increasing numbers of HIV-infected women [9]. In Eastern Europe, there is a higher prevalence of HIV infection (1.0 versus 0.3% in the West), more infected women of childbearing age (34% of total HIV-infected population versus 26% in the West) and health care systems have only limited capacity to cope with the worsening HIV epidemic [1,10]. In Ukraine, 2115 infants were born to women with identified HIV-infection in 2004, a 40% increase since 2003 (Dr N Zhilka, personal communication, 2005). The approach to PMTCT in Ukraine currently follows models used in other resource-limited settings: single dose nevirapine (sdNVP) to mothers intrapartum and to the neonate and/or short course antenatal zidovudine (ZDV) prophylaxis, with formula feeding [11–13]. Similar approaches are being used in other Eastern European countries with high HIV prevalence and/or incidence [14].

The European Collaborative Study (ECS) is an ongoing cohort study which started nearly 20 years ago in Western Europe and extended to Ukraine in 2000. This provided the opportunity for an epidemiological analysis of the situation in Ukraine, comparison with Western Europe and assessment of whether the experience in the West has relevance for the younger epidemic in the East.

Methods

HIV-1 infected pregnant women were enrolled and their infants prospectively observed in accordance with standard protocols [6,15]. Pregnant women are screened for HIV infection within standard antenatal care, with all but one centre having a universal antenatal HIV screening policy [16] and those infected invited to enrol; pregnant women identified as HIV-infected from before pregnancy were also invited to participate. Informed consent was obtained before enrolment, according to local guidelines and ethics approval was granted. The ECS was set up in 1985. Centres from Spain, Italy, the United Kingdom, Germany and Belgium have participated since the study started, with centres from Sweden (1986), the Netherlands (1987), Poland (1989), Denmark (1995) and Ukraine (2000) joining subsequently.

Information collected included maternal CD4 cell count (since 1992), most likely maternal mode of HIV acquisition and antiretroviral prophylaxis/treatment. In the Ukraine centres, CD4 cell counts were available for a small, selected group only [$n = 71$, of which 69 were receiving antiretroviral therapy (ART), two-thirds from one centre]. Delivery and infant characteristics recorded included mode of delivery, sex, birthweight, gestational age and infection status. Maternal CD4 cell counts nearest the time of delivery were used here.

Infants with persistence of antibody beyond 18 months of age and/or a positive virological marker of infection on two separate blood samples regardless of age were included as infected [6,15]. If a child from a Western/Central European centre was HIV antibody-negative and no virus or antigen had ever been detected, (s)he was classified as uninfected, regardless of age. In the centres in Ukraine, due to a lack of virological diagnostic laboratory facilities, dried blood spot filter papers were collected and sent to Amsterdam for testing (Retina^{TM} Rainbow HIV-1 RNA assay; Primagen, Amsterdam, The Netherlands). As diagnosis of HIV infection based on clinical symptoms may occur earlier than exclusion of infection in settings with limited access to virological tests [17], only children born 18 months or more before the time of the analysis were included in the estimation of MTCT rates in the Ukraine centres to reduce likelihood of bias; definition of HIV infection here was based on the development of AIDS and HIV-associated mortality ($n = 12$), persistence of antibody beyond 18 months ($n = 24$) or detectable virus in two or more blood samples taken on different occasions ($n = 6$).

Elective CS was defined as delivery before rupture of membranes and onset of labour, premature delivery as occurring before 37 weeks of gestation, with gestational age confirmed by ultrasound and reported to the nearest completed week, and IDU in pregnancy according to self-report, clinical report or neonatal drug withdrawal symptoms. Women with CD4 cell counts < 200 cells/μl were classified as severely immunosuppressed. Multiple births (32 twin pairs, one triplet) were treated as separate mother–child pairs.

Univariable comparisons for categorized variables were tested with the χ^2 test or χ^2 test for trend. Univariable and multivariable logistic regression analysis was used to obtain odds ratios (OR), adjusted odds ratios (AOR) and 95% confidence intervals (95% CI). All probability values were two-tailed. Data entry was carried out using MS Access 2000 (Microsoft Corp., Redmond, Washington, USA) and analyses using SAS statistical software (v8.02; SAS Institute, Cary, North Carolina, USA).

Results

HIV-infected pregnant women

Of the 5967 mother–child pairs enrolled by December 2004, 1251 (21%) were from Ukraine, 179 (3%) from Poland and 4537 (76%) from Western European centres. Poland was the only country included without a universal antenatal HIV testing policy at this time [18] and most (136; 76%) of the pregnant women enrolled here had an IDU history; all the women were white, all but one had been born in Poland and they had a median age at delivery of 26.5 years (range, 17–43 years).

Maternal and delivery characteristics of women enrolling in the eight Western European countries and in Ukraine are presented in Table 1. Western European centres were ethnically heterogeneous, reflecting recent migration from Africa: 84% (863/1025) of black women came from sub-Saharan Africa, increasing from 4% (35/838) in 1985–1989 to 37% (478/1309) in 2000–2004 ($P < 0.002$). Women from sub-Saharan Africa enrolling in 2000–2004 were older (median age 30.3 versus 27.8 years), less likely to be married or cohabiting [71% (326/457) versus 78% (574/733); $\chi^2 = 7.42$; $P = 0.006$] and more likely to be identified as HIV-infected through antenatal testing [44% (211/267) versus 26% (220/831); $\chi^2 = 42.9$; $P < 0.0001$] than other women enrolling in Western European centres at this time. Overall, women enrolling in the Western European centres in 2000–2004 were more likely to know their HIV diagnosis before pregnancy, independent of ethnicity, mode of acquisition and parity, with an AOR of pre-pregnancy diagnosis of 10.1 (95% CI, 7.95–12.8) in 2000–2004 with 1985–1989 as baseline. Women from Ukraine were 83% less likely to be diagnosed before pregnancy than women in Western Europe (AOR, 0.17; 95% CI, 0.15–0.21) overall.

Women enrolling in the Ukraine centres were more similar to those enrolling in Western European centres in the first 5 years of the study than those enrolling more recently. In the Western centres in 1985–1989, 93% (783/846) of women were white, 35% (294/846) aware of their infection before pregnancy and 79% (668/846) IDUs. Median age in the Western European centres was 28.5 years (range, 10–47 years) overall, 25.1 years (range, 10–41 years) in 1985–1989 and 31.7 (range, 15–47 years) in 2000–2004, compared with 25.4 years (range, 14–43 years) in Ukraine. Prevalence of very young maternal age was higher in Ukraine than in Western

Table 1. Maternal and delivery characteristics.

	Western Europe		Ukraine
	Whole period $n = 4537$	2000–2004 $n = 1384$	2000–2004 $n = 1251$
Ethnicity	$n = 4364$	$n = 1326$	$n = 1234$
Black	1025 (23)	559 (42)	1
White	3158 (72)	702 (53)	1212 (98)
Other	181 (4)	65 (5)	21 (2)
Timing of first positive HIV test	$n = 4537$	$n = 1384$	$n = 1251$
Before pregnancy	2582 (57)	916 (66)	248 (20)
During pregnancy	1535 (34)	419 (30)	759 (61)
At delivery	420 (9)	49 (4)	244 (19)
Parity at enrolment	$n = 4128$	$n = 1294$	$n = 1219$
0	2191 (53)	571 (44)	710 (58)
1	1155 (28)	412 (32)	381 (31)
2	492 (12)	210 (16)	90 (7)
≥ 3	290 (7)	101 (8)	38 (3)
History of pregnancy termination	$n = 4190$	$n = 1313$	$n = 1220$
No	2565 (61)	805 (61)	692 (57)
Yes	1625 (39)	508 (39)	528 (43)
Living outside country of birth	$n = 4269$	$n = 1309$	$n = 1243$
No	2935 (69)	611 (47)	1024 (99)
Yes	1334 (31)	698 (53)	9 (1)
Reported risk factors for acquisition of HIV infection	$n = 4537$	$n = 1384$	$n = 1251$
IDU	1062 (23)	135 (10)	111 (9)
Sexual	2320 (51)	1031 (74)	318 (25)
IDU and sexual	928 (20)	139 (10)	239 (19)
Other	51 (1)	15 (1)	8 (1)
No risk factors specified	176 (4)	64 (5)	575 (46)
Gestational age	$n = 4456$	$n = 1350$	$n = 1251$
< 34 weeks	235 (5)	91 (7)	32 (3)
34–36	634 (14)	240 (18)	89 (7)
≥ 37	3587 (81)	1019 (75)	1130 (90)
Birth weight (g)	$n = 4459$	$n = 1262$	$n = 1251$
Median (range)	2932 (420–5190)	2900 (420–5190)	3085 (1200–5000)

IDU, injection drug user.

Europe [9% (114/1250) were aged < 20 years compared with 4% (150/4209); $\chi^2 = 64.7$; $P < 0.0001$] and Ukrainian young women were more likely to be married [39% (43/110) versus 19% (19/100); $\chi^2 = 10.1$; $P = 0.001$], less likely to have had a pregnancy termination [10% (11/113) versus 16% (29/140); $\chi^2 = 4.87$; $P = 0.027$] and less likely to report any IDU [13% (14/104) versus 26% (53/149); $\chi^2 = 14.3$; $P = 0.0002$].

Temporal trends in maternal mode of acquisition of HIV are presented in Figs 1a and 1b. In Western Europe this has shifted from IDU-related to heterosexual transmission. Since 2000, IDU has gradually declined in Ukraine, with an increase in women reporting no risk factors; that is, this latter group did not report current or past use of injecting drugs, having an IDU or other high-risk sexual partner, blood transfusions or any other high-risk sexual behaviour (e.g. multiple sex partners). Women not reporting specific risk factors in Ukraine were largely married or cohabiting (87%; 479/553), of similar age to those reporting heterosexual risk factors (respective medians, 24.7 and 25.6 years, with 52 and 45% aged < 25 years) and were significantly younger than IDUs (median age 27.2 years; 12% aged < 25 years; $\chi^2 = 370.5$; $P < 0.0001$). Prevalence of current IDU was 17% (757/4537) in Western European centres and 10% (125/1251) in Ukraine; in the West this prevalence decreased from 30% (257/846) in 1985–1989 to 5% (71/1384) in 2000–2004 ($\chi^2_{\text{trend}} = 316.5$; $P < 0.0001$). Although in Ukraine, former or current IDUs were older than other women (see above), in Western Europe women without an IDU history were older than those with (median age 29.1 versus 27.9 years, respectively), reflecting the proportional increase in older African women in recent years [19].

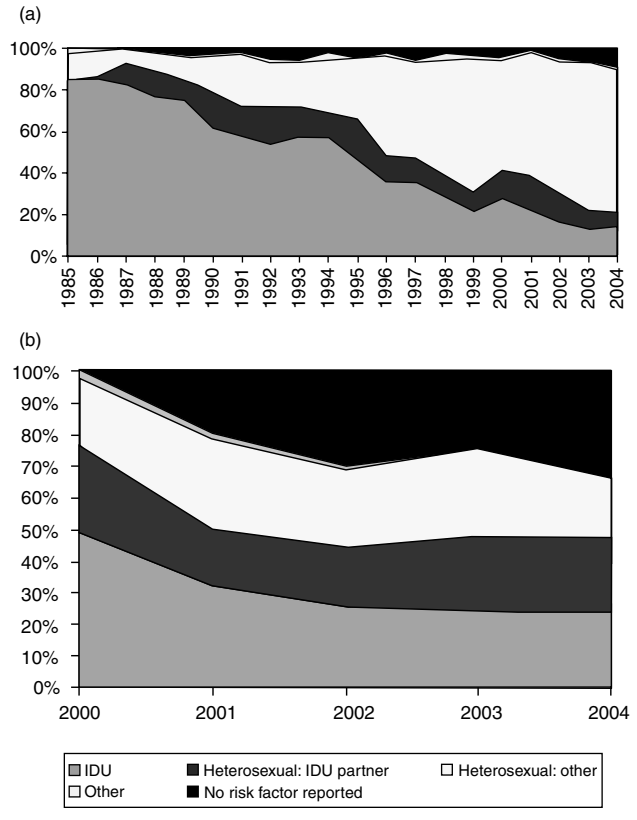

Fig. 1. Trends over time in mode of acquisition of HIV infection: (a) Western European centres and (b) Ukraine. IDU, injection drug user.

Median maternal CD4 cell count was 420 cells/μl (range, 0–2350 cells/μl) ($n = 3009$, including 71 from Ukraine). In 1135 (38%) women CD4 cell count was ≥ 500 cells/μl, in 1471 (49%) it was 200–499 cells/μl and in 403 (13%) < 200 cells/μl. Black women were more likely to be severely immunosuppressed than white (141/787 (18%) versus 240/2016 (12%); $\chi^2 = 16.9$, $P < 0.0001$). Limiting a multivariable logistic regression to data on 2205 Western European women from the period when CD4 cell count measurements were routinely recorded and allowing for ethnicity and time period (the latter used to assess maturity of the epidemic and as a proxy for trends in therapeutic management), black women remained at increased risk of severe immunosuppression than white women (AOR, 1.47; 95% CI, 1.13–1.91); women delivering in 2000–2004 were significantly less likely to be severely immunosuppressed than women delivering in 1992–1995 (AOR, 0.66; 95% CI, 0.48–0.89), independent of ethnicity.

Mother-to-child transmission

In the following analyses, data from the Polish centres were combined with those from the Western European centres, due to small numbers in the former and very similar access to PMTCT interventions. Overall MTCT rates were 6.7% (95% CI, 4.9–8.9) (42/628) in Ukraine and 9.1% (95% CI, 8.3–10.0) (373/4092) in Western/Central Europe. The MTCT rate declined significantly in the latter centres from 16.1% in 1992–1993 to 1.7% in 2002–2003 ($\chi^2 = 70.6$; $P < 0.0001$), with no significant trend in Ukraine over 2000–2004 ($P = 0.76$).

Of the 2441 (52%) women in Western/Central European centres receiving no antenatal ART, most enrolled before 1994; 1021 (22%) women received monotherapy or dual therapy and 1252 (26%) received HAART, in 610 (49%) cases initiated before pregnancy. The 739 women in Western/Central Europe on ZDV monotherapy started this at a median of 26 gestational weeks, with most delivering before 1997. In Ukraine, two-thirds ($n = 793$) of women received sdNVP, of whom 63% ($n = 503$) also received ZDV monotherapy, as a short-course regimen, initiated at a median 35 gestational weeks; a further fifth of women received either ZDV monotherapy ($n = 208$) or HAART ($n = 27$). Table 2 includes the crude MTCT rates in Western/Central Europe and in Ukraine, stratified by ART. Although no adjustment was made for other variables, the MTCT rate in Western/Central

Table 2. Mother-to-child transmission (MTCT) rates by antiretroviral prophylaxis or treatment group.

	Western Europe 1985–1994		Western Europe 1995–1999		Western Europe 2000–2004		Ukraine 2000–2004	
Antenatal ART	*n* (%)	Crude MTCT rate (95% CI)	*n* (%)	Crude MTCT rate (95% CI)	*n* (%)	Crude MTCT rate (95% CI)	*n* (%)	Crude MTCT rate (95% CI)
None	2008 (96)	15.4% (282/1830; 13.8–17.1)	283 (24)	12.8% (30/234; 8.8–17.8)	148 (10)	4.65% (6/129; 1.7–9.9)	223 (18)	19.8% (19/96; 12.4–29.2)
sdNVP: mother–infant		–		–		–	290 (23)	6.8% (9/131; 3.2–12.6)
Monotherapy	73 (4)	12.3% (8/65; 5.5–22.8)	550 (47)	6.1% (28/459; 4.1–8.7)	116 (8)	4.0% (4/101; 1.1–9.4)	–	–
without sdNVP	–	–	–	–	–	–	208 (17)	7.8% (6/77; 2.9–16.2)
with sdNVP	–	–	–	–	–	–	503 (40)	4.2% (8/192; 1.8–8.0)
Dual nucleotide therapy	2	0/2	134 (11)	0.83% (1/121; 0.02–4.5)	147 (10)	0.8% (1/127; 0.02–4.3)	–	–
HAART	–	–	205 (18)	2.69% (5/186; 0.9–6.2)	1046 (72)	1.0% (8/838; 0.4–1.9)	27 (2)	0/2

ART, antiretroviral therapy; sdNVP, single dose nevirapine; CI, confidence interval.

Europe in the IDU-driven era (1985–1994) among women not receiving antenatal ART was not significantly different from that in the same group in Ukraine currently (15.4 versus 19.8%; $\chi^2 = 1.02$; $P = 0.31$).

In Western/Central Europe the elective CS rate increased from 17% (204/1220) in 1990–1994 to 47% (543/1156) in 1995–1999 and 63% (909/1438) in 2000–2004 ($\chi^2 = 589.2$; $P < 0.0001$), whereas in Ukraine the elective CS rate was 33% (418/1251), with no trends over the 5 years of data collection.

Logistic regression analyses of MTCT risk were carried out separately for Western Europe and Ukraine (Table 3). As only 71 mother–child pairs from Ukraine had CD4 cell counts, we were unable to include CD4 cell count in the analysis for this area. In Ukraine, only 170 women in the analysis had an elective CS, and although the AOR indicated a reduced transmission risk versus vaginal delivery, this did not reach statistical significance (Table 3). Overall, use of abbreviated regimens in Ukraine was associated with a 70% reduced MTCT risk. Use of sdNVP alone was associated with a 66% reduced MTCT risk after adjusting for prematurity and mode of delivery compared with no antiretroviral prophylaxis (AOR, 0.34; 95% CI, 0.15–0.82), with a similar AOR for ZDV monotherapy only (AOR, 0.44; 95% CI, 0.16–1.22), although this did not reach statistical significance probably owing to small numbers; sdNVP-boosted short-course ZDV was associated with the greatest reduction in risk (AOR, 0.23; 95% CI, 0.09–0.63) compared with no prophylaxis; however, this combination was not statistically significantly more effective compared with sdNVP alone or short-course zidovudine alone. In Western/Central Europe, maternal CD4 cell count was an important risk factor, with severe maternal immunosuppression independently associated with a doubled risk and elective CS with a two-thirds reduced risk. Women taking HAART were more than 90% less likely to transmit infection than those untreated (Table 3) and 75% less likely than women on mono or dual therapy (AOR, 0.25; 95% CI, 0.12–0.54).

Discussion

We present findings from the first epidemiological study of HIV-infected pregnant women in Ukraine. The Ukrainian HIV epidemic has been dominated by IDU [5,13], with high IDU prevalence, young age at IDU initiation, high-risk behaviours (drug use-related and sexual), low HIV prevention awareness and the intersecting epidemics of IDU and commercial sex work accelerating the epidemic [4,20,21]. By 2004, 50% of women enrolled in our Ukraine centres were IDU or reported an IDU sexual partner. Although nearly 80% women in Western European centres enrolling in 1985–1989 were IDUs

Table 3. Risk factors for mother-to-child transmission (MTCT) for Western Europe and Ukraine.

	Western Europe			Ukraine		
	N 2544	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	*N* 498	Unadjusted OR (95% CI)	Adjusted OR[b] (95% CI)
Maternal CD4 cell count						
≥ 500 cells/μl	957	1.00	1.00	–	–	–
200–499 cells/μl	1240	1.48 (1.05–2.09)	1.88 (1.32–2.68) $P < 0.001$	–	–	–
< 200 cells/μl	347	1.76 (1.12–2.76)	2.07 (1.29–3.32) $P = 0.003$	–	–	–
Mode of delivery						
Vaginal delivery	1082	1.00	1.00	311	1.00	1.00
Emergency CS	338	0.51 (0.32–0.80)	0.69 (0.42–1.13) $P = 0.14$	17	0.55 (0.07–4.25)	0.59 (0.07–4.85) $P = 0.62$
Elective CS	1124	0.18 (0.12–0.28)	0.34 (0.22–0.52) $P < 0.001$	170	0.49 (0.22–1.05)	0.86 (0.36–2.00) $P = 0.72$
Maternal ART						
None	1052	1.00	1.00	96	1.00	1.00
sdNVP and/or SC ZDV	–	–	–	402	0.25 (0.13–0.47)	0.30 (0.14–0.64) $P = 0.002$
Mono/dual	631	0.25 (0.17–0.39)	0.34 (0.21–0.53) $P < 0.001$	–	–	–
HAART	861	0.07 (0.04–0.13)	0.09 (0.04–0.17) $P < 0.001$	–	–	–
Prematurity						
≥ 37 weeks	2066	1.00	1.00	452	1.00	1.00
< 37 weeks	478	1.31 (0.92–1.87)	1.78 (1.20–2.63) $P = 0.004$	46	3.65 (1.66–8.01)	2.19 (0.93–5.16) $P = 0.08$

[a]Adjusting for antenatal antiretroviral therapy (ART), mode of delivery, prematurity, maternal CD4 cell count and sex.
[b]Adjusting for antenatal ART, mode of delivery, prematurity and sex. OR, odds ratio; CI, confidence interval; CS, caesarean section; SC, short course; sdNVP, single dose nevirapine; ZDV, zidovudine.

this had declined to 20% in 2000–2004. The relatively low prevalence of IDU history (less than 30%) reported among pregnant women in Ukraine may reflect under-reporting due to social desirability bias or alternatively, pregnant IDU women may not have been enrolled due to a lack of antenatal care. However, these biases are likely to have remained constant over time, suggesting that the decline in IDU seen is real.

Our findings regarding mode of acquisition in Ukraine are consistent with reports of the evolving epidemic there, with evidence that HIV is spreading to bridging populations (sexual partners of IDU and sex workers) and beyond to the general population, with one-third of new HIV diagnoses in Ukraine heterosexually acquired in 2003 [1,13,21,22]. Most women not reporting any risk factors in our Ukraine centres probably acquired HIV heterosexually through unprotected sexual intercourse with casual or regular partners, including their husbands (87% were married or cohabiting), but were unaware of their exposure to HIV. Similarly, 48% of new HIV infections in women in Eastern Europe in 2004 reported to the European HIV/AIDS Monitoring Centre were in the 'other/undetermined' category [9]. Our finding of more very young women in Ukrainian versus Western European centres probably reflects their tendency to start child-bearing at younger ages, but also suggests that Ukrainian women may acquire HIV infection at younger ages [23].

The overall MTCT rate in the Ukrainian centres was 6.7% during 2000–2004. The Ukraine government implemented the use of short-course ZDV and/or sdNVP for mother and infant with formula feeding in 2001 [22]. We lacked statistical power to show a significant difference between either short-course ZDV boosted with sdNVP compared to sdNVP alone or to short-course ZDV alone, although the crude MTCT rates suggest that there might be a benefit in combining sdNVP and short-course ZDV in this population, consistent with trial findings elsewhere and WHO guidelines [11]. The overall MTCT rate in the ZDV monotherapy group in Western Europe was marginally less than that in Ukraine (6.4 versus 7.8%), despite shorter ZDV duration in Ukraine, starting at 35 weeks. The Ukrainian government recently updated its national policy, which now recommends that short-course ZDV is started at 28 weeks gestation. The in utero/intrapartum MTCT rate in the sdNVP only group here was similar to that in the HIVNET 012 trial (6.9 versus 8.1%) [24]. However, the rate at which short-course ZDV was boosted by sdNVP was somewhat higher in our non-trial situation than in the Thai PHPT2 trial [11] with longer antenatal ZDV, but lower than in the West African Ditrame Plus study where just over half were breastfed [25].

National guidelines in Western Europe now recommend HAART in pregnancy as prophylaxis for PMTCT, with

continuation post-partum determined by maternal virological and immunological status, although there is still a place for ZDV monotherapy combined with elective CS for some pregnant women (e.g. those with low viral loads not requiring HAART for their own health) [26,27]. The upper 95% CI of the 1% MTCT rate among women on HAART in the Western European centres was 1.9%, just overlapping with the lower 95% CI of the 4.2% MTCT rate among Ukrainian women receiving sdNVP and short-course ZDV, at 1.8%. This highlights that a reduction in MTCT rates to very low levels without widespread access to HAART is possible in non-breastfeeding, non-trial settings.

However, access to HAART for eligible HIV-infected mothers should be prioritized, and is not only important for maternal health, but also for the children's future health and social care, regardless of infection status [28,29]. Although expanding access to antiretroviral drugs in Ukraine is underway, only an estimated 13% of HIV-infected people eligible for ART are currently treated [13]. As we only had limited CD4 cell count data in Ukraine and no viral load information, we cannot make any conclusions regarding the need for HAART in our study population.

In terms of generalizability of our results to elsewhere in Ukraine, an estimated 20% of all pregnant HIV-infected women delivering nationally in 2001–2004 were enrolled in the ECS (Ukrainian AIDS Center, 2005 unpublished data). The ECS centres were in Southern Ukraine, the national epicenter [30], in Odessa, Micolaiev and Simferopol and include those at the forefront of the PMTCT programme in Ukraine [31]. Uptake of and access to prophylactic interventions in these centres may be somewhat greater than in areas with lower prevalence and/or less experience. In particular, the 33% elective CS rate in the Ukrainian centres here was higher than that reported for HIV-infected women in Ukraine overall, at around 14% (Dr N Zhilka, personal communication, 2005). Furthermore, our findings regarding mode of acquisition of HIV may be specific to Southern Ukraine, as the first affected region of Ukraine, particularly regarding the trends over time. With regard to generalizability to other Eastern European countries, although IDU remains a driving force in many countries' epidemics, an increasing proportion (up to 45% or more) of new reported HIV infections are due to unprotected sex in Russia, Belarus, Moldova and Kazakhstan [32].

There are similarities between the early HIV epidemics in Western Europe and Ukraine, notably the importance of IDU and sexual contact with IDUs. Access to and uptake of PMTCT prophylaxis was rapid in Western Europe [33,34], and antenatal HAART use has contributed to the very low rates of MTCT there [35–37]. Although knowledge of effective PMTCT interventions pre-dated the Ukraine epidemic, application of these is an enormous challenge here, as in all low-income settings [38–40]. These challenges include transforming national PMTCT strategies from a medically-focused vertical approach towards a horizontal approach and integrating prevention activities into maternal and child health services [41]. The Ukraine government has addressed PMTCT with substantial success, with a decreasing MTCT rate from over 25% prior to 2000 to 8% in 2002 [22] (Dr N Zhilka, personal communication, 2005). However, increasing HIV incidence and lack of widespread access to HAART is likely to result in an increasing burden of paediatric HIV infection in Ukraine. The juxtaposition between this situation and the discussion in Western Europe of the potential elimination of vertically-acquired HIV infection underscores the urgent need to scale-up the response to the epidemic in the most affected regions of Eastern Europe, including Ukraine, remembering that PMTCT not only includes application of antenatal and perinatal prophylaxis and prevention of unwanted pregnancies in HIV-infected women, but also primary prevention.

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Time to Undetectable Viral Load after Highly Active Antiretroviral Therapy Initiation among HIV-Infected Pregnant Women

European Collaborative Study[a]

Background. There have been no clinical trials in resource-rich regions that have addressed the question of which highly active antiretroviral therapy (HAART) regimens are more effective for optimal viral response in antiretroviral-naive, human immunodeficiency virus (HIV)–infected pregnant women.

Methods. Data on 240 HIV-1–infected women starting HAART during pregnancy who were enrolled in the prospective European Collaborative Study from 1997 through 2004 were analyzed. An interval-censored survival model was used to assess whether factors, including type of HAART regimen, race, region of birth, and baseline immunological and virological status, were associated with the duration of time necessary to suppress viral load below undetectable levels before delivery of a newborn.

Results. Protease inhibitor–based HAART was initiated in 156 women (65%), 125 (80%) of whom received nelfinavir, and a nevirapine-based regimen was initiated in the remaining 84 women (35%). Undetectable viral loads were achieved by 73% of the women by the time of delivery. Relative hazards of time to achieving viral suppression were 1.54 (95% confidence interval, 1.05–2.26) for nevirapine-based HAART versus PI-based regimens and 1.90 (95% confidence interval, 1.16–3.12) for western African versus non-African women. The median duration of time from HAART initiation to achievement of an undetectable viral load was estimated to be 1.4 times greater in women receiving PI-based HAART, compared with women receiving nevirapine-based HAART. Baseline HIV RNA load was also a significant predictor of the rapidity of achieving viral suppression by delivery, but baseline immune status was not.

Conclusions. In this study, nevirapine-based HAART (compared with PI [mainly nelfinavir]-based HAART), western African origin, and lower baseline viral load were associated with shorter time to achieving viral suppression.

Plasma HIV RNA load is the preeminent risk factor for mother-to-child transmission (MTCT) of HIV infection [1, 2]. In resource-rich regions, HAART (typically composed of 3 antiretroviral agents from 2 drug classes) has substantially reduced MTCT rates through successful suppression of HIV RNA load [2, 3]. Although an increasing proportion of HIV-infected pregnant women in these regions are identified and treated before pregnancy, a substantial minority receive a diagnosis antenatally and start antiretroviral therapy (ART) for the first time during pregnancy to delay disease progression and/or to prevent MTCT [2]. In many Western European countries, these women are increasingly likely to have acquired HIV infection through heterosexual contact and to be from countries with generalized epidemics (mainly countries in sub-Saharan Africa) [4]. No clinical trials in resource-rich regions have addressed the question of which regimens are more effective for optimal viral response in ART-naive, HIV-infected pregnant women. Using data from a multicenter prospective cohort, this study was conducted to determine whether choice of initial HAART regimen for HIV-infected pregnant women is associated with the duration of time that is necessary to achieve undetectable viral load by delivery.

METHODS

The European Collaborative Study is an ongoing observational cohort study that was established in 1985,

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in which HIV-1–infected pregnant women are enrolled and their infants are prospectively observed according to standard protocols [2]. Informed consent was obtained, and local ethics committee approval was granted. Information collected included timing and type of ART, maternal CD4 cell count, HIV RNA load, and sociodemographic characteristics.

Laboratory tests were performed locally in laboratories located in tertiary care centers and university hospitals. For HIV RNA load quantification, Amplicor HIV-1 Monitor Test, versions 1.5 and ultrasensitive (Roche Diagnostic Systems); Quantiplex HIV-1 RNA (b-DNA) assay, version 3.0 (Chiron Diagnostics); or nucleic acid sequence–based amplification/nuclisens (Organon Teknika) were used. Classification of undetectable viral load (viral suppression) was based on the lower limit of quantification of the assay. Of the 759 antenatal HIV RNA load measurements available, 561 (74%) were measured with ultrasensitive assays (quantification limit, $\leqslant$50 copies/mL). HAART was defined as a regimen of $\geqslant$3 antiretroviral drugs, including a nucleoside reverse-transcriptase inhibitor backbone and nevirapine (NVP)—a nonnucleoside reverse-transcriptase inhibitor (NNRTI)—or a protease inhibitor (PI).

We restricted the analysis to HIV-infected women who were ART-naive at conception; 153 women were first identified as having HIV-infection during pregnancy, and 87 women were known to have HIV infection before pregnancy (46 of these women were known not to have previously received prophylaxis for prevention of MTCT, either because of no prior pregnancies or documented nonreceipt of ART during previous pregnancies that occurred while enrolled in European Collaborative Studies, and 41 were not receiving ART at conception and had no documented prior ART use). Other eligibility criteria were a detectable HIV RNA load measurement within 6 weeks before initiation in the study and at least 1 subsequent measurement.

Statistical methods. To determine the association of maternal factors and initial HAART regimen with viral suppression, we examined the duration of time from treatment initiation to the achievement of undetectable HIV RNA load until the time of delivery. For women not achieving an undetectable HIV RNA load, the time was right-censored at the last measurement at or before delivery. For those achieving an undetectable HIV RNA load, the end point was known only to have occurred between initiation and the first measurement (left-censoring) or between any 2 measurements following initiation (interval-censoring).

A parametric survival model based on the Weibull distribution, incorporating left-, right-, and interval-censoring, was used [5]. Estimates from Weibull models can be represented either as relative hazards (RHs) or as the acceleration factor between 2 levels of a covariate. Because most women reached viral suppression, the RH reflects the rapidity with (rate at) which viral suppression occurred, rather than solely the probability of the event occurring; larger RHs are associated with more rapid attainment of viral suppression.

After adjusting for baseline viral load, race, type of HAART, baseline CD4 cell count, maternal age, trimester at initiation of HAART, timing of HIV diagnosis, history of injection drug use, and year of delivery were considered in the Weibull model. A stepwise model selection procedure was used to choose salient prognostic variables, with a variable retained if its inclusion resulted in a significantly improved log likelihood. The propensity of being treated with NVP-based HAART was estimated by a logistic regression model that included the covariates mentioned above; the propensity score was then included in all adjusted models after stratification into quintiles [6].

Stratified survival curves and associated survival probabilities were obtained with Turnbull's generalization of the Kaplan Meier estimate, allowing for interval-censored data [5, 7], with 95% CIs calculated using the adjusted bootstrap percentile method with 1000 replications. Turnbull estimates of the proportion of women achieving undetectable viral loads, by treatment group and race, were calculated separately for women with baseline viral loads $\geqslant$4 $\log_{10}$ copies/mL or <4 $\log_{10}$ copies/mL. Skewed continuous variables were compared using the Mann-Whitney *U* test, and categorical variables were compared using the χ^2 test. Analyses were performed using Stata software, version 9.1 (StataCorp), and R, version 2.2.0 (R Development Core Team).

RESULTS

Of 1346 women receiving antenatal HAART who delivered a newborn from 1997 through 2004, 240 pregnant, HIV-infected women met our inclusion criteria. The characteristics of these women are shown in table 1. Most women (59%) were black, 90% of whom were born in sub-Saharan Africa (table 1). PI-based HAART was initiated in 156 women (65%), with the remaining 84 women (35%) receiving an NNRTI-based regimen (all including NVP). Most regimens had a zidovudine and lamivudine combination nucleoside reverse-transcriptase inhibitor backbone (table 2). One hundred twenty-five PI-based regimens (80%) included nelfinavir (NFV), with the remaining containing a lopinavir and ritonavir combination (4 regimens), ritonavir (13 regimens), indinavir (8 regimens), or saquinavir (6 regimens). The proportion of women receiving NVP-based HAART increased from 16 (25%) of 64 women during 1997–2000 to 37 (35%) of 105 women and 31 (44%) of 71 women during 2001–2002 and 2003–2004, respectively ($P = .02$). There were no differences between black and non-black women with respect to type of HAART received (48 [34%] of 141 black women vs. 34 [35%] of 96 non-black women received an NVP-based HAART regimen; $P = .94$), the distribution of baseline median HIV RNA load (4.13 $\log_{10}$ copies/mL [interquartile range (IQR), 3.54–4.60] vs. 4.20 $\log_{10}$ copies/mL [IQR, 3.76–

Table 1. Characteristics of 240 treatment-naive, HIV-infected pregnant women at initiation of HAART and the number of women acheiving the end point of an undetectable HIV RNA load by delivery.

Characteristic	All women	Women acheiving an undetectable viral load
Race		
Non-black	96 (41)	63 (66)
Black	141 (59)	110 (78)
Unknown	3	2
Region of birth[a]		
Europe	75 (32)	46 (61)
The Americas	23 (10)	18 (78)
Asia	8 (3)	7 (88)
Northern Africa	6 (2)	5 (83)
Eastern Africa	44 (19)	33 (75)
Southern Africa	2 (1)	1 (50)
Central Africa	40 (17)	31 (78)
Western Africa	39 (16)	32 (82)
Unknown	3	2
Age at delivery		
Median years (IQR)	29 (25–33)	
15–19 years	10 (4)	6 (60)
20–29 years	113 (48)	86 (76)
30–39 years	107 (45)	74 (69)
⩾40 years	8 (3)	7 (88)
Unknown	2	2
History of IDU		
Non-IDU	215 (91)	159 (74)
IDU	21 (9)	12 (57)
Unknown	4	4
Timing of diagnosis of HIV infection		
Antenatal	153 (64)	113 (74)
Prepregnancy	87 (36)	62 (71)
Stage of pregnancy at initiation of HAART		
Median weeks of gestation (IQR)	23 (18–27)	
First trimester	14 (6)	12 (86)
Second trimester	168 (70)	129 (77)
Third trimester	58 (24)	34 (59)
HIV RNA viral load, $\log_{10}$ copies/mL		
Median (IQR)	4.16 (3.62–4.58)	
⩾5	20 (8)	10 (50)
4–4.99	125 (52)	88 (70)
3–3.99	74 (31)	58 (78)
<3	21 (9)	19 (90)
CD4 cell count		
Median cells/μL (IQR)	328 (210–480)	
<200 cells/μL	48 (22)	29 (60)
200–499 cells/μL	124 (56)	95 (77)
⩾500 cells/μL	48 (22)	38 (79)
Unknown	20	13

NOTE. Data are no. (%) of patients, unless otherwise indicated. IDU, injection drug user.

[a] Regions of Africa were defined according to the United Nations groupings.

Table 2. Characteristics of study patients by HAART category.

Characteristic	PI-based HAART (n = 156)	NVP-based HAART (n = 84)	P[a]
Received NRTI backbone			
No. of patients receiving zidovudine and lamivudine (%)	139 (89)	72 (86)	
No. of patients receiving another dual combination (%)	17 (11)	12 (14)	.58
Median time of initiation of HAART, weeks of gestation (IQR)	23 (18–27)	21.5 (16–28)	.57
Median baseline HIV RNA load, $\log_{10}$ copies/mL (IQR)	4.18 (3.60–4.58)	4.08 (3.71–4.54)	.58
Median baseline CD4 cell count, cells/mm^3 (IQR)	305 (190–452)	355 (277–506)	.02
Median no. of viral load measurements (IQR)	3 (2–3)	3 (2–3)	.77
Median interval between successive HIV RNA load tests, weeks (IQR)	7.5 (4–10)	6 (4–10)	.07
Median duration of gestation at delivery, weeks (range)	38 (25–42)	37 (23–41)	<.01

NOTE. NRTI, nucleoside reverse-trascriptase inhibitor; NVP, nevirapine; PI, protease inhibitor.

[a] P values were calculated with the χ^2 test or Mann-Whitney U test, as appropriate.

4.56]; $P = .18$), and baseline median CD4 cell count (313 cells/mm^3 [IQR, 210–449] vs. 370 cells/mm^3 [IQR, 231–528]; $P = .12$). However, black women tended to start treatment later than non-black women (median of 24 weeks of gestation [IQR, 20–28] vs. median of 20.5 weeks of gestation [IQR, 15–27]; $P \leqslant .01$).

The median number of virological measurements per woman was 3 (range, 2–7 measurements), with a similar interval between successive tests for the 2 treatment groups (table 2). Figure 1 shows the distribution of HIV RNA load measurements at initiation, by weeks of gestation, together with baseline median viral load and baseline median CD4 cell count. Thirty-nine (24%) of 165 women starting HAART during the first or second trimester had a CD4 cell count <200 cells/mm^3, compared with 9 (16%) of 55 women starting HAART during the third trimester ($P = .35$).

Although time of HAART initiation and baseline HIV RNA loads were similar between treatment groups, the PI-based group had significantly lower baseline CD4 cell counts (table 2). Among the NVP group, 61 women (73%) had a CD4 cell count >250 cells/mm^3 at initiation; of these women, 57 (93%) delivered a newborn before February 2004 (when NVP prescribing information changed [8]).

The median gestational age at delivery was 38 weeks (range, 23–42 weeks), and 175 (73%) of 240 women had undetectable viral loads by this time; table 1 shows the number of women reaching this outcome, by maternal characteristics. The proportion of women achieving an undetectable viral load did not differ between treatment groups (111 [71%] of 156 women receiving PI-based regimens and 64 [76%] of 84 women receiving NVP-based HAART regimens; $P = .49$). Figure 2 displays estimated proportions of women achieving undetectable viral loads—beginning at the time of initiation of therapy—stratified by HAART category and baseline viral load. For women with a baseline HIV RNA load $\geqslant 4$ $\log_{10}$ copies/mL, 35.2% (95% CI, 20%–51.1%) of the PI group and 53.0% (95% CI, 20%–94%) of the NVP group achieved an undetectable HIV RNA load by 5 weeks, 56.4% (95% CI, 45%–71%) of the PI group and 76.4% (95% CI, 56%–94%) of the NVP group achieved an undetectable viral load by 10 weeks, and 59.4% (95% CI, 43%–755) in the PI group and 93.4% (95% CI, 66%–99.0%) of the NVP group achieved an undetectable viral load by 15 weeks, indicating a differing response by treatment category (figure 2*B*). Stratifying by race and baseline viral load, 58.7% (95% CI, 44%–75%) of non-black women and 66.3% (95% CI, 53%–79%) of black women with baseline HIV RNA loads $\geqslant 4$ $\log_{10}$ copies/mL achieved an undetectable viral load at 10 weeks, and 64.1% (95% CI, 38%–85%) of non-black women and 79.3% (95% CI, 65%–93%) of black women achieved an undetectable viral load at 15 weeks, suggesting possible race-associated differences.

RHs from univariable survival analyses adjusting for baseline viral load confirmed the race differences described above (RH for black women vs. non-black women, 1.40; 95% CI, 1.00–1.97) and were explored in further models through examination of region of birth. Table 3 shows the RHs for time from initiation of HAART to achievement of an undetectable viral load for the full-adjusted model. Twenty-three women with information missing on race or CD4 cell count were excluded from the model; these women had characteristics that were similar to those of women who were included (data not shown). Including 2-way interaction terms between any of the variables in the final model did not significantly improve the fit. The rate of women achieving an undetectable viral load in the NVP group was estimated to be almost 1.5 times than that in the PI group (table 3). Baseline viral load and being of Western African origin were also significant factors affecting the rate of achieving undetectable viral load by delivery. A sensitivity analysis including women receiving a PI-based regimen including NFV only revealed similar RHs among women receiving NVP-

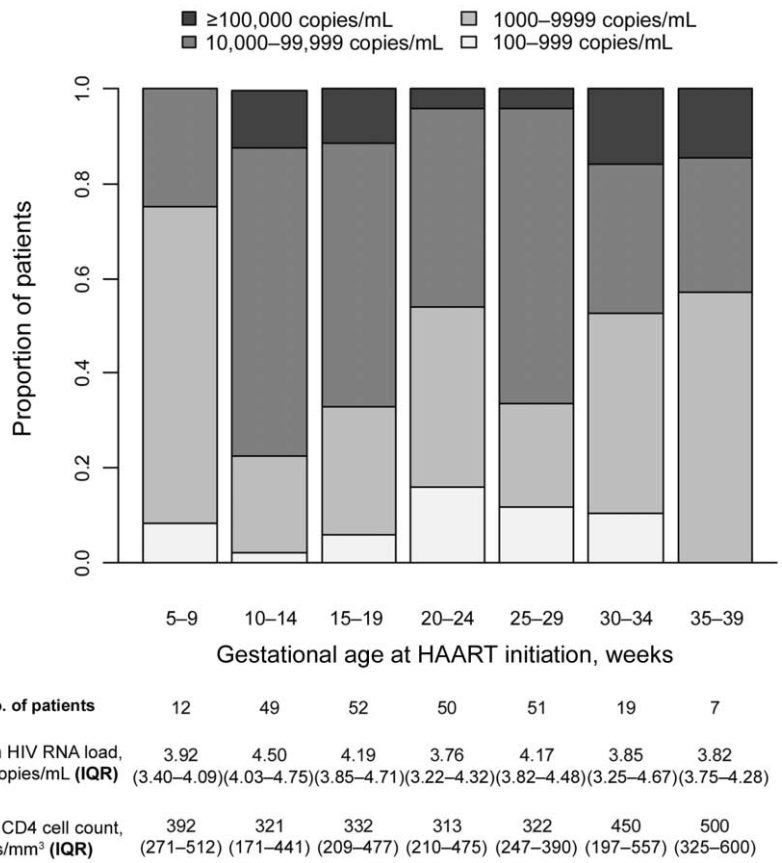

Figure 1. Distribution of baseline HIV RNA load measurements, median baseline HIV RNA load, and median CD4 cell count, by timing of initiation of HAART during pregnancy. IQR, interquartile range.

based HAART (RH, 1.56; 95% CI, 1.05–2.32), as did including only women initiating therapy in the first and second trimesters (RH, 1.82; 95% CI, 1.18–2.84).

Using the acceleration factor format, the median time to achievement of an undetectable viral load for a woman receiving PI-containing HAART was estimated to be 1.38 times (95% CI, 1.04–1.83 times) that for a woman receiving NVP-containing HAART. The predicted median time to achievement of an undetectable viral load for non-African women with baseline CD4 cell counts of 200–499 cells/mm^3 and HIV RNA viral loads of 3.81–4.39 $\log_{10}$ copies/mL who initiated treatment during the second trimester was 7.1 weeks (95% CI, 3.60–10.53 weeks) for the NVP group and 9.8 weeks (95% CI, 5.38–14.16 weeks) for the PI group; for Western African women with similar characteristics, these times were 4.4 weeks (95% CI, 2.1–6.7 weeks) and 6 weeks (95% CI, 3.2–8.9 weeks), respectively.

Viral response in 70 women eligible for NVP, according to current prescribing advice [8] (i.e., with baseline CD4 cell counts <250 cells/mm^3) was explored; the median baseline viral load was 4.35 $\log_{10}$ copies/mL (IQR, 4.05–4.69), the median duration of gestation at initiation of HAART was 22 weeks, and 16 women (24%) received NVP. The percentage of women reaching an undetectable viral load at 5 weeks was 34.8% (95% CI, 20.4%–46.7%) for those receiving PI-based regimens and 52.9% (95% CI, 22.1%–71.6%) for those receiving NVP regimens; the percentages increased to 50.4% (95% CI, 34.0%–62.8%) and 82.4% (95% CI, 50.7%–93.7%), respectively, at 8.5 weeks. In adjusted analyses, these treatment group differences were not statistically significant (RH for NVP-containing HAART, 1.89; 95% CI, 0.87–4.12).

The 65 women (27%) who delivered with a detectable viral load were similar to those achieving undetectable viral loads with regard to regard to race and type and timing of HAART (data not shown). However, more of the women with detectable viral loads at delivery were severely immunosuppressed (defined as a CD4 cell count <200 cells/mm^3; 19 [33%] of 58 women with a detectable viral load vs. 29 [18%] of 162 women with an undetectable viral load; $P = .03$), and more of these women had baseline viral loads >5 $\log_{10}$ copies/mL (10 [15%] of 65 women with a detectable viral load vs. 10 [6%] of 175 with an undetectable viral load; $P = .03$); the median viral load at delivery for women with a detectable viral load was 2.48 $\log_{10}$ copies/mL (IQR, 2.26–3.11), and only 20 of these women (31%) had a viral load >1000 copies/mL.

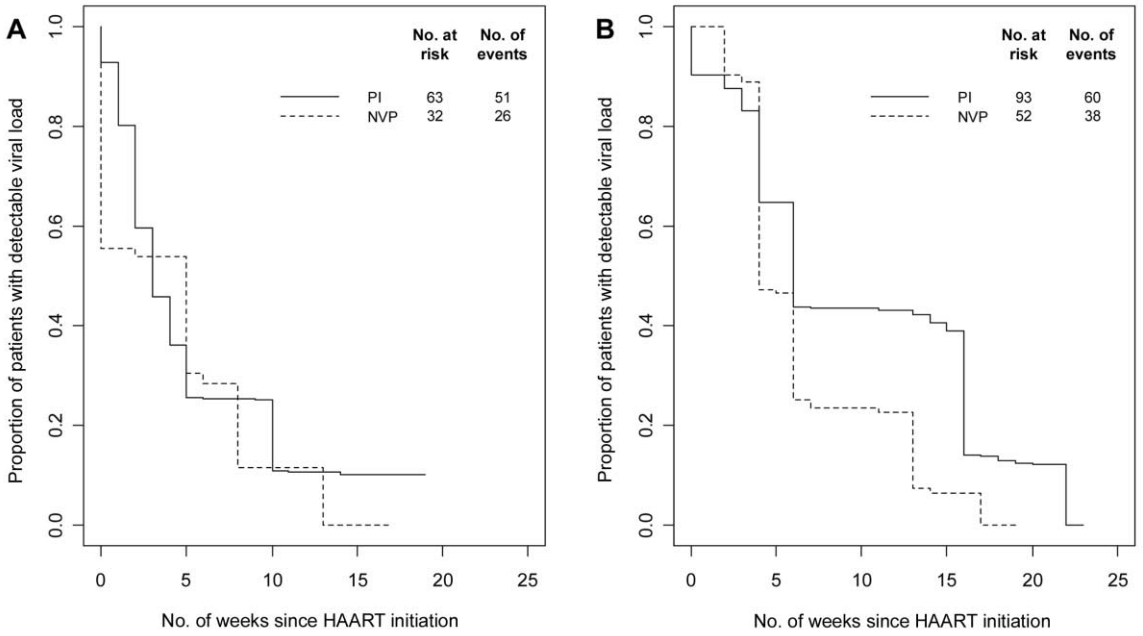

Figure 2. Survival curves for the time from initiation of HAART to achievement of an undetectable viral load, by initial treatment category: *(A),* women with a baseline viral load <4 $\log_{10}$ copies/mL, and *(B),* women with a baseline viral load ⩾4 $\log_{10}$ copies/mL. NVP, nevirapine; PI, protease inhibitor.

DISCUSSION

Suppressing plasma HIV RNA load below detectable limits is one of the goals for effective treatment of HIV-infected women during pregnancy and for prevention of MTCT [9, 10]. In our study, most pregnant women (73%) initiating HAART antenatally delivered with an undetectable viral load, and the remaining women delivered with a detectable but generally very low viral load. Less than one-quarter of the women had immunological indications for treatment [9, 10]. Most women were prescribed PI-containing HAART, with a highly homogenous approach, with 76% of these women receiving a combination of NFV, zidovudine, and lamiduvine. NFV has the most extensive data on pharmacokinetics and safety during pregnancy among all of the PIs and is currently preferred for use in antenatal HAART, especially in patients in whom there are no maternal indications for treatment [11]. One-third of women received NVP-containing HAART, with increasing use over time; this trend is unlikely to continue because of updated NVP prescribing advice [8]. The predominance of zidovudine- and lamiduvine-containing regimens in our study reflects current recommendations for this nucleoside reverse-transcriptase inhibitor combination to be the backbone treatment for pregnant women [10, 11] and is consistent with prescribing patterns in Europe for nonpregnant individuals [12].

To our knowledge, this study is the first to suggest that choice of initial HAART regimen has implications for timely achievement of undetectable viral load during pregnancy. Adjusting for baseline prognostic factors, the hazard of achieving an undetectable viral load was greater for women receiving NVP-containing HAART, with women in the PI group requiring an average of 1.4 times longer to achieve viral suppression. Findings conflict with regard to the relative effectiveness of PI-containing versus NNRTI-containing HAART regimens in nonpregnant adults. A recent direct meta-analysis of "head-to-head" randomized trials suggested that NNRTI-based HAART (predominated by efavirenz) was 60% more effective for virological suppression than was PI-based HAART (50% boosted PIs), although no difference in clinical outcomes was reported [13]. However, an indirect meta-analysis yielded contradictory results (i.e., NNRTI-based HAART was less effective than PI-based HAART for virological suppression [13]); these discordant results may be a result of differences in population, study design, or type of nucleoside reverse-transcriptase inhibitor backbone, highlighting the difficulties of translating trial findings into clinical recommendations and the importance of direct comparisons [13].

Therapeutic decision-making during pregnancy is complicated by unique factors, including the need to consider prevention of MTCT, safety and toxicity, and physiological changes, which may affect pharmacokinetics [8, 14]. Accumulating data on NFV pharmacokinetics suggest that drug levels during the third trimester may frequently be subtherapeutic [15–17]. This may explain why we found a superior virologic response with NVP-containing versus PI-containing (mostly

Table 3. Factors associated with time to achieving undetectable HIV RNA load after initiation of HAART during pregnancy among 217 study women.

Variable	No. of patients	Univariate analysis		Multivariable analysis	
		RH (95% CI)	*P*	RH (95% CI)	*P*
Region of birth					
Non-African	96	1.00		1.00	
Eastern Africa	42	1.15 (0.73–1.83)	.55	1.61 (0.84–3.11)	.15
Central Africa	33	1.58 (0.96–2.60)	.07	1.24 (0.70–2.21)	.46
Northern or southern Africa	7	0.82 (0.31–2.13)	.68	1.25 (0.38–4.07)	.72
Western Africa	39	1.58 (1.0–2.50)	.05	1.90 (1.16–3.12)	.01
HAART regimen					
PI-based	141	1.00		1.00	
NVP-based	76	1.62 (1.14–2.31)	<.01	1.54 (1.05–2.26)	.02
Baseline HIV RNA load, $\log_{10}$ copies/mL[a]					
⩾4.40	72	1.00		1.00	
3.81–4.39	73	1.75 (1.15–2.66)	<.01	1.70 (1.08–2.68)	.02
<3.81	72	2.54 (1.69–3.80)	<.001	2.76 (1.68–4.52)	<.001
Baseline CD4 cell count, cells/mm^3					
<200	47	1.00		1.00	
200–499	123	1.39 (0.89–2.15)	.14	1.25 (0.69–2.24)	.46
⩾500	47	1.42 (0.83–2.43)	.20	1.40 (0.71–2.76)	.33

NOTE. Univariate and multivariable estimates were adjusted for baseline viral load. Multivariable estimates were adjusted for all covariates listed in the table, with the addition of the treatment propensity score and trimester of initiation during pregnancy. NVP, nevirapine; PI, protease inhibitor; RH, relative hazard.

[a] Baseline HIV RNA load was categorized according to their tertiles.

NFV) HAART—in contrast to the Combine Study, in which an equivalent response was reported among ART-naive nonpregnant individuals randomized to a zidovudine and lamiduvine backbone with NVP or NFV [18].

African and non-African pregnant women had similar baseline immune and virological status—in contrast to previous findings based on the whole cohort [4]—probably reflecting eligibility criteria for this analysis. The median baseline CD4 cell count among black women in our study was marginally lower than those reported in African prevention of MTCT trials (335–363 cells/mm^3 among ART-naive pregnant women) [19, 20]. Univariably, black women in our study responded to HAART more favorably than did non-black women; further investigation, stratifying by region of birth, revealed that this effect was limited to women of western African origin. Limited information is available regarding response to HAART among African populations, and even less is available for pregnant African women. In the Drug Resource Enhancement against AIDS and Malnutrition pilot in Mozambique, 26 (65%) of 40 pregnant women starting HAART, with a median baseline HIV RNA load of 4.2 $\log_{10}$ copies/mL, achieved viral suppression (viral load, <400 copies/mL) by delivery after an average of 12 weeks [21]; these data are consistent with our results. An impact of race on disease progression or response to HAART has been suggested by several studies of pregnant and nonpregnant individuals, which generally revealed poorer virological responses among black and/or African groups; these findings were suggested to be a result of coinfections or adherence [22–24]. We did not have adherence data available, but it seems unlikely that differing adherence levels could explain our findings, because the better virological response to HAART was limited to the western African group only. Little is known about the impact of different HIV subtypes on the effectiveness of HAART [25, 26]. Differences in underlying maternal subtype may possibly explain our findings, although host biological and genetic differences may also play a part [27].

We found no significant difference in time to attaining undetectable viral load between severely immunosuppressed women and those with greater immunocompetence. This is consistent with other studies that have found a significant association between baseline viral load and subsequent virological response after HAART initiation but not an association between baseline CD4 cell count and virological response [28, 29]. Few studies have examined the latter association among ART-naive pregnant women, in whom treatment effect and the relationship between baseline CD4 cell count and viral load may differ from that in nonpregnant adults [30, 31].

Our data are limited by their observational nature [32]; however, we allowed for interval-censoring [5], adjusted for timing of initiation of therapy during pregnancy, and minimized confounding by ART experience through our selection criteria. Additionally, we used a treatment propensity score to reduce

bias in the comparison of a treatment group to a nonrandomized control group [6]. We could not account for additional factors potentially influencing response to HAART, such as adherence, biological differences in drug activity arising from variations in body weight and pharmacokinetics between groups, HIV subtypes, and other genetic factors [14, 33, 34]. A disadvantage of cohort data is their limited contemporary relevance when therapeutic practices have changed over time. A case-in-point is the NVP prescribing changes after the association of the drug with hepatotoxicity in women with moderate to high CD4 cell counts [8]. If clinicians comply with prescribing advice, one would expect the future group of ART-naive women starting NVP-containing HAART to have lower CD4 cell counts than the women in our study. However, our subanalysis of women with CD4 cell counts <250 cells/mm^3 indicates that our results may be generalizable but had limited statistical power.

Although guidelines state that pregnancy should not preclude use of optimal ART regimens [11], in reality, there are limited options. For nonpregnant adults, NNRTI-containing HAART is recommended as a first-line regimen, preserving PI-containing HAART for later treatment, with efavirenz as the preferred agent. Because efavirenz is contraindicated during the first trimester of pregnancy, NVP-containing HAART has been increasingly used for ART-naive pregnant women in Europe, but this is no longer recommended for women with relatively good immune functioning. The potential option of initiating efavirenz-based HAART during the second or third trimester, if contraception can be assured after delivery, has been suggested in current World Health Organization recommendations for resource-limited countries [35], but whether this approach will be used in Europe is uncertain.

To date, NFV has been the overwhelming choice to accompany zidovudine and lamivudine in the treatment of ART-naive women in our study, but was less effective with regard to virological suppression than NVP-containing HAART. Boosted PI regimens appear to offer superior virological suppression in ART-naive adults, compared with PI alone [36], and lopinavir and ritonavir combination therapy is identified as a preferred PI regimen for initial HAART during pregnancy in current US guidelines—albeit, with limited pharmacokinetic and safety data [11, 37]. As more information becomes available, including boosted PIs in initial HAART regimens during pregnancy may become increasingly common, and the question of the equivalence of such regimens to other HAART regimens warrants investigation (in addition to research of new agents, such as integrase inhibitors). In the absence of clinical trials of HAART among pregnant women, our findings add to the evidence base to assist therapeutic decision-making for ART-naive, HIV-infected pregnant women. Our results strongly suggest that an ART-naive pregnant woman with a CD4 cell count <250 cells/mm^3 should begin receiving NVP-containing HAART rather than NFV-containing HAART. In addition, our results highlight the urgent need for further research of the pharmacokinetics, efficacy, and safety of ART during pregnancy.

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HIV-infected pregnant adolescents and youth: results from a European cohort study

EUROPEAN COLLABORATIVE STUDY*

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Abstract
Globally the HIV epidemic mainly affects young people, particularly young females who are vulnerable to acquisition of HIV as a result of their biological and social susceptibility. Women represent an increasing proportion of newly diagnosed HIV cases in Western and Eastern Europe, reaching 35% and 40%, respectively, in 2004. In the European Collaborative Study (ECS), HIV-infected pregnant women are enrolled and their infants followed-up prospectively. By the end of 2005, 5956 women had enrolled, of whom 1912 (32%) were aged<25 years at delivery. Enrolments of youth declined in Western European centres from 59% in 1985 to 18% in 2005. In Ukraine, youth enrolments declined from 52% in 2001 to 43% in 2005. Median ages of the young and older sub-cohorts were 22.3 and 29.9 years, respectively. Injecting drug use (IDU) was more common in the older than younger sub-cohorts [42% (n=1684) vs. 35% (n=675), p<0.001]. However, young IDUs were more likely to be currently using injecting drugs than older IDUs (59% vs. 38%, p<0.001), to report current sharing of injecting equipment (22% vs. 13%, p=0.001) and to report an IDU sex partner (59% vs. 48%, p<0.02). Young HIV-infected pregnant women in Europe are a heterogeneous group, possibly less identifiable as being at risk of HIV infection. They will have diverse needs for services during and after pregnancy, including harm reduction services and psychosocial support, in addition to a universal need for prevention of mother-to-child transmission services.

Keywords: *HIV, youth, injecting drug use, hetero sexual transmission, pregnancy*

Introduction

Nearly half the world's population are aged less than 25 years, and young people between the ages of 15 and 24 years account for half of the new cases of HIV infection each year (UNAIDS, 2006). An estimated 10 million youth (15–24 years) were living with HIV infection by the end of 2003, of whom two-thirds lived in sub-Saharan Africa. In contrast, an estimated 6% (or 600,000) were living in Eastern Europe and Central Asia and less than 1% in Western Europe.

Women are both biologically and socially more susceptible to acquisition of HIV than men (Johnson & Laga, 1988). Young women are especially vulnerable, due not only to

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their increased biological susceptibility to acquisition of infection as a result of an immature genital tract, but also because, in many settings, the patterns of sexual networking (for example, with young women commonly having older sexual partners) and gender inequalities also put them at increased risk (UNAIDS, 2006). It is estimated that young women worldwide aged 15–24 years are 1.6 times more likely than young men to be HIV-infected (UNFPA, 2005).

In Western Europe, 3.5% of the 55,103 female cases of HIV infection reported to date have been in women aged 15–19 years and 42% in those aged 20–29 years. In Eastern Europe the HIV epidemic is more recent but has accelerated rapidly, particularly in the Russian Federation and Ukraine (Hamers & Downs, 2003). In these settings, 19% of the 112,500 HIV infections to date in women have been in those aged 15–19 and 61% in those aged 20–29 years (EuroHIV, 2005).

The European Collaborative Study is an ongoing prospective study of HIV-infected women and their children. In this paper we describe the sub-group of young, pregnant HIV-infected women aged < 25 years enrolling in the study, noting trends over time and geographic patterns with regard to their sociodemographic, obstetric and HIV-disease related characteristics.

Methods

The European Collaborative Study (ECS) is a cohort study, in which HIV-infected women are identified during pregnancy and their infants followed prospectively according to standard clinical and laboratory protocols (European Collaborative Study, 2005). The ECS was set up in 1985 and includes 33 centres from 10 European countries (Spain, Italy, the United Kingdom, Germany, Belgium, Sweden, the Netherlands, Poland, Denmark and Ukraine).

HIV-infected women identified in pregnancy are invited to participate in the study, with informed consent obtained; pregnant women already known to be HIV infected as the result of earlier testing are also invited to take part. Local ethical approval has been granted. Information collected at enrolment and during pregnancy includes sociodemographic characteristics, obstetric history, mode of acquisition of HIV infection and injecting drug use (IDU) history.

Women aged less than 25 years by the time of delivery of their infant were considered as 'youth', according to the current UNAIDS definition of youth as those people falling between the ages of 15 and 24 years inclusive (www.unaids.org/youth). The small number of pregnant adolescents enrolled in the study aged < 15 years were considered together with the youth sub-group. All other women (i.e. those aged 25 years or more at delivery of their infants) were classified as the older sub-cohort, and used in specific comparative analyses. IDU was determined by self-report, the presence of drug withdrawal symptoms in the neonate and clinical observation; toxicology screening was not carried out. Women enrolling in Ukraine were classed as living in Eastern Europe and the remaining women were classified as living in Western Europe (including the 2% of women enrolled in the Polish centres).

Statistical analysis

Univariable comparisons were assessed with the χ^2 test for categorical variables. Univariable and multivariable logistic regression analysis was used to obtain unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI). Data entry was carried out

using Microsoft Access 2000 and analyses using SAS statistical software (version 9.01, SAS Institute, Cary, NC, USA).

Results

Trends in enrolment of youth over time

By the end of December 2005, 5956 HIV-infected women had enrolled in the ECS, 1912 (32%) of whom were aged < 25 years at the time of delivery. Median ages of the young and older sub-cohorts were 22.3 years and 29.9 years, respectively. The proportion of young women aged < 25 years enrolling in the study declined substantially in the Western European centres over the study period (Figure 1), from nearly 60% in 1985 to a plateau of 12–14% in 1999–2002; however, since then the proportion of young women enrolling began to increase, reaching just under a fifth of enrolments in 2005. In the centres in Ukraine, the rate of youth enrolments gradually declined since enrolments began in 2000, but the absolute level was substantially higher than that in the Western European centres, at around 40–50% of total enrolments.

Characteristics of young sub-cohort

Most (1646, 86%) of the younger cohort were white, 209 (11%) were black (186 of whom were born in Africa) and 29 (3%) of other ethnic groups. Just over half the youth sub-cohort (1111, 58%) were living in Western Europe and 801 (42%) in Ukraine, whereas a greater proportion of the older sub-cohort lived in Western Europe 78% ($n=3078$, $\chi^2=211.6$, $p<0.001$). Figure 2 shows the most likely mode of acquisition of HIV infection of 1481 women with identified transmission risk factors. The remaining 431 women

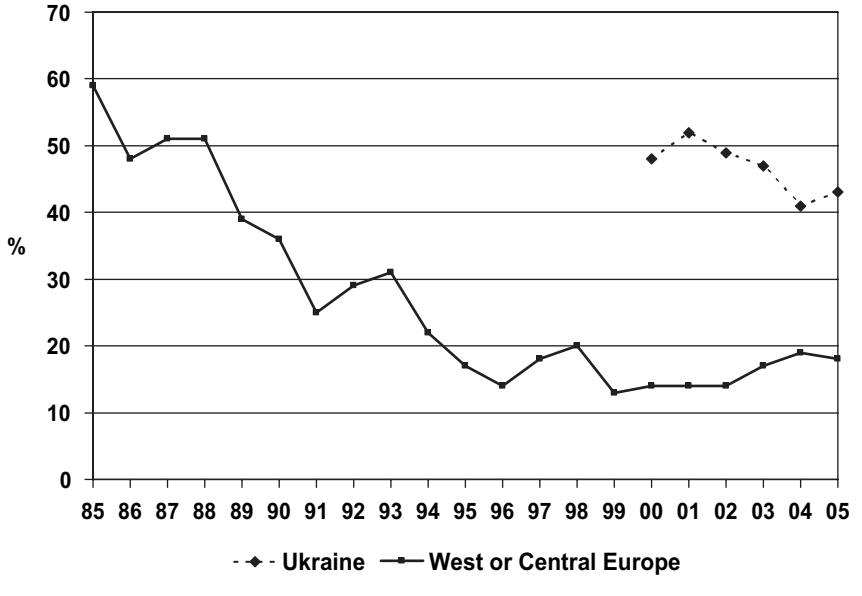

Figure 1. Trends over time in enrolment of youth (as a percentage of total new enrolments in the cohort), by geographic area.

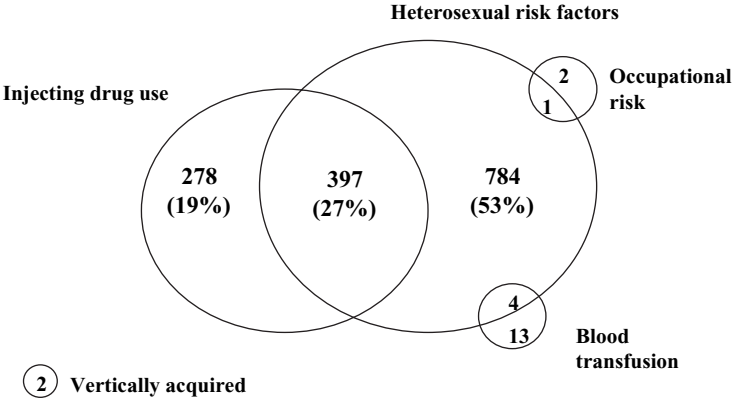

Figure 2. Most likely mode of acquisition of HIV infection in 1481 young women with identified risk factors.

reported no specific risk factors for acquisition of HIV infection – they did not report current or past use of injecting drugs, having an IDU or other high-risk sexual partner, blood transfusions or any other sexual risk (e.g. multiple sex partners or origin from an area where heterosexual transmission is common); most (95%, 409) of this group of young women were living in Ukraine, were married or cohabiting (88%, 395) and had never been pregnant previously (59%, 255).

Injecting drug use

There were 675 (35%) women with a history of or current IDU in the young sub-cohort (540 in Western Europe and 135 in Ukraine), significantly fewer than in the older sub-cohort (42%, $n=1684$, $\chi^2=21.8$, $p<0.001$). However, young IDUs had substantially more risky behaviours than older IDUs, with a higher proportion injecting illicit drugs in the current pregnancy [59% (273/460) vs. 38% (476/1251), $\chi^2=62.0$, $p<0.001$] and reporting an IDU sex partner [59% (354/605) vs. 48% (736/1525), $\chi^2=18.2$, $p<0.001$]. Of those currently using injecting drugs at enrolment, younger women were significantly more likely to report sharing injecting equipment than older women [22% (59/273) vs. 13% (64/467), $p=0.001$]. Among 288 young IDUs who were using injecting drugs at enrolment, having an IDU sex partner and not being married or cohabiting were significantly associated with an increased likelihood of sharing injecting equipment in the current pregnancy, while knowledge of HIV status before the current pregnancy was protective against this high-risk behaviour with borderline significance (Table I).

The proportion of young women with current or past IDU has declined significantly over the study period in the Western European centres, from 74% (245/396) in 1985–1989, to 48% (162/336) in 1990–1994, 26% (48/188) in 1995–1999 and 14% (37/257) in 2000–2005 ($p<0.05$). The prevalence of risky sexual behaviour among young IDU women in Western Europe was relatively stable over the study period, with 58% (142/245) reporting an IDU sex partner in 1985–1989 and 54% (20/37) in 2000–2005. However, the prevalence of sharing injecting equipment declined from 30% (34/113) to 11% (1/9) in the same time period among the sub-group with current use in pregnancy and information available. In the Ukraine centres, where enrolment started in 2000, 20 (43%) of the 47 young women currently using injecting drugs reported sharing injecting equipment.

Table I. Risk factors for sharing injecting equipment in young IDU women.

	% Sharing	OR (95% CI)	AOR [95% CI], *p* value
Timing of HIV diagnosis			
During pregnancy/at delivery	24% (41/173)	1.00	1.00
Before pregnancy	14% (16/115)	0.54 (0.29–10.01)	0.53 (0.27–10.1), 0.054
IDU sex partner			
Never	9% (7/75)	1.00	1.00
Ever	23% (50/213)	3.00 (1.30–6.95)	2.73 (1.16–6.38), 0.02
Marital status			
Married or cohabiting	15% (27/178)	1.00	1.00
Single or divorced	27% (30/110)	2.20 (1.23–3.93)	2.12 (1.16–3.87), 0.01

Non-IDU youth with identified sexual risk factors

There were 789 young women without a history of IDU who had sexual risk factors for acquisition of HIV infection (Figure 2). A total of 185 (23%) were refugees or migrants from Africa (primarily sub-Saharan Africa); origin in Africa was considered a risk factor for acquisition of HIV infection, although no information was available on when these women arrived in Europe from Africa. Of the remaining 604 women, the most common risk factor was having an IDU sexual partner (399, 66%).

Identification in relation to pregnancy

Overall, young women were less likely to know of their HIV infection status before pregnancy than older women [31% (599/1912) vs. 53% (2143/4044), $\chi^2=244.6$, $p<0.001$], with the majority of youth identified through testing during pregnancy ($n=1083$) or intrapartum ($n=230$). This age-related finding applied within risk groups; for example, 46% (308/675) of IDU youth had been diagnosed before pregnancy compared with 78% (1097/1683) of older drug-using women ($\chi^2=76.4$, $p<0.001$) and among the African-born women, 28% (52/184) of the young women knew of their infection before pregnancy vs. 48% (336/707) of those older ($\chi^2=22.0$, $p<0.001$). However, in the case of IDUs, this association was confounded by the increasing trend towards earlier diagnosis over time, and since 2000, 84% (36/43) of young IDUs in Western Europe have been aware of their infection before becoming pregnant.

Within the youth sub-cohort, IDUs were nearly twice as likely to have received their HIV diagnosis before their pregnancy as non-IDUs [46% (308/675) vs. 24% (284/1191), $\chi^2=94.3$, $p<0.001$]. However, this association was only of borderline significance among the sub-group of Ukrainian women [24% (32/135) vs. 17% (107/638), $\chi^2=3.63$, $p=0.057$]. Among the young women without an IDU history, the likelihood of knowledge of HIV status before the current pregnancy varied between groups. For example, 34% (125/371) of non-African women living in Western Europe knew of their HIV status before their pregnancy compared with only 17% (107/638) of non-IDU women in Ukraine.

Discussion

The proportion of young HIV-infected women enrolling in the Western European centres of the ECS has declined over time. As only pregnant women are eligible to enrol, this may partly reflect the ageing of the antenatal population in Europe over the study duration

(Goldstein et al., 2003). In Ukraine, the proportion of youth enrolments is substantially higher than in study centres in Western Europe, at just under a half of total enrolments; this is due most probably to the HIV epidemic in Ukraine predominantly affecting youth, with 80% of reported HIV infections among individuals aged less than 30 years, twice that in Western Europe (UNAIDS 2004), in addition to the younger age at initiation of childbearing in Ukraine (Goldberg et al., 2001).

In our cohort, just over a third of young HIV-infected women enrolling were past or current IDUs, with most of these women enrolled in the earlier years of the study. Our finding that young IDUs were more likely to report risky behaviours than older IDUs appears to reflect partly their timing of enrolment, as more young IDUs were enrolled at a time when such behaviours were more prevalent, prior to the implementation of harm reduction activities in Western Europe, including needle and syringe exchanges, voluntary counselling and testing (VCT), drug substitution programmes and outreach education (Des Jarlais & Friedman, 1988; Hunter et al., 2000; Amundsen et al., 2003). However, other studies have identified youth as being particularly vulnerable to risk-taking behaviours in relation to both sex and injecting drug use (Fennema et al., 1997; Herlitz & Ramstedt, 2005; Krall et al., 2000; Smith et al., 2005). O'Connell and colleagues reported that being female, being young and having fewer years experience of injecting drugs were significantly associated with requiring help with injecting, which itself was an independent predictor of HIV seroconversion (O'Connell et al., 2005). Among the IDU youth here, sharing of injecting equipment has declined substantially over time in Western Europe, indicating that harm reduction messages have reached at least some of this group of young women and their injecting partners. It was more difficult to investigate trends in Ukraine, as enrolment has been over a shorter period.

Among the young IDUs in this study, those most vulnerable to risky injecting behaviour, as indicated by current injecting equipment sharing, were those unaware of their HIV infection status before pregnancy, those having sex with other IDUs and those who were single. There is evidence from elsewhere that IDUs who are aware of their HIV status have less risky injecting behaviours than other IDUs, which is consistent with our finding (Wood et al., 2001). There is increasing evidence of gender differences with regard to risk behaviours among IDUs, for example, with women more likely to share injecting equipment than men, more likely to need help injecting and more likely to have multiple sexual partners as a result of exchanging sex for drugs or money to buy drugs (Kyrychenko & Polonets, 2005; Montgomery et al., 2002; O'Connell et al., 2005); these findings indicated that gender-specific strategies for harm reduction may be appropriate and possibly more effective than other approaches.

Being the sexual partner of an IDU and not consistently using condoms are well-recognized risk factors for sexual acquisition of HIV, and compounds the existing risk of parenteral acquisition in women who themselves inject drugs (Estebanez et al., 2000; Wilson et al., 1999). Here, the proportion of young IDU women who had sexual partners who also injected drugs remained relatively stable over time. We did not collect information on sexual practices, including condom use, but the fact that this is a cohort of pregnant women indicates that unprotected sexual intercourse has occurred. Other research has indicated that sexual risk-taking among IDUs is often more resistant to change than injecting behaviours (White et al., 1993) and given the reported reductions in injection equipment-sharing here, it could be hypothesized that young IDUs in the more recent years of the study were more likely to have acquired infection sexually than parenterally. These findings reiterate the importance of delivering harm reduction messages to young IDUs that encompass both sexual and injecting behaviours.

The role and impact of 'bridging populations' between high-risk groups such as IDUs and sex workers and the general population is critical in the evolution of HIV epidemics (WHO/UNAIDS, 2000). In our study population, the impact of IDU on the acquisition of HIV was considerable among young women with no IDU history as a result of sexual contact with male IDU; for example, two-thirds of non-African, non-IDU women here reported having an IDU partner. We found that young women who were sexual partners of IDUs but who did not inject drugs themselves were no more likely to know of their HIV status before pregnancy than non-IDU women who did not report an IDU sex partner. This could be interpreted in a number of ways – for example, the former group may not have known that having sexual intercourse with an IDU put them at risk of HIV infection and had therefore not sought HIV testing previously; alternatively, they may not have accessed VCT services, despite an appreciation of the risk, for a variety of personal and/or structural reasons.

There is evidence to show that consistent condom use by IDUs is usually less common with their primary sexual partners than with casual sexual partners (Rosengard et al., 2004), and thus women with IDU sex partners are at risk from IDUs' risk behaviour with both sexual and injecting partners. In a nested study within the ECS, investigating the prevalence of and risk factors for sexually transmitted infections, HIV-infected pregnant women who reported having had an IDU sex partner were at a three-times increased risk of bacterial sexually transmitted infections (STIs) than those with no previous sexual contact with an IDU (European Collaborative Study, 2006).

Nearly a quarter of young women in our study did not report any specific risk factors for HIV acquisition, most of whom were living in Ukraine. Most women probably acquired infection heterosexually through unprotected sexual intercourse with casual or regular partners, including their husbands (88% were married or cohabiting), but were unaware that they had been exposed to HIV. Although social desirability bias may have resulted in some under-reporting of risk factors, our finding is consistent with the rapidly evolving epidemic in Ukraine and the shift towards a generalized epidemic with at least 1% of the population estimated to be HIV infected, with most infections concentrated in the youngest age groups (UNAIDS, 2004); furthermore, 48% of new HIV infections in women in Eastern Europe in 2004 reported to the European Centre for the Epidemiological Monitoring of HIV/AIDS were in the 'other/undetermined' category (EuroHIV, 2005).

Regarding the timing of identification of HIV infection, our results are reassuring with regard to young IDUs enrolling in Western Europe. Although initial findings indicated that young IDUs were less likely to know their HIV status than older IDUs, over time there was an improvement in ascertainment of infection status among all IDUs regardless of age. This suggests that access to VCT in the group of drug-using young women is relatively good (although the observation is limited by a relatively small sample size). Knowledge of HIV infection status prior to pregnancy among IDU youth in Ukraine was substantially lower than in the Western European centres, with only 25% of IDUs enrolling since 2000 diagnosed prior to pregnancy; scaling-up VCT in Ukraine, particularly among vulnerable groups, is a priority of the Ukraine HIV programme.

UNAIDS has called for an intensification of HIV prevention worldwide, with a focus on prevention among young people one of the essential programmatic actions required (UNAIDS, 2005). This is in line with the UN General Assembly Special Session on HIV/AIDS youth-specific targets, including to ensure that by 2005 at least 90% by 2005, and by 2010 at least 95% of young men and women aged 15–24 have access to the information, education and services necessary to develop the life skills required to reduce their vulnerability to HIV infection. Our results not only highlight the need for primary prevention and

harm reduction services targeted at youth already using injecting drugs and those at risk at initiating IDU, but also at other groups with high-risk sexual behaviours. Young HIV-infected pregnant women in Europe, and those vulnerable to acquisition of HIV as a result of their own behaviour and that of their partners, are a relatively heterogeneous group and will have diverse needs for services during and after pregnancy, including harm reduction services and psychosocial support, in addition to a universal need for services for prevention of mother-to-child transmission of HIV infection.

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CHAPTER 3.
TOXOPLASMA GONDII INFECTION

3.1. Scientific background: toxoplasmosis in pregnancy

Toxoplasma *gondii* is a common parasitic infection acquired by ingesting oocysts excreted by cats and contaminating soil or water, or by eating tissue cysts which remain viable in undercooked meat of infected animals (1). The life cycle of T. gondii cysts consists of two stages asexual and sexual: the asexual takes place in the intermediate hosts (mammals and birds), the sexual stage takes place in the intestine of the definitive host (members of feline family, predominantly domestic cats).

T. gondii has been shown to migrate over long distances in the host's body; crossing biological barriers, actively enter the blood stream, invade cells and cross substrates and non-permissive biological sites such as the placenta.

Clinical manifestations and severity of illness following infection are affected by features of the interaction between the parasite and the host and include strain virulence, inoculum size, route of infections, competence of the of the host's immune response, integrity of the host's mucosal and epithelial barriers, host's age and genetic background. In particular genetic background plays a significant role in increasing susceptibility to T. gondii in humans: for example HLA-DQ3 appears to be more frequently associated with toxoplasma-dependent encephalitis. Thus in this area, further research is mandatory to clear the role of genes on foetal susceptibility to intrauterine infection (2).

The rates of positive seroprevalence in women at child-bearing age in the period 1990-2000 were 58% in Central European countries, 51-72% in several Latin-American countries and 54-77% in West African countries (3). Low seroprevalence, 4-39%, was reported in southwest Asia, Chine and Korea as well as in cold climate areas as Scandinavian countries. It should be noted that seropositive prevalence in the same country may differ among populations or geographical regions.

Congenital toxoplasmosis

Congenital infection occurs only when a woman becomes infected during pregnancy. The prevalence of T. gondii and its incidence of human infection vary widely amongst various countries. Worldwide, 3-8 infants per 1000 live births are infected in utero. Multiple factors are associated with the occurrence of congenital toxoplasmosis (CT) infection, including route of transmission, climate, cultural behaviour, eating habits and hygienic standards. This combination leads to marked differences even among developed nations.

Only a few cases of CT transmitted by mother who were infected prior to conception have actually been reported (4-6). Such cases could be attributed to re-infection with a different, more virulent strain or by reactivation of a chronic disease.

Chronically infected women, who are immunodeficient, may also transmit the infection to their foetuses; the risk of this occurrence is difficult to quantify, but it is probably low. Latent T. gondii infection may be reactivated in immunodeficient individuals (such as HIV-infected women) and result in MTFT of the parasite.

The risk of foetal infection is multifactorial, depending on the time of maternal infection, immunological competence of the mother during parasitemia, parasite load and strain's virulence (3). The probability of foetal infection is only 1% when primary maternal infection occurs during the preconception period but increases as pregnancy progresses; infection acquired during the first trimester by women not treated with anti-T. gondii drugs results in congenital infection in 10 to 25% of cases. For infections occurring during the second and third trimesters, the incidence of foetal infection ranges between 30–54% and 60–65%, respectively (7). The consequences are more severe when foetal infection occurs in early stages of pregnancy, when it can cause miscarriage (natural abortion or death occurs in 10% of pregnancies infected with T. gondii (8), severe disease, intra-uterine growth retardation or premature birth. The likelihood of clinical symptoms in the newborn is reduced when infection occurs later. Clinical manifestations in newborns with congenital toxoplasmosis vary and can develop at different times before and after birth. Most newborns infected with T. gondii are asymptomatic at birth (70–90%). When clinical manifestations are present they are mainly non-specific and may include: a maculopapular rash, generalized lymphadenopathy, hepatomegaly, splenomegaly, hyperbilirubinemia, anemia and thrombocytopenia (9). The classic triad of chorioretinitis, intracranial calcifications and hydrocephalus is found in fewer than10% of infected infants. The most prevalent consequence of congenital toxoplasmosis is chorioretinitis. Late-onset retinal lesions and relapse can occur many years after birth (8), but the overall ocular prognosis of congenital toxoplasmosis seems satisfactory, when infection is identified early and appropriately treated. Early diagnosis and treatment are believed to reduce the risk of visual impairment.

Overall most children with CT are developmentally normal but up to four per cent die or have evidence of permanent neurological damage or bilateral visual impairment during the first years of age with a wide spectrum of clinical diseases (8,10).

Maternal serology

Toxoplasma infection in immunocompetent adults is usually asymptomatic. Consequently, infected mothers can only be detected by serological testing. Prenatal testing for toxoplasmosis involves testing of women with undetectable antibodies at their first visit. To detect infection early in pregnancy, women who are IgG and IgM positive at their first prenatal test undergo further tests (e.g. for high or rising IgG or low IgG Avidity). None of these tests reliably determines the timing of infection and most women identified will have acquired infection prior to conception and hence are unlikely to transmit the infection to the foetus. When serology alone is insufficient, other evidence for toxoplasma infection should be sought. Replacing foetal blood analysis, which is a high risk procedure for the foetus, with molecular evaluation of amniotic fluid has provided a low risk diagnosis of congenital toxoplasmosis. Polymerase chain reaction (PCR) is currently the most common molecular technique routinely used for diagnosis of toxoplasmosis, although, it has not yet been standardized. The most popular target gene for PCR diagnosis of T. gondii is the 35-fold repetitive gene B1. Different protocols influence the sensitivity and specificity of PCR assays. The specificity and positive predictive value of PCR tests on am-

niotic fluid samples is close to 100% (11,12). However, the sensitivity of these PCR tests varies and estimated, based on a large number of studies, to be 70–80%. One report showed that the sensitivity of PCR from amniotic fluid is affected by the stage of pregnancy in which maternal infection occurs: best sensitivity was detected when maternal infection occurred between 17 and 21 weeks of pregnancy (11-14). In addition, treatment with anti-toxoplasma drugs may also affect the sensitivity. However, the reliability of a PCR test performed on amniotic fluid prior to the 18th week of pregnancy requires further evaluation. It should also be noted, that testing amniotic fluid for T. gondii was found to be effective about 4 weeks following infection, which is already during the parasitemic stage in the infected mother. Therefore, PCR test should not be performed in the absence of serologic or other clinical/sonographic data indicative of infection.

Treatment

Anti T. *gondii* treatment initiation generally requires confirmatory laboratory tests in a reference centre, followed by consultation with experts.

Once maternal infection has been confirmed, treatment with spiramycin is prescribed to reduce the risk of MTCT, the woman is referred for amniocentesis for foetal diagnosis and, if foetal infection has occurred, treatment with pyrimethamine-sulfadiazine combination is prescribed to reduce impairment in the child.

Spiramycin is used to prevent placental infection; it is used in many European countries as Italy but in the US it is currently not approved by the FDA.

The combination of pyrimethamine, (adult dosage 25–100 mg/d×3–4 weeks), sulfadiazine adult dosage 1–1.5 g qid×3–4 weeks) and folinic acid (leucovorin, 10–25 mg with each dose of pyrimethamine, to avoid bone marrow suppression) is the basic treatment protocol recommended by the WHO (15) and CDC (16).

Treatment with pyrimethamine and sulfadiazine to prevent foetal infection is contraindicated during the first trimester of pregnancy due to concerns regarding teratogenicity, except when the mother's health is seriously endangered. During the first trimester sulfadiazine can be used alone.

As recently reviewed by Montoya and Liesenfeld (14), treatment protocols vary among different centres. The effectively of anti-T. gondii treatment is evaluated based on two criteria: rate of mother to child transmission and prevalence and severity of sequel. The majority of the studies are retrospective or cohort studies of various populations and case definitions. The difference in study patterns and methodologies affects the reliability and validity of the results and thus prevents issuing further recommendations.

3.2. Our Experience: Materials and methods

- **Type of study**: This is a prospective cohort study;
- **Inclusion criteria**: pregnant women with suspected infection by Toxoplasma *gondii* are enrolled and followed up;
- **Data collection for pregnant women**: Each pregnant woman enrolled in the study is assigned a unique coded serial number and this number is used to follow her and her child anonymously. Clinical and laboratory information is collected from enrolled women and during pregnancy; the amount of follow-up information depending on the trimester at which the women were enrolled and the number of routine visits they underwent during pregnancy. Initial information collected includes mother's full obstetric history, marital status, ethnic group, likely route Toxoplasma infection (eating raw or undercooked meat, contact with soil etc.), treatment details. Clinical and ultrasonographyc information (biometry, Doppler velocimentry, nuchal translucency) information is collected during pregnancy as well as every complication during pregnancy or after delivery. Serum samples from all pregnant women referred to our Department for suspected toxoplasma infection were submitted for confirmatory testing from outside sources to our laboratory. Confirmatory serologic testing includes IgG and IgM enxyme-linked immunosorbent assay (ELISA) and IgG Avidity test. Results of the toxoplasma serologic profile help to distinguish recently acquired infections, termed recent infection, from those acquired in the more distant past, termed distant infection. Toxoplasma serologic profile results, the gestational age of the patient and the results of previous serologic testing, when available, are routinely used to assess whether infection was acquired during pregnancy. When the toxoplasma serologic profile suggests an infection acquired during pregnancy, treatment with spiramycin is recommended. To establish whether the foetus is infected we recommended PCR examination of amniotic fluid at 18 weeks gestation or more and always after at least 4 weeks form maternal infection. When amniocentesis is positive for T. gondii or the parents refuses the invasive prenatal diagnosis, we recommend pyrimethamine -sulfadiazine treatment. Delivery information includes gestational age in completed weeks, birthweight in grams, the administration of therapy during pregnancy including type of therapy and dose, mode of delivery and perinatal problems. Each child is followed-up by a specific team of paediatricians up to 12 months of age and documents the child's care and treatment as well as their clinical status and evidence of disease progression. If the foetus is judged infected, he will be followed up as long as possible according to parents compliance.

3.3. The effectiveness of the screening

There are two principal motivations for maternal screening and diagnosis of foetal infection with toxoplasmosis. The first objective is to provide clinicians with information that would allow them to make optimal decisions regarding treatment. Specifically, repeated screening of pregnant women has been used as a strategy to allow early diagnosis of maternal infection. An early detection of maternal infection and prenatal diagnosis of congenital infection is also advocated for making a more optimal choice of the medications.

The second objective of prenatal testing is to provide women with the opportunity for making an informed decision regarding their pregnancy. Women can make informed decision with respect to pregnancy termination if foetal infection is confirmed and/or the foetus is found to have significant anomalies on ultrasound scan.

However there is no consensus about the most effective screening strategy or the best type of treatment. Uncertainty about the benefits of prenatal treatment (17-18) and concerns about adverse treatment effects and costs required to prenatal screening have led to diverse policies including no screening (19-20) neonatal screening and prenatal screening with monthly or 3-monthly re-testing scheduled.

So far, some authors have evaluated the effected of prenatal treatment on mother to child transmission. Wallon et al.(17) reviewed studies comparing treated and untreated concurrent groups of pregnant women with proved or likely acute toxoplasma infection. The results showed treatment to be effective in five studies but ineffective in four. Gras et al.(21) reported that the effect of prenatal pyrimethamine–sulfadiazine combination treatment on the cerebral and ocular sequelae of intrauterine infection with T. *gondii* was not beneficial. Neto et al. (22) suggest that for patients with sequelae, because of the delay in anti-toxoplasma treatment (6–14 months post diagnosis), the disease was not prevented. Gilbert et al. (23) reported the effect of prenatal treatment in 554 infected women and their offspring. In this study comparison of early versus late treatment and of combination treatment (pyrimethamine, sulfadiazine) with spiramycin or no-treatment, were all statistically insignificant. Another European multicenter study comparing transmission rates and clinical outcomes in 856 mother–infant pairs, found no significant association between the outcome and the intensity of treatment protocol in pregnancy (24). In the same way a systematic review of cohort studies based on universal screening for congenital toxoplasmosis found that among 1438 mothers and 550 infected infants there was no evidence that prenatal treatment significantly reduced the risk of clinical manifestations (25).

Bessieres et al (26) found that cases could be identified during pregnancy as well as during the neonatal period. Foulon et al (27) reviewed the measures of prevention of congenital toxoplasmosis and concluded that treatment during pregnancy significantly reduces sequelae and treatment of infected children has a beneficial effect when therapy is begun soon after birth.

In conclusion, the efficacy of anti-T. gondii treatment in pregnancy is still an unsettled matter. It is difficult to find the effect of treatment when comparing the different studies because of: different treatment regimes and timing (for

small groups of patients), the pharmacokinetics patterns of drugs (concentration in amniotic fluid and foetal CSF), patient compliance with treatment and different methodologies of follow-up in each study.

Notwithstanding these uncertainties, there are many potential risks and psychological costs associated with prenatal screening and diagnosis for CT. The possible psychological consequences include parental anxiety due to both false positive results and the uncertainties related to the prognosis of children with prenatal diagnosis of CT. False positive results are likely to have negative psychological repercussions even after the birth of an unaffected child. Moreover, true positive results in unaffected or mildly affected children may also constitute a source of long-term anxiety and parental concern.

Whatever may be the benefits of screening for maternal toxoplasmosis is discussed in the above letter published in an international journal.

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We declare that we have no conflict of interest.

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Author's reply

Although many of the panellists in our study have extensive experience with older patients in many different settings (eg, emergency, surgery, medicine, oncology, rehabilitation, and physical therapy), none was specifically a geriatrician, as Marianne Falconer and Desmond O'Neill point out.

The issue of assigning priority in terms of support for activities of daily living was discussed in great detail. Although perhaps more pervasive among older people, this issue, of course, is not restricted to them. It is of similar concern to almost all acutely ill patients, irrespective of baseline health or abilities. Thus the need to consider debility and provision of support for activities of daily living outside the hospital setting in the event of early discharge is independent of age.

Certainly, the panellists did not imply that support for activities of daily living is not important. However, one of the underlying assumptions, as detailed in our report, was that basic care would exist for those discharged to the community, and that such support would therefore be met in other settings. This tempered the assigned priority, since withdrawal of hospital-based support for activities of daily living was not, in itself, felt to result in an adverse medical outcome requiring a critical intervention.

In the next phase of the study, we will have the ability to determine whether the panellists' assigned priorities for all critical interventions were reasonably accurate, by means of empirically derived data.

I declare that I have no conflict of interest.

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Patient safety and patient error: the carer's perspective

In their Viewpoint on patient safety and patient error (Jan 13, p 158),[1] Stephen Buetow and Glyn Elwyn mention carers (caregivers) only in their opening paragraph. Although patient error has received some attention in published studies, the relevant activities and experiences of carers have almost totally been ignored. The contribution of carers to health care in the UK has been valued at £57·4 million annually[2] and medicine-related tasks have been shown to be an integral part of the wider caring role with their own associated carer-burden.[3]

Carers can undertake a wide range of medicine-related activities, each of which presents its own potential for error:[4] monitoring supplies and ordering repeat prescriptions; assisting with administration of complex regimens, frequent dosing, and multiple forms of dosage and administration; and "clinical roles", including advising on as-needed and regular medicines and management of side-effects, for which they might have limited access to, and problems interpreting, information.

Carers operate in a partnership with their care-recipient[5] and often other carers. When differing perspectives regarding the risks, benefits, and value of medicines arise, this will affect the medicine-related behaviours of carers and care-recipients—eg, dilemmas relating to the sharing or concealment of information or their control. The relationship between the patient and health professional, and issues of confidentiality and autonomy, take on a further dimension when carers are included, since patients and carers have their own independent needs and agendas.

A conceptually valid model or taxonomy of patient error must embody the dimensions and effect of caring.

I declare that I have no conflict of interest.

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Screening for toxoplasmosis in pregnancy

The Syrocot Study Group (Jan 13, p 115)[1] states that it is unclear whether prenatal antitoxoplasma treatment has any benefit. This result needs urgent confirmation since it poses the basis for a radical change in prenatal management.

Although some researchers have raised doubts about the effect of

prenatal treatment for toxoplasma infection,[2] there has never been a definitive consensus. Nevertheless, mass screening for toxoplasmosis is mandatory in some European countries, including Italy, with a consequent "cost" to both the public and patients. In theory, screening can only be done if there is a reliable and valid test to detect the disease in its preclinical stage and if advantages of interventions are clear.[3] But there is evidence that serological screening in pregnancy is not reliable.[4,5]

Between 1995 and 2006, 542 mother-child pairs were counselled in our department for suspected toxoplasmosis. Repetition of serology and reinterpretation of the serological profile (of all previous results) revealed a false-positive rate of 90% obtained in previous IgM tests and a seroconversion rate of 12% (63/542).

The rationale behind the decision to continue screening was that benefits to the fetus outweigh the consequences of a false-positive result (anxiety, invasive prenatal diagnosis, treatment of uninfected women). However, given the uncertainty about the benefits of prenatal treatment, is it ethically correct to carry on?

We declare that we have no conflict of interest.

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Early child development in developing countries

"Finally I have data to convince my Minister of Finance to invest in early child development" reported the Minister of Women and Child Development, Malawi, at a meeting held at the Institute of Child Health, London, UK, to mark *The Lancet*'s series on child development in developing countries (Jan 6–Jan 20).[1–3]

At the meeting, hosted by the Centre for International Health and Development, presenters explained that more than 200 million children younger than 5 years are not developing to their potential owing to poverty, poor health, and nutrition. Although effective interventions are available, coverage is low. Representatives from WHO, UNICEF, and the World Bank expressed a strong commitment to strengthening programmes and research to move the *Lancet* recommendations forward. Other agencies including UNESCO, the Bernard van Leer and Aga Khan Foundations, and many non-governmental organisations explained how they are putting the recommendations into practice.

The *Lancet* steering group will become the International Child Development Committee. This year, we plan to advocate for early child development programmes through presentations at meetings in Turkey, India, Spain, Venezuela, and Bangladesh and at the Society for Research in Child Development and the Pediatric Academic Societies. We will meet at the Rockefeller Foundation's Bellagio Study and Conference Center to develop implementation strategies and establish priorities in collaboration with the Child Health and Nutrition Research Initiative. We will provide guidance in assessment of existing programmes, development of new models for delivering services, and integration of child development activities into health and nutrition services. In 2 years, we will report on global progress in early child development programmes.

The International Child Development Committee comprises S Grantham-McGregor, P Engle, M Black, M Cabral de Mello, J Meeks Gardner, B Lozoff, N Ulkuer, T Wachs, S Walker, and M Young. I declare that I have no conflict of interest.

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Risk factors for adverse outcomes in developing countries

In their child development series article, Susan Walker and colleagues (Jan 13, p 145)[1] cite two of our recent publications:[2,3] "Two small Brazilian studies suggest an association between incidence of diarrhoea in the first 2 years of life and impaired cognitive performance in later childhood.[89,90] However, a larger cohort study in Peru with control for

3.5. The diagnostic procedures

The diagnostic evaluation of T. gondii is part of routine pregnancy follow-up and differential diagnosis of intrauterine infection. Intrauterine ultrasonographic findings of T. gondii infection are usually non-specific and in most cases no pathological evidences are revealed. In certain cases the ultrasonographic findings may include: intracranial calcifications, echogenic streaks, microcephalus, ventricular dilatation and hydrocephalus (1). Gay-Andrieu et al (2) described two cases of intrauterine infection in which the diagnosis was based upon hydrocephalus in foetal ultrasound, even though PCR of amniotic fluid was negative in both cases. Additional ultrasonographic findings may include hepatomegaly, splenomegaly, ascitic fluid, cardiomegaly and placental abnormalities (3).

Safadi et al (4) followed 43 children with congenital toxoplasmosis for a period of at least 5 years. Most of them (88%) had sub-clinical presentation at birth. The most common neurological manifestation was a delay in neuropsychomotor development. Half of the children developed neurological manifestations, 7 children had neuroradiologic alterations in skull radiography, and 33 children in tomography. Notably, cerebral calcifications were not associated with an increased incidence of neurological sequelae. Chorioretinitis was the main ocular sequelae, found in almost all children and noted years after birth, despite specific therapy in the first year of life.

Starting from our experience in foetal ultrasound, we have analysed the efficacy of targeted ultrasonographic examinations to predict the effect of foetal infection by T. gondii. As reported above, this paper was presented as an oral communication at the World Congress on Ultrasound in Obstetrics and Gynecology.

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Abstract preview

The role of targeted fetal ultrasound in women with acute toxoplasmosis in pregnancy

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Objective: To assess the role of targeted fetal ultrasound in pregnant women with acute toxoplasmosis.
Methods: 187 pregnant women with seroconversion or fourfold increase of IgG antibody titer for Toxoplasma gondii in 2 weeks represent the study population. All women underwent monthly ultrasound monitoring; amniocentisis was proposed for the PCR test, treatment with spiramicin or pirimetamine/sulfadiazine according to the risk was planned, unless the development of adverse effects of therapy. Ultrasound examinations were carried out by expert operators looking for lesions/ signs possibly related to fetal infection. In all cases, gestational age at referral, at seroconversion and at the ultrasound evidence of fetal lesions, type of maternal treatment, result of PCR, feto-neonatal outcome were analysed.
Results: Prenatal ultrasound showed one or more pathological features in 7/187 (3%) cases (ventriculomegaly 4/7; splenomegaly 2/7, intracranial calcifications 2/7). Median time to evidence of the first abnormal finding was 15 weeks (range 2-29). In no case, was termination of pregnancy considered because of the advanced gestational age at ultrasonographic diagnosis. At birth, 18/187 neonates resulted infected (10%). As for the outcome, there were 186 livebirths (1 twin), 1 intrauterine death and 1 neonatal death. In both deaths, ultrasonography had shown more than one sign of infection. All neonates with negative prenatal ultrasound had a normal development, all the remaining 5 neonates with abnormal fetal ultrasound were infected (median follow-up time 58 months, range 5 months - 11 years).
Conclusion: Our results demonstrate that, in fetuses at high risk of congenital infection (CT), ultrasound may have higher prognostic significance than amniocentesis. In fact, in this series a negative PCR could not rule out CT. In addition, we have confirmed the high specificity of ultrasound though the small numbers relatively reduce the significance of this finding.

CHAPTER 4
FOETAL MALFORMATIONS: DIFFERENTIAL DIAGNOSIS

4.1 Introduction

Major congenital malformations occur in 2–4% of all live births. Up to 15% of all diagnosed pregnancies will result in foetal loss. The cause of these adverse pregnancy outcomes is understood in only a minority of the incidents. In particular, ultrasound is commonly used during pregnancy to recognise findings that may be indicative of foetal infection. If foetal infection is suspected, suggestive sonographic findings should be sought. Although not specific, common sonographic findings of foetal infection include ventriculomegaly, intracranial calcification, hydranencephaly, microcephaly, cardiac anomalies, hepatosplenomegaly, echogenic bowel, intraabdominal calcifications, hydrops, placentomegaly, IUGR and abnormal amniotic fluid volume. It is important to note that most affected foetuses with infection appear sonographically normal. Although a normal anatomic survey can be reassuring, it cannot predict a normal anatomic outcome. Findings also change or resolve over time so serial scanning can be very important.

The recognition of minor or major foetal anomalies is of crucial importance in the prenatal era because it may lead to one of the subsequent scenarios:

1. The couple opt for pregnancy termination,
2. The baby is delivered in a high specialized centre with specific ability for the disease,
3. The baby takes advantage of intrauterine therapy or surgery,
4. The couple go on with the pregnancy and is prepared to welcome a baby affected by a health problem.

In ancient times congenital abnormalities were only apparent at birth, on the contrary with the advent of obstetric ultrasound the full range of foetal disease could be diagnosed and this led to the concept of the foetus as a patient. Over the last 20 years obstetric ultrasound has revolutionised the clinician's approach to the foetus and we have learnt an enormous amount about foetal physiology and also the natural history of foetal malformations and foetal disease. Using this information an accurate prenatal diagnosis can be made and this diagnosis together with the likely prognosis can be conveyed to the parents.

For the parents the diagnosis of a foetal abnormalities is usually an unexpected and shattering experience. The approach to the parents is now truly multidisciplinary involving many different clinicians including obstetricians, radiologists, paediatricians, paediatric surgeons and clinical geneticists. In this way each expert in his or her field can help to give the parents as much information as possible.

It is clear therefore that obstetric ultrasound has come a long way from the early days of static scanners and has also introduced the possibility of foetal therapy.

During the years we observed many cases of different foetal malformations. Some of these were not related to foetal infection but, because of their atypical features, were considered for publications.

Above a select list of papers published in international journals is reported.

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SHORT COMMUNICATION

Early prenatal diagnosis of concordant posterior urethral valves in male monochorionic twins

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The association between monozygotic twins and posterior urethral valves (PUV) in postnatal life has been thoroughly described. In the fetus, the prenatal recognition of PUV is feasible. However, it has been repeatedly reported in singletons but never in monochorionic twins. We describe two cases of early prenatal diagnosis of concordant PUVs in monochorionic twins. In one of the sets, the expression of the disease was different for each twin. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: posterior urethral valves; prenatal diagnosis; monochorionic twins; prune-belly syndrome

INTRODUCTION

The association between posterior urethral valves (PUV) and monozygotic twins is well established in the pediatric literature, with at least 13 cases reported so far (Morini *et al*., 2002). In the fetus, both the complete and the incomplete form of PUV can be diagnosed prenatally by ultrasound on the basis of a distended bladder, severe hydronephrosis and oligohydramnios. Although the ultrasound diagnosis of PUV has been reported as early as the 13th week of gestation in singletons (Oga *et al*., 1994), the prenatal recognition of PUV in twins has yet to be described.

We report the early prenatal diagnosis of concordant PUV in two sets of male monochorionic twins.

CASE REPORTS

Case 1

A 31-year-old Caucasian woman, in her first pregnancy, was referred to our unit at 14 weeks' gestation owing to a suspected case of PUV in one twin of a monochorionic diamniotic pregnancy. Family history was negative for congenital anomalies and for twinning. On ultrasound, we confirmed the monochorionicity of the pregnancy, but detected anomalies in the urinary tracts of both twins. In particular, one twin showed complete PUV: an extremely distended and ruptured bladder with urinary ascites (Figure 1A) and severe bilateral hydronephrosis with hyperechoic dysplastic kidneys. In addition, the urinary ascites acted as a contrast medium, enabling us to detect the extremely reduced thickness of the abdominal wall. The other twin was diagnosed with an apparently less severe form of PUV: moderately distended bladder with evidence of the bladder neck, bladder wall thickening, severely dysplastic hydronephrotic kidneys (Figure 1B), and distended distal urethra (Figure 1C). The amount of amniotic fluid was only moderately reduced in both sacs owing to the early gestational age. No other malformations were detected. Karyotyping by CVS was proposed but was declined by the patient. During the prenatal counseling session, the couple was informed of the type of malformation and of its different manifestation in each twin. An assessment of fetal renal function by repeated cystocentesis was proposed to be carried out in the least affected twin in order to assess the renal function (Evans *et al*., 1991; Nicolini and Spelzini, 2001), but the couple refused the procedure and opted for termination of pregnancy. The pathology report confirmed the prenatal diagnosis of PUV in both twins (Figure 3A,B). The twin with the severest form had developed prune-belly syndrome (PBS) (Figure 3A).

Case 2

A 35-year-old woman, gravida 2,1,0,0, was referred to our unit at 15 weeks of gestation with a suspected case of megacystis in both fetuses of a monochorionic diamniotic pregnancy. On ultrasound, both fetuses showed signs of complete PUVs: both had severely distended bladders with evidence of the bladder neck (Figure 2) and hyperechoic dysplastic kidneys. These findings led us to suspect the development of secondary PBS in both twins. The karyotype, obtained by CVS, showed a 46,XY complement. In this case as well, after a thorough prenatal counseling session, the couple opted for termination of pregnancy. The necropsy confirmed the prenatal diagnosis of PUVs. Both the specimens at birth showed secondary PBS (Figure 3C).

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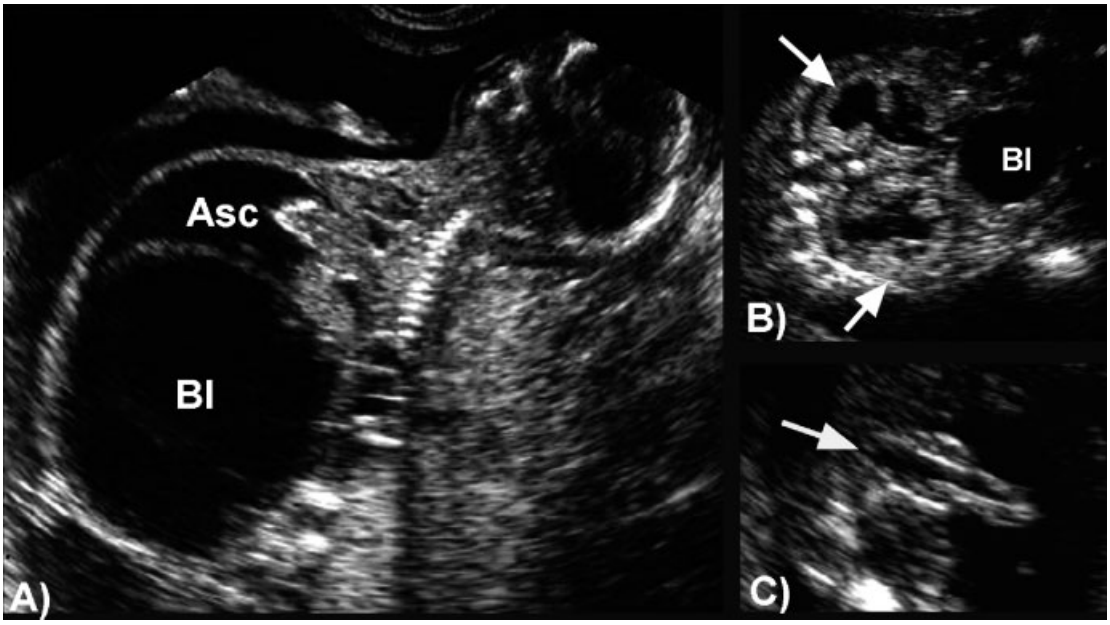

Figure 1—Case 1. (A) shows a longitudinal view of the twin with an extreme form of posterior urethral valves: an extremely distended bladder (Bl) and urinary ascites (Asc); note also the thin abdominal wall, consistent with the diagnosis of secondary prune-belly syndrome; (B) shows that the other twin had severely dysplastic and hyperechoic kidneys (arrows), a moderately enlarged bladder (Bl) and (C) a distended urethra (arrow)

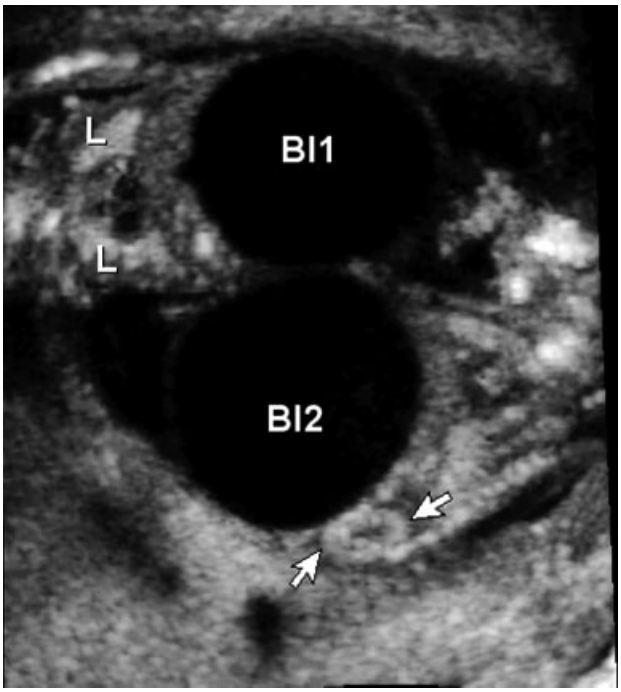

Figure 2—Case 2. Three-dimensional ultrasound—VCI-C rendering. The image shows a coronal view of both fetuses with severely dilated bladders (Bl1 and Bl2) and severe oligohydramnios. In the upper fetus, the lungs (L) and the heart in between are visible. In the lower fetus, the arrow indicates a hyperechoic dysplastic kidney

DISCUSSION

PUVs represent the most common cause of severe obstructive uropathy in children, with an incidence of approximately 1 in 1500 to 1 in 8000 boys (Cendron *et al*., 1994), and they appear to be the cause of about 9% of cases of urinary obstruction in fetuses (Elder, 1997). PUV is a sporadic disorder whose etiology is unknown, though its association with twinning has suggested a genetic cause. It may be related to failure of the complete disintegration of the urogenital membrane, which leaves membranous tissue within the posterior urethra responsible for the obstruction of the bladder outlet (Dinneen *et al*., 1993).

The prenatal ultrasound diagnosis of PUV is feasible and has already been reported as early as the 13th week of gestation (Sweeney *et al*., 1981; Dibbins *et al*., 1985; Turner, 1985; Helin and Persson, 1986; Silver *et al*., 1990; Oga *et al*., 1994; Cochat *et al*., 1996; Rani *et al*., 1997; Cohen *et al*., 1998; Yerkes *et al*., 2001); since the urethral obstruction is not directly detectable through an ultrasound, the diagnosis of PUV depends, in most instances, upon the recognition of sonographic signs of the lower urinary tract obstruction in a male fetus (dilated bladder, hydronephrosis and renal dysplasia (Figures 1 and 2). Additional inconstant findings are represented by a dilated posterior urethra and bladder wall hypertrophy (Glazer *et al*., 1982; Hayden *et al*., 1988). The most important prognostic indicator is represented by the presence of a hyperechoic kidney with cortical cysts, which is indicative of severe renal dysplasia (Romero, 1988). The occurrence of renal dysplasia was formerly believed to be uniquely dependent upon the degree and length of the urinary tract obstruction due to misexpression of the Pax-2 gene (Edouga *et al*., 2001); however, this concept has recently been partially revised and the onset of renal dysplasia is currently considered, at least in some instances, to be primary and synchronous with the

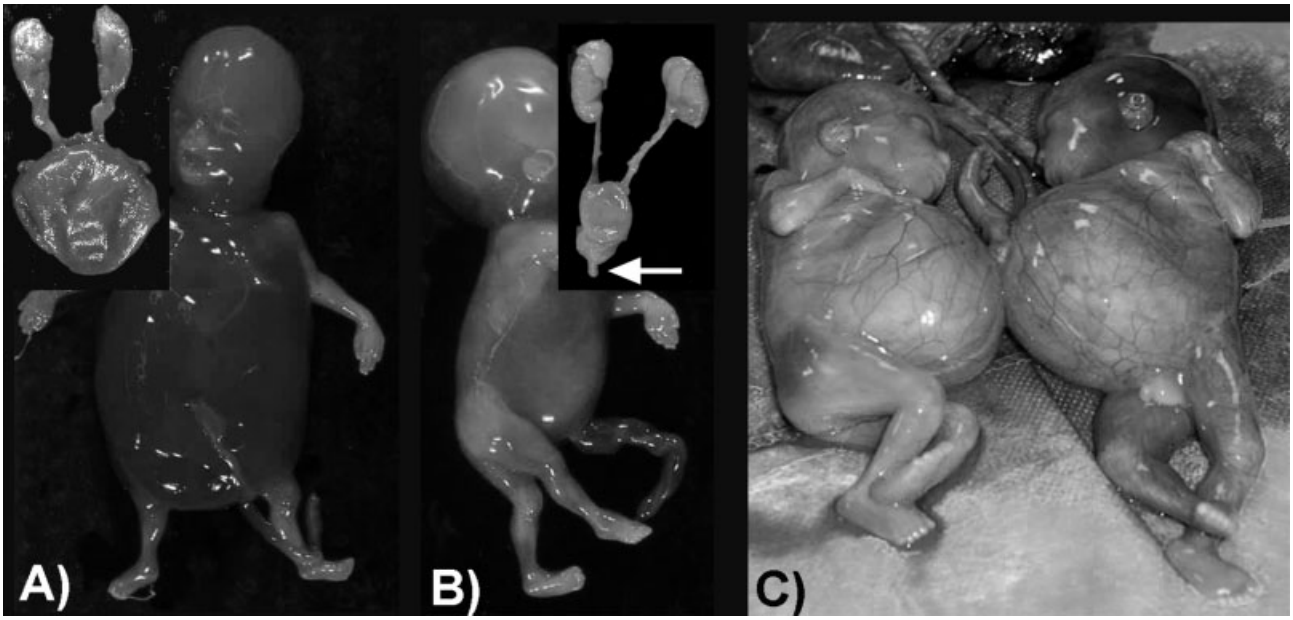

Figure 3—Postmortem examination of the specimens. (A) Case 1, same twin of Figure 1A: the extreme abdominal distention is evident; in the upper left insert, the specimen of the kidneys and the urinary tract with the extremely dilated bladder is shown; (B) Case 1, same twin of Figure 1B: note the lesser abdominal distention in comparison with (A) and the dilated distal urethra (arrow) on the excised specimen (upper right insert); (C) the set of twins of case 2, both showing massive abdominal distention

urinary tract obstruction owing to the misexpression of not only the Pax-2 but also of other genes such as Bcl2 and the transforming growth factor b1 (Winyard *et al*., 1996; Yoo *et al*., 2000). Another prognostic factor worth considering is certainly the amount of amnionic fluid: an early onset and severe oligohydramnios is a reliable indicator of impaired renal function and, therefore, such a finding should be part of the prenatal prognostic evaluation. However, it should be underlined that the oligohydramnios sequence starts developing only after 16 weeks of gestation, since until this time the amniotic fluid is mainly produced by filtration through the membranes, and therefore during the first 16 weeks of pregnancy it cannot be considered as a reliable indicator of normal renal function.

The ultrasound differential diagnosis includes other causes of lower urinary tract dilatation, such as massive vesico-ureteral reflux and the megacystis, microcolon intestinal hypoperistalsis (MMIHS) syndrome (Kohler *et al*., 2004). However, the amount of amniotic fluid is severely decreased in complete PUV, normal or moderately reduced in severe vesico-ureteral reflux and increased in the MMH syndrome. As for the differential diagnosis between PUV and urethral atresia, this is often impossible though the latter instance is a great deal rarer than PUV (Hurwitz *et al*., 1984).

Whether complete PUV has led to the development of PBS, cryptorchidism and abdominal wall hypoplasia should be detected. In fact, the triad identifying the PBS is represented by the megacystis, cryptorchidism and hypoplastic abdominal wall. However, cryptorchidism can be detected only after the 32nd week of gestation when the testicles are expected to descend in the scrotum in 97% of male fetuses (Achiron *et al*., 1998), whereas the laxity and thinning of the abdominal wall musculature are much more difficult to detect; in this case, we could detect it in one fetus of the first set of twins owing to the presence of the urinary ascites which acted as a natural contrast medium (Figure 1A). Therefore, the prenatal diagnosis of PBS may be confidently hypothesized only in the third trimester of pregnancy. The overall prognosis of the condition remains guarded despite the possibility of *in utero* intervention by vesico-amniotic shunting (Gnirs *et al*., 1988; Perez-Brayfield *et al*., 2001).

The overall prevalence of structural defects is 1.2–2 times higher in fetuses from twin pregnancies compared to singletons, with most of the excess risk due to increased rates in monochorionic twins (Baldwin, 1994). This is true also for PUV, whose association with twins has repeatedly been reported in postnatal life (Kroovand *et al*., 1977; Grajewski and Glassberg, 1983; Livne *et al*., 1983; Romero, 1988). However, despite this association, we were not able to find any case of concordant PUV diagnosed prenatally in twins in a MEDLINE search from 1966 using the key words 'posterior urethral valve, fetus, and twins'. Hence, to our knowledge, the present cases represent the first two cases of concordant PUV in monochorionic twins to be reported prenatally. Of interest is also the different manifestation of the anomaly in each twin of the first set: one presented with a classic PBS secondary to a full-blown form of PUV, whereas the second one presented with an apparently less severe form.

In conclusion, we have documented the early prenatal diagnosis of PUV in two sets of monochorionic twins and described the various manifestations of the disease in the first set of twins. This latter finding should be considered when assessing monochorionic twins with one affected fetus, for the involvement of the other

twin may be subtle and escape ultrasound diagnosis if a detailed examination is not performed.

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CASE REPORT

Split notochord syndrome variant: prenatal findings and neonatal management

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Spilt notochord syndrome is an extremely rare form of spinal dysraphism characterized by a complete cleft of the spine and a persistent communication between endoderm and ectoderm. A variant of split notochord syndrome was diagnosed in a 25-week-old fetus showing a prolapsed congenital colostomy and a spinal cystic lesion. The final diagnosis included protruding colon segment, imperforate anus with a rectourethral fistula and lipomyelomeningocele. The ultrasound features of the condition and the post-natal management are discussed. The neonate was successfully treated with a posterior sagittal anorectoplasty, while the lipomyelomeningocele was resected at a later stage. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: split notochord syndrome; prenatal diagnosis; congenital colostomy; dorsal enteric fistula; lipomyelomeningocele

INTRODUCTION

Split notochord syndrome (SNS) represents an extremely rare and pleomorphic form of spinal dysraphism characterized by a wide spinal defect and a persistent communication between endoderm and ectoderm (Almog *et al*., 2001, Gilbert-Barness and Luise, 1997). In its basic form, it consists of a neural tube defect with an endoectodermal fistula opening in the dorsal area, but several variants differing for the type and the site of the associated anomalies, which involve the gastrointestinal tract, the central nervous system (CNS) and, less often, the urogenital tract have been described by pediatric surgeons and neonatologist (Faris and Crowe, 1975; Flower, 1998). Owing to its pleomorphism, this condition has rarely been identified prenatally, with only two cases reported in the last 20 years (Almog *et al*., 2001). We present here the prenatal findings leading to the putative diagnosis of SNS and the post-natal surgical management of this condition.

CASE REPORT

A 29-year-old obese woman, gravida 2 para 0, was referred to our centre at 25 weeks of gestation with a suspicion of a neural tube defect involving the distal part of the sacrum. The family history was unremarkable, with no consanguinity reported. The transabdominal ultrasound examination showed a small round hypoechoic apparently cystic mass involving the lower part of the sacrum and an oblongated perineal structure closely resembling an intestinal loop, possibly originating from the lateral perineal region. None of the cerebellar and ventricular abnormalities usually associated with an open spinal defect were present. Since the fetus was in breech presentation and the patient significantly overweight, trans-vaginal ultrasound was performed. A much clearer view of the spinal mass, the intestinal fluid-filled structure and their relationship was achieved, the intestinal structure originating from the lateral aspect of the gluteal region (Figure 1A). The patient was informed about the likely presence of a combined defect of the caudal embryonal area involving both the spine and the lower intestinal tract, consistent with the split notochord spectrum of anomalies. Karyotyping was offered, but was declined by the patient. Two subsequent scans did not reveal any change in the volume of the masses or any other abnormality possibly overlooked at the first scan. At term, a male neonate weighing 3400 g was delivered by caesarean section.

Post-natal physical examination showed an intestinal loop originating in the lateral perineal area, with the serosa on its external surface (Figure1B,C and Figure2), a dorsal enteric fistula opening at the base of the loop, an imperforate anus and a skin-covered lump in the sacral area (Figure1B and Figure2). The left thigh was mildly hypoplastic and the right testicle was undescended. An X ray of the abdomen and spine showed the rectum ending blindly 3 cm from the perineal surface and the splitting of the sacrum and the coccyx with a scimitar-shaped sacral defect. A decompressive colostomy and a partial resection of the protruding colon segment were performed as initial treatment. Histology confirmed that the protruding structure was of intestinal origin and that the external layer was the serosa. The post-operative course was uneventful. Magnetic resonance imaging (MRI) and computed tomography (CT) of the

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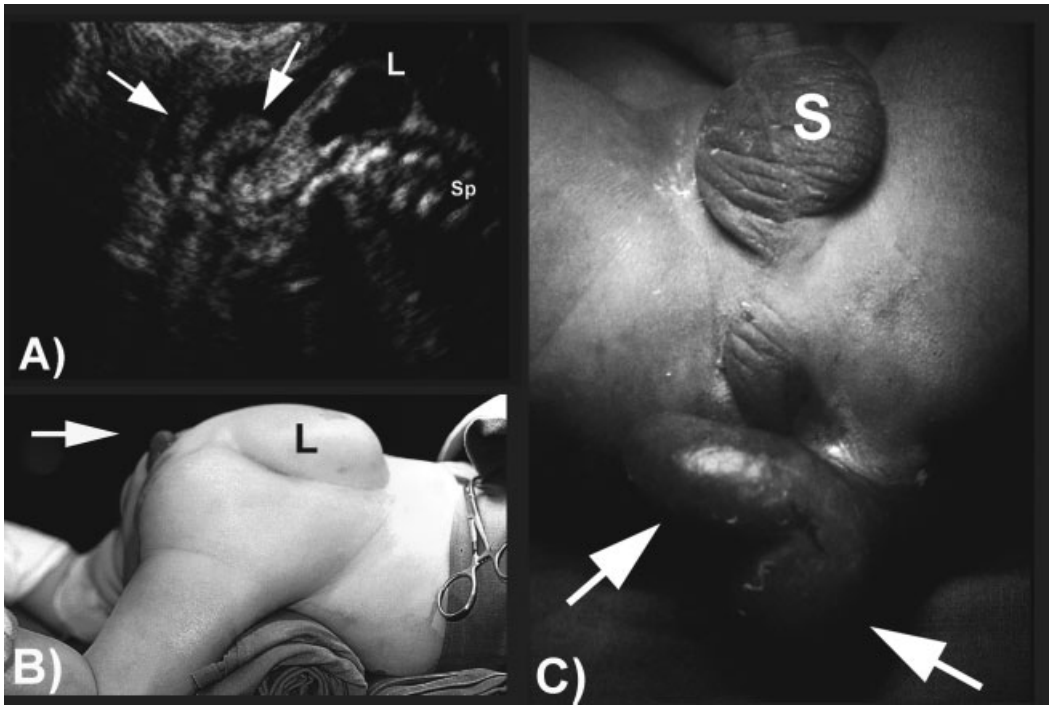

Figure 1—(A) At trans-vaginal ultrasound, the relationships between the protruding intestinal segment (arrows) and the sacrococcygeal lipomyelomeningocele (L) are demonstrated (Sp, spine). (B) For comparison, a lateral view of the lower trunk after the excision of the protruding intestinal segment and creation of the colostomy (arrow) is shown. Note the lumbar mass represented by the lipomyelomeningocele (L). (C) A frontal view of the neonate at birth showing the relationships between the intestinal segment (arrows) still in place and the scrotum (S)

brain and spine confirmed the normality of the former and the presence of a complete cleft below L_4 with a tethered cord associated with a lipomyelomeningocele. After counselling with neurosurgeons, it was decided to give priority to the repair the gastrointestinal malformation, and to untether the conus at a later stage. At the age of two months, the baby underwent posterior sagittal anorectoplasty via a combined dorsal and anterior approach. The double route approach was needed for the difficulties in locating the exact branching point of the protruding intestinal segment from the colon. During the intervention, a rectourethral fistula, demonstrated by the pre-operative distal colostogram, was also identified and closed. The protruding loop was then removed and the anoplasty completed in the standard way. The post-operative course was uneventful. The colostomy was closed at six months of age and the baby was discharged with a well-functioning anus. The colon was of normal length and size, whereas the ectopic sphincter showed a reduced para-sagittal muscular component, especially on the left side. The intestinal function was relatively good, with moderate constipation. As for the bladder function, the neurogenic bladder was managed by intermittent catheterization. At three years of age, the baby underwent the neurosurgical excision of the lipomyelomeningocele, in the hope that hopefully an improvement of bladder and intestinal function would ensue, but the situation remained basically unchanged after the operation.

DISCUSSION

Spinal dysraphisms consist of a wide spectrum of congenital anomalies resulting from defective embryogenesis of the spinal cord and vertebrae (Gilbert-Barness and Luise, 1997). The pathogenesis of the condition is unknown. One hypothesis refers to the persistence or partial obliteration of an accessory neuroenteric canal that connects the yolk sac and the amniotic cavity in the third post-conceptional week (Rosselet, 1995). However, the most recepted theory suggests that an anomalous splitting or duplication of the notochord would allow the endoderm or primitive gut to stick in and adhere to the dorsal ectoderm (Saundres, 1943). As for the lipomyelomeningocele, it is considered to be the result of the premature dysjunction of neuroectoderm and ectoderm (Sigh and Sigh, 1982).

The malformations featuring spinal dysraphisms can occur as isolated lesions or in combination with a wide range of anomalies of the spinal canal, the CNS and/or of other systems, most often the gastrointestinal tract. There are two varieties of spinal dysraphism, open and closed. In the former type (*spina bifida cystica*), the membrane and/or the cord may be exposed; in the latter type (occult spinal dysraphism), the spinal defect is covered with skin and can be associated or not with a skin-covered backmass (Byrd *et al*., 1991). The SNS, together with the caudal regression syndrome, belongs to the group of complex occult spinal dysraphism (Tortoli-Donati *et al*., 2000). Therefore, SNS represents an exceedingly rare

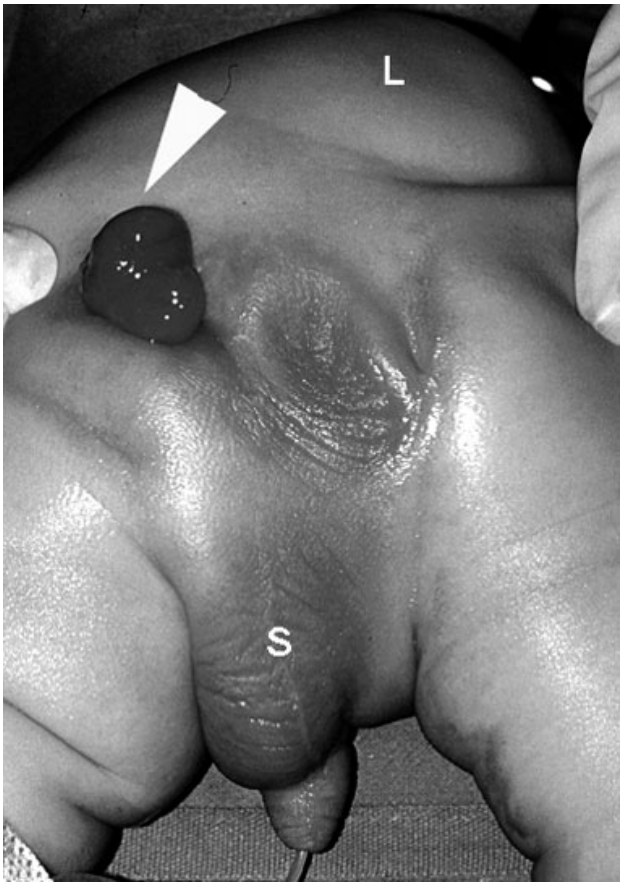

Figure 2—The dorsal view of the neonate shows the relationship between the protruding intestinal segment (arrowhead), the lipomyelomeningocele (L) and the scrotum (S)

malformation characterized by a persistent connection between the endoderm and ectoderm, resulting, in its most severe form, in a midline communication between the intestinal cavity and dorsal skin. In the most severe form, the so-called dorsal enteric fistula, the newborn has a bowel ostium on the back. Of note, SNS most commonly occurs in the lower cervical or upper thoracic spine, much less frequently in the lumbosacral region (Meller *et al*., 1989), as in the index case.

The difficulties encountered in the effort to correctly classify the SNS variant of the index case are easily understood if a MEDLINE search is attempted. We performed a search in the English language literature (1966–2004) for the terms 'split notochord syndrome' and 'dorsal enteric fistula' and retrieved only nine affected children. However, the lesions are so different from each other with such a wide range of associated anomalies that each one might be considered a unique variant (Table 1).

With respect to prenatal diagnosis of SNS, there are only two such reports, and both describe SNS with thoracic openings (Almog *et al*., 2001). To the best of our knowledge, this represents the first case of prenatal recognition of an SNS located in the lower spinal area. In our case, at ultrasound the occult spinal dysraphism (OSD) appeared as a sacro-coccygeal skin-covered cystic mass, thought to represent a simple spinal defect, but post-natally recognized as a lipomyelomeningocele, while the perineal mass showed all the features of an intestinal loop, hence the putative diagnosis of a complex malformation involving tissues of ectodermal and endodermal origin.

The differential diagnosis of lipomyelomeningoceles includes the cystic variant of sacrococcygeal teratomas and *spina bifida*. However, the latter is associated in the majority of cases with secondary modifications of the central nervous system, and, in addition, the bony vertebral defect is almost always detectable. As for sacrococcygeal teratomas, they are generally much larger and are not associated with other perineal or gluteal anomalies. Isolated lipomyelomeningoceles can be detected prenatally (Seeds and Powers, 1988), but not in the context of spinal dysraphisms. Since SNS is often associated with tethered cord, neurological deterioration may ensue, even in asymptomatic children, if prophylactic surgery is not performed. Although the anomaly is grossly evident at birth, the prenatal recognition of the condition allows *in utero* transport to tertiary referral centres. Whenever unusually appearing structures of intestinal origin are detected in association with cystic lesions of the lower spine, SNS and, in general, open spinal dysraphisms are the most likely diagnoses.

A last comment about the prenatal diagnostic issue regards the possible advantages of three-dimensional ultrasound. Since this anomaly alters dramatically the anatomy of the fetal breech, it is likely that the use of surface-rendering reconstruction might have significantly contributed to the diagnosis; in addition, the employment of the multiplanar imaging technique might have allowed for a detailed study of the anatomic relationship between the various elements of the complex malformation.

The management of SNS is basically tailored on the different anomalies present in each case. It aims primarily at the restoration of the intestinal function with separation of the neuroenteric fistulas, necessary to avoid fecal contamination of cerebrospinal fluid, if required (this did not apply to the index case for there was no neuroenteric communication in this case). The second issue is the excision of the lipomyelomeningocele, which should ensue as soon as possible, even in asymptomatic neonates, to avoid or reduce irreversible neurological complications (sensory loss, bladder dysfunction, motor loss, foot deformities and leg pain) associated with delayed release of a tethered cord. In our case, the surgical approach consisted of a two-stage procedure: the initial operation, at two months of age, included the excision of the protruding colon segment and the posterior sagittal anorectoplasty; the neurosurgical intervention, performed at three years of age, consisted of the excision of the lipomyelomeningocele and the release of the tethered cord. However, in this case, the moderately severe bladder and intestinal dysfunctions persisted unchanged after the operation.

In conclusion, we have reported the prenatal findings and the post-natal surgical management of a complex form of spinal dysraphism, the split notochord syndrome, featuring a very rare cluster of anomalies

Table 1—Cases of split notochord syndrome associated with dorsal enteric fistula and/or prolapsed natural colostomy reported to date

Author, year	Spinal defect	Congenital colostomy	DEF	Anus	Associated anomalies	Surgery	Status
Akgür *et al*., 1998	T10-L5	No	Yes	Incontinent	Meningomyelocele, dorsal enteric diverticulum near to dorsal enteric fistula	Yes	Dead
Alrabeeah *et al*., 1988[a]	Lumbosacral	No	Yes	Imperforate	NO	No	Dead
Asghar *et al*., 2002[b]	Double split (thoracic and lumbar)	Yes	Yes	Normal	Skin-covered meningocele with intestinal loop, enteric fistula and a fully developed lower limb	No	TOP
Bentley and Smith, 1960[c]	L2-sacrum	No	Yes	Imperforate	NO	No	Dead
Burrows and Sutcliffe, 1968	L2-sacrum	Yes	Yes	Normal	Meningocele, colonic duplication	Yes	Alive
Faris and Crowe, 1975	T10-sacrum	Yes	Yes	Imperforate	Meningomyelocele, lumbosacral mass containing small intestine and stomach	Yes	Dead
Gupta and Deodhar, 1987	L5-sacrum	Yes	Yes	Normal	Meningomyelocele	Yes	Alive
Hoffman *et al*., 1993	T10-sacrum	Yes	Yes	Normal	Meningocele, cloacal extrophy	?	Dead
Kanmaz *et al*., 2002	Lumbosacral	No	Yes	Normal	Dysgenetic *corpus callosum*, lipomyelomeningocele, gastrointestinal malrotation, wandering spleen, right inguinal hernia	Yes	Alive
Keen and Coplin, 1906	L3-sacrum	No	Yes	Normal	Sacrococcygeal teratoma	Yes	Alive
Kiristioglu *et al*., 1998	L1-sacrum	Yes	Yes	Imperforate	Meningocele, foreshortened colon	Yes	Alive
Kramer *et al*., 1988	T10-sacrum	No	Yes	Imperforate	Meningomyelocele, sacral agenesis	No	Dead
Meller *et al*., 1989	Lumbosacral	Yes	Yes	Anteriorly displaced	No	Yes	Alive
Pathak *et al*., 1988	Double split (C1-D5; D12-sacrum)	Prolapsed stomach and small intestine	No	Imperforate	Short colon	No	Dead
Rembe[4]	L1-sacrum	Small intestine and blind pouches	Yes	Normal	?	?	?
Singh and Singh, 1982	T10-sacrum	No	Yes	Normal	Hydrocephalus, depressed nasal bridge, ear anomalies	No	Dead
Our case	L4-sacrum	Yes	Yes	Imperforate	Lipomeningocele; tethered cord; rectourethral fistula	Yes	Alive*

DEF, dorsal enteric fistula.
[a] one of a series of seven;
[b] one of a series of four;
[c] one of a series of five;
[4] cited by Faris and Crow, 1975

(SNS, colostomy-like dorsal opening without exposed intestinal mucosa, anal atresia, rectourethral fistula, lipomyelomeningocele). This report demonstrates that SNS can be suspected prenatally whenever a spinal defect is detected together with an unusual gastrointestinal one, the congenital colostomy in our case, though it has to be emphasized that SNS represent extremely rare malformations. The prenatal recognition of this malformative cluster is important for a proper prenatal counselling and *in utero* transport to tertiary referral centres.

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CHAPTER 5
OTHER CHALLENGING WOMEN CONDITIONS AND COMPLICATION

5.1 Introduction

There is no formal or universally accepted definition of a "high-risk" pregnancy. Generally, however, a high-risk pregnancy involves at least one of the following: the woman or baby is more likely to become ill or die than usual, or complications before or after delivery are more likely to occur than usual. The term high-risk pregnancy is a catch-all term that's used to describe women who are on the risk continuum at any point during their pregnancy.

A pregnancy may be treated as high risk if:

- The woman is over 35 years or under 17 years;
- It is a multiple pregnancy;
- The woman has a chronic health condition such as diabetes, heart problems, or a blood-clotting disorder that has the potential to affect your pregnancy;
- The woman has a history of gynecological problems such as pelvic inflammatory disease (PID), endometriosis, or large symptomatic fibroids;
- The woman has a history of pregnancy loss (miscarriage, ectopic pregnancy, or stillbirth) or premature birth;
- The woman has infectious diseases, including HIV, that could be transmitted to the baby during pregnancy or at the time of birth;
- The pregnancy is the result of assisted reproductive technologies;
- The woman had two or more second-trimester abortions;
- The woman was exposed by her mother to DES during pregnancy (which may increase the chances of having difficulty carrying a pregnancy to term);
- The woman conceived while using an IUD;
- The woman has a child with a genetic disorder or is a carrier for a genetic disorder (something that may increase the risk of giving birth to a child with that particular genetic disorder).

Any pregnancy can become high risk for maternal or fetal complications as:

- vaginal bleeding or spotting;
- swelling in the face or fingers;
- a leakage of fluid or increased vaginal discharge;
- persistent vomiting that is not related to morning sickness;
- fetal malformations or non reassuring foetal conditions.

5.2 Our Experience: Materials and methods

Advances in obstetrical medicine have made motherhood a possibility for large numbers of women who might have been discouraged from starting a family a generation ago.

The Department of Prenatal Care of the University of Naples Federico II is a highly specialized centre for high risk pregnancy. This highly specialised approach to various maternal and foetal conditions is guarantee by the presence of collaborative multidisciplinary teams depending on the diseases and of the Intensive Care Neonatal Department.

Every year we follow pregnancies complicated by multiple conditions as diabetes, high blood pressure, maternal infectious diseases, genetic conditions with possible foetal transmission, endocrine disorders, maternal cardiac diseases, foetal malformations, foetal anemia, ect.

In particular some of these conditions have been so challenging in their clinical management that we have judged it was worth to publish our experience.

5.3. Essential Thrombocythaemia

Essential Thrombocythemia (ET) is a clonal hematopoietic disorder (1) characterized by increased red blood cell mass. The resultant hyperviscosity of the blood predisposes such patients to thrombosis.

Polycythemia vera should be suspected in patients with elevated hemoglobin or hematocrit levels, splenomegaly, or portal venous thrombosis. Secondary causes of increased red blood cell mass (e.g., heavy smoking, chronic pulmonary disease, renal disease) are more common than polycythemia vera and must be excluded. Diagnosis is made using criteria developed by the Polycythemia Vera Study Group; major criteria include elevated red blood cell mass, normal oxygen saturation, and palpable splenomegaly.

Pregnancies in women affected by ET can be complicated by recurrent abortion, foetal growth restriction, stillbirth and placenta abruption. The increasing number of young people affected and the slight female preponderance suggest the elaboration of appropriate guide-lines for pregnant women. Present therapeutic approaches in pregnant patients vary from no treatment to treatment with platelet reductive agents as monotherapy or in combination with antithrombotic medications (acetylsalicilic acid – ASA).

Our experience with pregnant women affected by this conditions allowed us to publish a paper in an international journal. From 1991 to 2001 they had nine pregnancies; during 4 of them they were left untreated or under ASA treatment as a result of patient decision or resistance to the therapy, while in the course of the subsequent 5 pregnancies they received α Interferon (α-IFN) treatment, with or without ASA.

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Interferon alfa treatment for pregnant women affected by essential thrombocythemia: Case reports and a review

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Objectives: In the past essential thrombocythemia was considered a disease of the elderly. At present, the number of young people suffering from this disease is growing, with a slightly higher frequency in females. We investigated the effects of interferon alfa therapy in these patients.
Study design: We describe 9 pregnancies in 4 women affected by essential thrombocythemia.
Results: Four pregnancies were carried out without interferon alfa therapy, and resulted in 2 intrauterine deaths, 1 spontaneous abortion, and 1 neonatal death. Interferon alfa was given during another 5 pregnancies; among them, 2 ended in preterm deliveries with normal infants, and 3 in full-term deliveries. The literature is reviewed.
Conclusion: Our cases and published series suggest that fetal outcome is improved by therapy, and that interferon alfa may be the best therapeutic option.

Essential thrombocythemia (ET) is a clonal hematopoietic disorder[1] that can be diagnosed in adherence to the Polycythemia Vera Study Group (PVSG) criteria.[2] Pregnancies in women affected by ET can be complicated by recurrent abortion, fetal growth restriction, stillbirth, and placental abruption.[2] The increasing number of young people affected, and the slight female preponderance suggest the elaboration of appropriate guidelines for pregnant women. Present therapeutic approaches in pregnant patients vary from no treatment to treatment with platelet reductive agents as monotherapy or in combination with antithrombotic medications (acetylsalicylic acid [ASA]).[2]

We describe 4 women affected by ET. From 1991 to 2001, they had 9 pregnancies; during 4 of them, they were left untreated or under ASA treatment as a result of patient decision or resistance to therapy, while in the course of the subsequent 5 pregnancies, they received interferon alfa (α-IFN) treatment, with or without ASA.

Case report

The clinical characteristics of our patients at diagnosis are described in Table I. Information about platelet count, type, dose, and length of therapy for all of our cases are summarized in Table II.

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Table I Patient characteristics at diagnosis of ET

Patient	Age	Plt $\times 10^3/\mu L$	Spleen volume by US scan*	Cytogenetics	bcr/abl	BM fibrosis
1	25	812	NA	46,XX	Absent	Absent
2	22	732	115 mL	46,XX	Absent	Absent
3	28	720	150 mL	46,XX	Absent	Absent
4	25	1200	1270 mL	NA	Absent	NA

Plt, Platelet; *US*, ultrasound; *BM*, bone marrow; *NA*, not available.
* Normal spleen volume is considered 60 to 200 mL.[32]

Table II Patient obstetric history

			Platelet count*								
Patient	Pregnancy	Age at pregnancy	Start of pregnancy	Peak	Nadir	At delivery	α-IFN therapy schedule	Length of therapy	ASA	GA at delivery	Pregnancy outcome
1	I	24	814	NA	NA	NA	None		Yes	36 wks	IUD
	II	25	467†	560	362	280	3 MU, 3/W	From preconception to delivery	Yes	34 wks	A&H
	III	30	825	NA	NA	875	None		Yes		SA
	IV	31	650	NA	NA	635	None		Yes	30 wks	NND
2	I	23	440†	399	328	315	3 MU, 1/W	From preconception to delivery	No	FT	A&H
	II	27	458†	399	263	334	3 MU, 1/W	From preconception to delivery	Yes	FT	A&H
3	II‡	28	310†	317	265	380	3 MU, 1/W	From 14wks to delivery	Yes	36 wks	A&H
4	I	30	800	NA	NA	NA	None		Yes	27 wks	IUD
	II	35	1742	1503	641	400	3 MU, 3/W	From 8wks to delivery	Yes	FT	A&H

GA, Gestational age; *NA*, not available; *IUD*, intrauterine death; *A&H*, alive and healthy; *SA*, spontaneous abortion; *NND*, neonatal death; *FT*, term.
* $\times 10^3/\mu L$.
† The first pregnancy occurred before ET diagnosis.
‡ Pregnancy started during α–IFN treatment.

Patient 1

This patient had thrombocytosis (platelet count 814 $\times$ $10^3/\mu L$) since 1992. At her first pregnancy (October 1992 to May 1993), intrauterine death occurred while she was receiving only ASA. In August 1993, a diagnosis of ET was made at our institution, and α-IFN therapy at the dose of 3 international megaunits (MU), 5 days per week, was started. In November 1993, still under α-IFN therapy, she became pregnant again. After counselling, α-IFN was continued, and ASA (100 mg daily) was added, starting from 12 weeks of gestation. At 34 weeks of gestation we opted for cesarean section because of mild asymmetric intrauterine growth restriction (IUGR) with reduced amniotic fluid, and pathologic umbilical artery Doppler with absence of diastolic flow. A healthy female newborn of 2150 g weight (below the 25th percentile) with Apgar score 8 and 9 at 1 and 5 minutes, respectively, and normal number of platelets was delivered. Post partum, a modest rebound from 440 $\times$ $10^3/\mu L$ to 651 $\times$ $10^3/\mu L$ after operation was recorded. α-IFN therapy was discontinued to allow the patient to breast-feed. After 6 months, α-IFN therapy was resumed because of increasing platelet count. It was then definitively stopped because of insufficient response (the platelet count was constantly over 800 $\times$ $10^3/\mu L$). The patient had 2 more pregnancies under treatment with only ASA (100 mg daily). The first pregnancy, in October 1998, resulted in a spontaneous abortion after a few weeks of gestation; the other pregnancy, in July 2000, was complicated by symmetric IUGR, severe oligohydramnios, and absence of diastolic flow in the umbilical artery Doppler. A cesarean section was performed at 30 weeks of gestation. The Apgar score was 3 and 6 at 1 and 5 minutes, respectively, and the infant died after a few days from prematurity.

Patient 2

This woman was diagnosed with ET in March 1997, and soon started α-IFN therapy (3 MU 5 days per week) because of her history of bleeding episodes. In June 1997, she became pregnant. After counselling, she agreed to continue α-IFN therapy, and the dose was

Table III Summary of pregnancy outcome according to treatment (our series included)

Treatment	Reference	No. of pregnancies	SA (%)	IUD (%)	NND (%)	Ectopic pregnancy (%)	Elective abortion (%)	Pregnancy outcome	
								FD (%)	A&H (%)
None	1,3–5,7,10–13, 16–20,22,25	88	40	4	1	1	4	50 (57)	38 (43)
ASA	1,5,10–13,15,19–20, 22,24–26	86	25	7	1			33 (38)	53* (62)
ASA + IFN	6,7,14	8			1			1 (13)	7 (88)
IFN	2,5,21,23,27–28	11	2					2 (18)	9† (82)
ASA + dipyridamole	12,26	1		1				1	
ASA + heparin	2,5,11,20,26,29	9	1					1 (11)	8 (89)
ASA + heparin + AT-III antagonist	20	2							2
Hydroxyurea	10,12	2					1	1	1
ASA + hydroxyurea	1	1							1
Platelet pheresis	12,16,18	3				1		1	2
ASA + platelet pheresis + IFN	4	1							1
ASA + platelet pheresis	11,12	4							4
Others	10	2							2
Total		218	68 (31)	12 (6)	3 (1)	2 (1)	5 (2)	90 (41)	128 (59)

SA, Spontaneous abortion; *IUD*, intrauterine death; *NND*, neonatal death; *FD*, fetal death; *A&H*, alive and healthy.

* Down syndrome (1 case).

† Bone and genital malformation (1 case).

reduced to 3 MU 3 days per week starting from 20 weeks of gestation. ASA was not prescribed because of patient's history of bleeding. She had an uneventful pregnancy and was delivered of a normal male newborn weighing 3070 g at 41 weeks of gestation, with Apgar scores 8 and 9 at 1 and 5 minutes, respectively. The baby's platelet count was normal. The patient did not breast-feed in order to continue the therapy with α-IFN (3 MU once per week). In July 2002, she became pregnant again and continued the maintenance treatment. After an uneventful pregnancy, she was delivered of a healthy male baby weighing 3000 g at 40 weeks of gestation, with Apgar scores 8 and 9 at 1 and 5 minutes, respectively, and normal platelet count. She interrupted the therapy to breast-feed; the follow-up of both children reports good health.

Patient 3

This patient was 28 years old when ET was diagnosed; she had had her first pregnancy before ET appearance in 1996, at the age of 25 years. Being symptomatic with headache and erythromelalgia, she was treated with α-IFN (3 MU 3 times per week). As soon as the second pregnancy was documented (at 14 weeks of gestation), the dose was reduced and ASA (100 mg daily) was added. After counselling, this treatment was continued throughout the pregnancy. The pregnancy was uneventful, with normal growth of the fetus. At 36 weeks of gestation she was delivered of a healthy female newborn, weighing 3050 g, with Apgar scores 8 and 9 at 1 and 5 minutes, respectively, and normal platelet count. Lactation was pharmacologically suppressed to continue the treatment with α-IFN. No rebound thrombocytosis was observed after delivery. After a few months, the therapy was stopped for patient's decision (platelets count 372 $\times$ $10^3/\mu L$). At present, the follow-up shows normal baby development without clinical complications.

Patient 4

This woman was diagnosed with ET in 1991 when she was 25, and was given α-IFN (3 MU 3 times per week) until 1995, when she decided to stop the therapy. In July 1996, intrauterine fetal death occurred at 27 weeks' of gestation. In August 2000, she became pregnant again, and after counselling, at 8 weeks of gestation, therapy with α-IFN was started again, combined with ASA, 100 mg daily. This treatment was continued throughout the pregnancy. The pregnancy was uneventful with normal fetal development. At 40 weeks of gestation the patient was delivered of a female newborn, weighing 3210 g, with Apgar scores 7 and 9 at 1 and 5 minutes, respectively. The baby's platelet count was normal. α-IFN was interrupted to allow nursing, but it needed to be resumed after 15 days because the platelet count was increasing ($896 \times 10^3/\mu L$). The patient is still under α-IFN therapy, and the baby is growing healthy.

Comment

The issue of when and how to treat patients with ET is of particular concern in young patients because they will

be receiving therapy for many years, with increasing risk for treatment-related side effects. ET in pregnancy has been reported to be complicated by recurrent abortion, intrauterine death, stillbirth, premature delivery, preeclampsia, and fetal growth restriction caused by placental infarction resulting from thrombosis. Maternal complications, such as bleeding or thrombotic events,[2] may also occur during pregnancy or post partum. To improve pregnancy outcome and reduce maternal complications, the use of antiplatelet drugs and/or cytoreductive agents is often considered, but the optimal treatment is still controversial. Some believe that pregnancy outcome is not therapy dependent, and thus no treatment is needed during pregnancy.[3] On the other hand, antiproliferative drugs such as hydroxyurea could have adverse effects on the fetus (abortion, congenital malformations, or intrauterine growth restriction).[2-4] Following early reports on safety and efficacy during pregnancy in women with ET,[5] α-IFN is increasingly being used during pregnancy, although pregnancy is still listed as a contraindication to α-IFN treatment. α-IFN is not mutagenic in vitro or teratogenic in animal studies; it does not reduce fertility.[4-7] The abortifacient effects observed in rhesus monkey occur at doses markedly higher than those used in therapy.[5,8] Some reports have shown that IFN and IFN-like molecules are produced by murine and human placenta (trophoblast interferons). They are expressed for a short period in high concentrations, and have antiluteolytic, antiviral, antiproliferative, and immunomodulatory effects through receptors on the endometrial epithelium.[9]

The treatment by ASA has a logical basis in order to reduce ischemic placental damage. It is reported to be effective in many vaso-occlusive manifestations, but its efficacy in reducing pregnancy complications has not been proven.[3] It may increase the risk of hemorrhage, thus being indicated in symptomatic ET pregnant women without history of bleeding. According to some authors, management of the ET patient should be based on risk stratification[3,10] because patients over age 60 years or who have a previous history of thrombosis are at high risk. We think that even pregnancy should be considered a risk factor, especially if the patient has experienced complications during a previous pregnancy. All 4 of our cases needed to be treated both because of their being symptomatic since the onset of disease, and because of their obstetric history, as summarized in Table II.

From a review of the literature and including our series, 218 pregnancies in 114 women affected by ET have been analyzed in 27 reports.[1-7,10-29] Fetal outcome was the following: 128 live births (59%) (including 16 preterm deliveries and 1 postterm delivery), 67 miscarriages, 12 stillbirths, 5 elective abortions, 2 ectopic pregnancies, 3 neonatal deaths, and 1 incompetent cervix. Seven babies suffered from IUGR, 1 from Down syndrome, and 1 from IUGR with bone and genital malformation. It is difficult to draw general rules from these data because of treatment heterogeneity and absence of risk stratification. Of the 114 women, 23 (19%) were symptomatic before pregnancy. α-IFN treatment was scheduled in 20 of the 218 pregnancies but was continued until delivery in only 17. As summarized in Table III, the percentage of live babies was higher (69%) in treated (any treatment) than in untreated pregnancies (43%). In all but 3 patients treated with α-IFN (as single agent or combined with ASA), pregnancy resulted in live births without complications for either fetus or mother, thus resulting in effective and safe deliveries in 85% of the pregnancies in which it was administered. In our own series, α-IFN treatment led to term delivery in the same women who experienced poor outcome when α-IFN was not or could not be given (Table II), a finding that can be considered as an internal control. Even though normal pregnancies are described in absence of treatment, we think that all pregnant women with ET should be treated because the real number of patients suffering from complications of ET may be underestimated.[2] As for metabolism and possible side effects of α-IFN in pregnancy, no conclusive data are available. Pons et al[30] studied the pharmacokinetics of α-IFN in pregnant women in 1995 and demonstrated that α-IFN was undetectable both in the amniotic fluid and in fetal blood, and that the pharmacokinetic parameters did not differ from that in nonpregnant women.[30] Thus, at the doses used, α-IFN does not seem to cross the placenta and cannot cause any damage to embriogenesis or to the developement of the immunologic system in the fetus.[2] As for safety of breast-feeding during IFN therapy, in our patients, we either stopped IFN treatment or suppressed lactation after delivery, although there is no evidence of breast-feeding danger during by IFN. A slight elevation of IFN concentration was reported by Kumar[31] in the milk of lactating women treated by high-dose (30 MU iv) IFN. In a patient of our series (#1) who continued breast-feeding during IFN treatment, a formal quantitative assay of IFN concentration in the milk was prevented by intrinsic milk toxicity on the cells used for the assay; however, the milk from the patient was more toxic to the target cells than the milk from a healthy control subject.

In conclusion, optimal management of ET patients is still poorly defined, and there are no established protocols. Normally, only patients belonging to the high-risk group need to be treated. However, we feel that during pregnancy all women are at risk of complication and should be treated. We suggest α-IFN to reduce platelet count, combined with ASA if no hemorrhagic complication is present.

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5.4. Budd-Chiari syndrome

One of the patients attended the Department of High Risk pregnancy was a patient with a porto-systemic shunt because of a previous Budd-Chiari Syndrome. Budd-Chiari syndrome (BCS) is an uncommon liver disease defined as an obstruction to hepatic venous outflow at any level from the small hepatic vein to the junction of the inferior vena cava and the right atrium. The characteristic clinical triad is abdominal pain, hepatomegaly, and ascites. Heart failure and sinusoidal obstruction syndrome (formerly known as veno-occlusive disease) also impair hepatic venous outflow and share many features with BCS, but are considered separately as causes and treatments are different (1). The obstruction of the hepatic venous outflow leads to sinusoidal congestion, centrilobular necrosis, fibrosis and portal hypertension (2) as consequences variceal bleeding, refractory ascites, hepatorrenal syndrome and hepatopulmonary syndrome are very severe complications of BCS, with a potentially fatal outcome if no treated appropriately (3,4). Although the natural history of BCS is not well known, mortality is highest at first 2 years of diagnosis and was found to be independent of surgical portosystemic shunting and is affected by several prognostic indicators as the severity of encephalopathy, ascites, prothrombin time and serum levels of bilirubin. The main standard of treatment is based in anticoagulation unless contraindicated.

Budd-Chiari syndrome is rare in pregnancy even though pregnancy tends to promote Budd-Chiari syndrome. Budd-Chiari syndrome usually presents in the last trimester or within several months postpartum (5). The serum bilirubin and alkaline phosphatase levels are typically moderately elevated, with normal to mildly elevated serum transaminase levels. Budd-Chiari syndrome is diagnosed by pulsed Doppler ultrasound, hepatic venography, or magnetic resonance angiography (6). Liver biopsy may reveal central venous congestion.

The woman, we followed during pregnancy, was also affected by a protein C deficiency and had a bicornute uterus. The experience of this complicated case was published in an international journal as reported above.

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Case Report

Pregnancy in a woman with a history of Budd-Chiari syndrome treated by porto-systemic shunt, protein C deficiency and bicornuate uterus

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Budd-Chiari Syndrome (BCS) is a rare disorder caused by the obstruction of hepatic venous outflow, mainly due to the thrombosis of hepatic veins or of the terminal portion of the inferior vena cava (1). Although many cases are considered "idiopathic", underlying inherited (deficiency of natural coagulation inhibitors, factor V Leiden and prothrombin G20210A mutations) or acquired (myeloproliferative disorders, antiphospholipid syndrome, paroxysmal nocturnal haemoglobinuria, pregnancy, oral contraceptive use), conditions leading to thrombophilic states are often detectable and concur to the pathogenesis of BCS (1–7). Due to the rarity of the disease and to the low number of women conceiving after BCS, few data are available concerning counselling, management and outcome of pregnancy in this setting.

Case report

We report the case of a 34-year-old woman admitted to our institution at 10 weeks of her first pregnancy. Ten years earlier, she underwent a porto-systemic shunt after BCS, and was then prescribed life-long oral anticoagulant therapy, as protein C deficiency (42%, normal values 70–120%; STA® functional Protein C, Diagnostica Stago, Asnières-sur-Seine, France) was diagnosed. Screening for other congenital (plasma antithrombin, protein S and homocysteine levels; activated protein C resistance; genotyping for factor V Leiden and prothrombin G20210A mutations) or acquired (lupus anticoagulant and anticardiolipin antibodies; objective and laboratory data suggesting haematological, immunological or chronic inflammatory disorders) thrombophilic conditions was negative. Type I protein C deficiency was then confirmed by repeated measurements, also including evaluation of antigen levels (46%, normal values 68–125%; ELISA with rabbit polyclonal antibodies, Dako A/S, Glostrup, Denmark) and ruling out possible causes of acquired deficiency (other indexes of liver biosynthesis and vitamin K-dependent proteins were within normal ranges).

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Warfarin had been withdrawn after the biochemical diagnosis of pregnancy (6th week) and substituted by nadroparin 5,700 UI twice daily. Ultrasonography showed a bicornuate uterus, with a gestational sac and vital embryo in the right uterine horn and a large intramural leiomyoma in the other uterine horn.

Ultrasound follow-up during pregnancy did not show abnormalities of the liver and of the hepatic vein flow, presence of fluid in abdomen or foetal malformations and growth restriction. Biochemical testing of liver enzymes, bilirubin, platelet count and coagulation parameters (PT, aPTT; activated protein C resistance; plasma fibrinogen, antithrombin, D-dimer) were in the normal range throughout the pregnancy. Ultrasound and laboratory follow-up was performed every 3–4 weeks until the 20th week, then every two weeks. Pregnancy was uneventful until 29 weeks of gestation, when the patient was admitted because of severe swelling of the legs, ascites and a premature rupture of membranes (PROM), which prompted labour induction and the need for urgent caesarean section because of podalic presentation. At the opening of the abdominal wall, about five liters of milky ascites were found. The male newborn (weight about 900 g; 1 and 5 minutes Apgar scores: 1 and 5, respectively; venous and arterial umbilical pH: 7.0–7.1) died after two days because of respiratory distress, although the lack of necroscopy did not enable to rule out other possible reasons for his death. As expected due to the neonate's prematurity, coagulation tests showed abnormally prolonged PT and aPTT and low plasma levels of coagulation factors and inhibitors, including protein C.

Discussion

BCS is a rare and serious thrombotic disease with significant morbidity and mortality. The underlying acquired and/or inherited thrombophilic conditions, often associated with this syndrome, contribute to the severity of prognosis, with the risk of thrombotic recurrences and co-morbidity in patients with haematological or immune disorders and cancer (1–7).

Early diagnosis and intervention to reduce portal hypertension, together with prolonged anticoagulation and the possibility of liver transplantation [a 68% overall survival at ten years has been recently reported (8)], have improved clinical outcomes and life expectancy of patients with BCS. Therefore, conceptional and pregnancy issues in fertile women should be carefully addressed.

The association of BCS with pregnancy is well recognised, several cases having been reported since the early 1970's (9–10).

On the other hand, very few data are available concerning pregnancy in women with a history of BCS (11–12).

It is known that pregnancy can result in fatal complications for the mother and/or for the infant in women with chronic liver disease (13). Fertility is often decreased in advanced liver disease, and this may provide a degree of protection for such patients who would be at increased risk; however, pregnancy may occur even in the presence of advanced liver disease, and it is necessary to anticipate and plan for possible complications of the specific liver disease. Counselling prior to pregnancy is the best policy, with consideration of transplantation prior to childbearing or of sterilization if it is more appropriate, based on the patient's general and liver function evaluation (13). In this respect, the presence of severe portal hypertension and high risk of variceal bleeding (further increased during pregnancy) may play a relevant role. In women with BCS, risks related to the possible coexistence of thrombophilia and to anticoagulation also have to be taken into account, as in the patient here reported. This woman was carrier of protein C deficiency, a severe inherited thrombophilic condition (14). Increased risk of venous thromboembolism is reported during pregnancy and puerperium in patients with inherited deficiency of coagulation inhibitors, and such risk is likely to be also greater in women with previous thromboembolic events than in asymptomatic carriers (15–16). Antithrombotic prophylaxis with low-molecular-weight heparins (LMWH) has been shown to be a safe and effective approach for women carrying thrombophilic abnormalities and/or with a history of venous thromboembolism in pregnancy (17–18). In this respect, preconceptional advice may include the withdrawal of coumarins prior to childbearing, also in order to prevent the risk of embryopathy (19).

Our patient started her pregnancy without such counselling, and the presence of uterine abnormalities (bicornuate uterus with a large intramural leiomyoma in the left horn) was unknown. Bicornuate uterus is the most frequent congenital uterine abnormality (20), whose prevalence is not negligible in the general female population (0.4–4%). In such a condition, the inadequate vascularity to the developing embryo and placenta, the reduced uterine intraluminal volume or cervical incompetence predispose to pregnancy complications [early miscarriage, preterm labour and breech presentations (20)].

To our knowledge, this is the first pregnancy described in a woman with a history of BCS treated by porto-systemic shunt and with a bicornuate uterus. The association of the two diseases is only coincidental and contributed to the unsuccessful outcome of pregnancy. The physiologic enlargement of the uterine horn during pregnancy caused a compression of the shunt, leading to the onset of severe ascites and, presumably, to the PROM with labour induction. However, the relative contribution of the anatomic uterine abnormalities and of the ascites as factors triggering PROM cannot be established. The sudden and severe presentation of ascites was rather unexpected in this patient, who underwent a careful laboratory and ultrasound follow-up since the 20th week, and hampered the induction of enhancement of foetal lung maturity planned at 30–32th week.

Despite the adverse outcome for the foetus, this report supports the concept that pregnancy in women with previous BCS and porto-systemic shunt is possible, and that prophylaxis with LMWH is safe and effective also in pregnant women with severe thrombophilia, although the optimal regimen for prophylaxis and its monitoring (LMWH would require anti-factor Xa levels, often not available, as in this patient) are still subjects of debate (21). Due to the low number of women conceiving after BCS, recommendations and guidelines regarding future conception and management during pregnancy in this setting have not yet been defined. Our experience highlights the need for a careful preconceptional counselling in these women, including advice for liver and vascular disease, antithrombotic prophylaxis, especially when thrombophilic abnormalities coexist, and the search for other maternal disease potentially affecting pregnancy outcome.

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5.5. Abnormal setting of pregnancy: ectopic pregnancy.

Ectopic pregnancy is one in which the products of conception develop outside the uterine cavity. By far the commonest site is the fallopian tube.

Ruptured ectopic pregnancy accounts for 10 to 15 percent of all maternal deaths (1,2). Fortunately, after the advent of transvaginal ultrasonography and beta subunit of human chorionic gonadotropin (beta-hCG) tests, the incidence of rupture and case-fatality rates declined from 35.5 deaths per 10,000 ectopic pregnancies in 1970 to 3.8 per 10,000 in 1989(2).

Risk factors most strongly associated with ectopic pregnancy include previous ectopic pregnancy, tubal surgery, and in utero diethylstilbestrol (DES) exposure. A history of genital infections or infertility

Ectopic pregnancy is most common in women of reproductive age who present with abdominal pain and vaginal bleeding approximately seven weeks after amenorrhea. These findings are nonspecific and are common in patients who may miscarry(1).

A normal or slightly enlarged uterus, vaginal bleeding, pelvic pain with manipulation of the cervix, and a palpable adnexal mass significantly increase the likelihood of an ectopic pregnancy. Significant abdominal tenderness suggests ruptured ectopic pregnancy, especially in a patient with hypotension who presents with guarding and rebound tenderness.

Diagnostic tests for ectopic pregnancy include a urine pregnancy test; ultrasonography; beta-hCG measurement.

Ultrasonography is the diagnostic test of choice, with limitations largely based on availability and the gestational age of the pregnancy (3). Ectopic pregnancy is suspected if transabdominal ultrasonography does not show an intrauterine gestational sac and the patient's beta-hCG level is greater than 6,500 mIU per mL (6,500 IU per L) or if transvaginal ultrasonography does not show an intrauterine gestational sac and the patient's beta-hCG level is 1,500 mIU per mL (1,500 IU per L) or greater (2).

Cervical pregnancy is a rare life-threatening form of ectopic pregnancy. In the past, hysterectomy was often the only choice available because of profuse hemorrhage that accompanied the attempts of removal of the cervical pregnancy.

We present a case series of (4) women with cervical cervix. Some had a good outcome but other had a bad outcome because of concomitant conditions which affected the therapeutic protocol.

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Is uterine artery embolization for cervical ectopic pregnancy always safe?

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KEYWORDS:
Cervical pregnancy;
Counseling;
Ectopic pregnancy;
Embolization;
Myoma;
Treatment

Abstract. The study objective was to assess the feasibility and the efficacy of bilateral uterine artery embolization (BUAE) for the treatment of cervical pregnancy. The design was a series of 3 cases of viable cervical pregnancy diagnosed by transvaginal ultrasonography and treated by means of BUAE and subsequent uterine curettage. Three women with viable cervical pregnancy underwent BUAE and subsequent uterine curettage in the department of obstetrics and gynecology, High Risk Pregnancy Center, University "Federico II" of Naples. Measurements included surgical outcomes and preservation of fertility. The treatment was effective in all cases. Two patients resumed normal menstruation about 1 month after the procedure, whereas 1 patient underwent a hysterectomy 2 weeks after embolization because of acute ischemic degeneration of a concomitant myoma. The conservative management of cervical pregnancy with angiographic BUAE is a feasible and effective option, even if subsequent hysterectomy may be required. Counseling is necessary.

Cervical pregnancy is a rare form of ectopic pregnancy in which the blastocyst implants and grows within the cervical canal. Cervical pregnancy occurs in less than 1% of all extrauterine gestations.[1] The incidence varies from 1/1000 to 1/18 000 pregnancies,[2] but recently it was reported to be 1/8628 pregnancies.[3] This condition has been associated with a high morbidity rate and, in the past, frequently led to hysterectomy as a life-saving procedure with consequent shattering effects on future fertility.

Early diagnosis of cervical pregnancy has substantially improved with the use of transvaginal ultrasonography (TV-US) and pelvic magnetic resonance imaging (MRI). Moreover, the combination of these 2 techniques permits better definition of disease evolution. Actually, conservative treatment with preoperative bilateral angiographic uterine artery embolization (BUAE) followed by curettage may be considered a valid approach. This strategy is effective both in terms of surgical outcomes, such as control of blood flow and prevention of severe hemorrhage, as well as in preserving fertility.

On the basis of these considerations, the aim of this study was to describe a series of 3 cases of viable cervical pregnancy diagnosed by TV-US and treated by means of BUAE and subsequent uterine curettage, underlining the safety of the technique but also its potential risk.

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

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Table 1 Summary of patients' characteristics

Patient	Age (yrs)	Spontaneous pregnancy	Potential causative factors	Gestational age at diagnosis (wks)	Live embryo
1	39	Yes	TC	11	Yes
2	40	Yes	Conization	12	Yes
3	43	Yes	Unknown	9	Yes

TC = cesarian section.

Description of the series

Table 1 shows the main characteristics of the 3 patients. Table 2 shows the management of cervical pregnancy in our series with related side effects and outcomes.

Patient No. 1

A 39-year old woman (gravida 3, para 0, cesarean sections 2) was admitted to our department with a diagnosis of cervical pregnancy at 9 weeks, 5 days' gestation. The patient reported a β-thalassemia carrier status. She described her last menstruation as poor vaginal bleeding. On speculum examination, the cervix had a bluish color. Transvaginal ultrasonography revealed a gestational sac with yolk sac and embryo with positive cardiac activity within the cervical canal having a crown-rump length (CRL) of 32 mm (Figure 1). The patient was hemodynamically stable with a hemoglobin level of 12.8 g/dL.

To better evaluate the cervix and the invasion of trophoblastic tissue, pelvic MRI was also performed. The MRI findings agreed with ultrasonography findings (Figure 2). Conservative management was planned because the patient wanted to preserve her fertility, although a detailed consent for hysterectomy in case of emergency was required and obtained. She did not receive medical therapy, such as administration of methotrexate. With the patient under general anesthesia, BUAE was performed, as detailed below, to prevent massive hemorrhage, and dilation and suction curettage was performed.

No blood transfusion was required, and the patient was discharged 4 days after the surgical intervention. The patient resumed menstruation 35 days after embolization, although her menses were irregular for the next 6 months.

Patient No. 2

A 40-year-old woman (gravida 2, para 1, cesarean section 1) was referred from another hospital to our department with diagnosis of cervical pregnancy at 11 weeks, 4 days' gestation. The patient did not report any dilation and curettage, but a history of conization for cervical intraepithelial neoplasia II.

During treatment in the previous hospital, the woman received 2-cycle methotrexate treatment. In particular, the first methotrexate administration was systemic, whereas the second was intra-amniotic.

Transvaginal ultrasonography performed on admission in our department revealed a still-viable embryo. The gestational sac was within the cervical canal, the CRL of the embryo was 45 mm, and the fetal heart rate was 155 beats/min. A T_2-weighted MRI confirmed the cervical pregnancy with the placenta-like mass and 1 fetus in the uterine cervix.

Because the patient wanted to preserve her fertility, conservative management was planned. Thus the patient was informed ofthe eventual risks related to the procedure and the possibility of undergoing hysterectomy, and informed consent was signed.

Bilateral uterine artery embolization, as detailed below, followed by a vacuum evacuation and curettage of the cervical canal was performed. The estimated blood loss was minimal, and the patient did not receive transfusion. She was discharged after 6 days, because we waited for a significant decline of serum β-human chorionic growth hor-

Table 2 Management of cervical pregnancy in our series, side effects, and outcome

Patient No.	Other therapy before UAE	Pre-embolization hemorrhage	Arteries embolized	Postembolization surgical treatment	Postembolization hemorrhage	Side effects and follow-up
1	None	None	Bilateral uterine	Dilation and curettage	None	None; regular menses
2	MTX	None	Bilateral uterine	Vacuum evacuation Dilation and curettage	None	None; irregular menses for 3 months
3	None	None	Bilateral uterine	Dilation and curettage + hysterectomy	None	Status febrile, degenerative leiomyomata, postoperative infertility

MTX = methotrexate; UAE = uterine artery embolization.

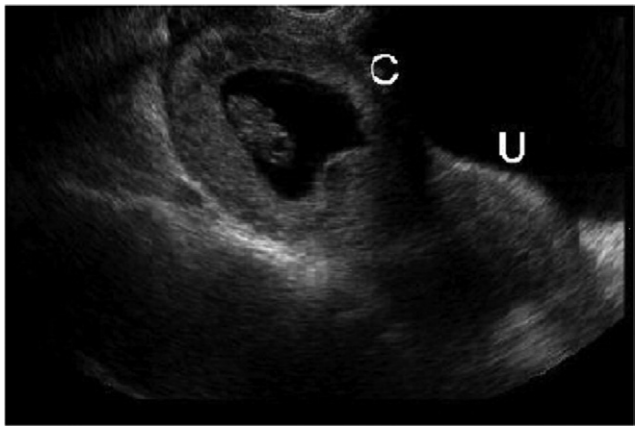

Figure 1 Patient No. 1. Transvaginal ultrasonographic scan of the cervical pregnancy. The gestational sac with the vital embryo is located in the cervical canal *(C)*. The uterine body is empty *(U)*.

mone (β-hCG) concentration, and normal menstruation resumed 38 days after embolization.

Patient No. 3

A 43-year-old woman (gravida 3, para 0, cesarean section 3) was admitted to our department with a diagnosis of cervical pregnancy at 8 weeks, 2 days' gestation. The patient had an unremarkable medical history (no previous intrauterine procedures or pelvic inflammatory disease). Transvaginal ultrasonography revealed a gestational sac below the closed internal cervical os and an intramural anterior uterine myoma with a main diameter of 62 mm.

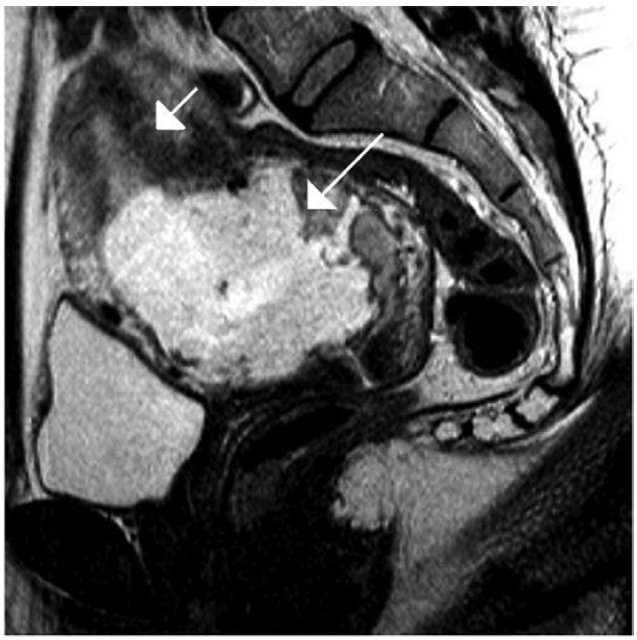

Figure 2 Patient No. 1. Magnetic resonance imaging findings include an empty uterus *(short arrow)* and a gestational sac in the cervical canal with trophoblastic invasion *(long arrow)*.

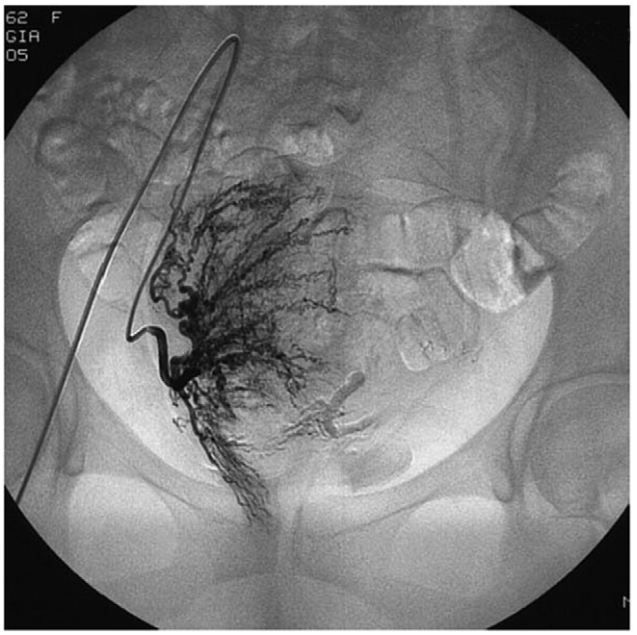

Figure 3 Patient No. 3. Pre-embolization angiogram of the right uterine artery. The right uterine artery before embolization showed tortuous arteries in its distal branches dilated because supporting the myoma.

The embryo was viable and had a CRL of 21 mm. The cervical pregnancy and the location and size of leiomyoma were confirmed by MRI. Treatment modalities, together with their benefits and potential risks, were discussed with the patient who signed an informed consent form. Conservative management was planned because patient desired future fertility. Dilation and suction curettage was performed with the patient under general anesthesia after BUAE was performed to prevent hemorrhagic complications. The BUAE is detailed below. Angiography of the right uterine artery before embolization showed tortuous and dilated arteries in the distal branches because they were supporting the myoma (Figure 3).

The estimated blood loss was less than 100 mL, and no transfusion was required. Blood pressure was 115/70 mm Hg. One day after the procedure, the patient developed a fever. Postoperative ultrasound scanning performed on the fourth day showed a normal cervix but ultrasonographic findings of degenerative myoma. The white blood cell count of 18×10^9/L and the cultures indicated a mixed aerobic and anaerobic infection. Antibiotic therapy was started.

Because of the persistence of the febrile status and the worsening of the ultrasonographic findings, we decided to perform a simple hysterectomy. The patient was monitored by daily white blood cell count and hemoglobin measurements and continued to receive antibiotic therapy for another week. Two days after hysterectomy the patient had a normal corporeal temperature, and the white blood cell count returned to the normal range.

Pathologic examination of the removed uterus revealed necrosis, degeneration, and inflammation of myoma tissue

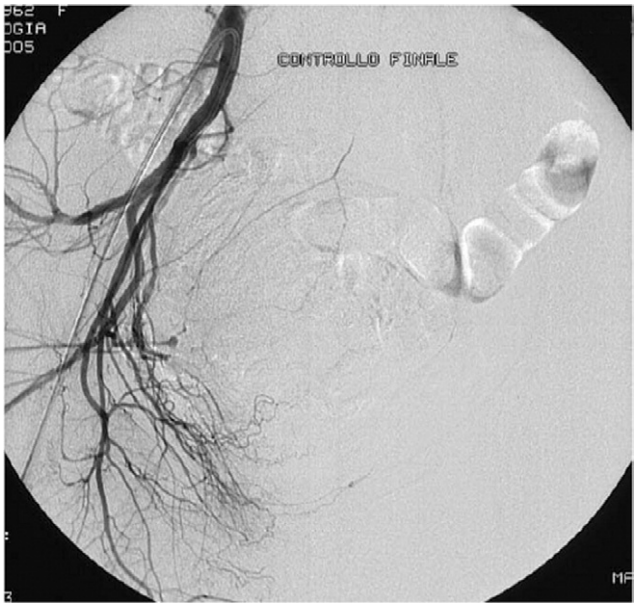

Figure 4 Patient No. 3. Postembolization angiogram of the right uterine artery. The vascularity of the mass and myoma was significantly reduced, and the distal arteries are not present.

caused by the temporary reduction of the uterine flow. The patient was discharged in stable condition after 3 weeks.

Description of the procedure

The BUAE was performed with patients under general anesthesia. The procedure consisted of a percutaneous catheterization of the right femoral artery and both hypogastric arteries via a 5F head hunter–shaped catheter. Selective embolization of the uterine arteries was performed with Spongostan/Surgifoam (gelatin-based absorbable hemostats; Johnson and Johnson Management, Berkshire, UK) for patients 1 and 2 and Embosphere microspheres 500 to 700 μm (acrylic polymer impregnated with gelatin; Biosphere Medical, Rockland, MA) followed by Bead Block Compressible Microspheres 700 to 900 μm (polyvinyl alcohol, water soluble synthetic resin; Terumo Medical Corp., Somerset, NJ) for patient 3.

Cessation of vascular uterine circle and complete absence of the myoma vascularization was provided by control angiography (Figure 4). No complication was observed immediately after each procedure.

Discussion

The use of in vitro fertilization, embryo transfer, and microsurgical techniques have recently increased the rate of ectopic pregnancies which are diagnosed earlier because of TV-US. However, cervical pregnancy remains a rare event with an unknown cause, although the frequency of this disease can be influenced by a combination of iatrogenic events, such as previous dilation and curettage, previous cesarean section, previous cervical surgery, or Asherman's syndrome.[2,4]

The first ultrasound report of this uncommon type of ectopic pregnancy was reported in 1978 by Raskin.[5] Even if no maternal death has been reported in the literature since 1953, patients with cervical pregnancy are considered a high-risk group for severe and potentially life-threatening hemorrhage, which may lead to hysterectomy and fertility loss.[6]

The early diagnosis of cervical pregnancy has improved with the use of ultrasound scanning, leading to a significant decline of complications. In particular, ultrasound scanning has been able to differentiate between cervical pregnancy and the cervical stage of miscarriage.[7] Transvaginal ultrasonography improves visualization and allows diagnosis by direct visualization of an intracervical ectopic gestational sac or trophoblastic mass. In cases of cervical pregnancy the implantation appears within the cervical canal and is not accompanied by decidual reaction. Therefore the trophoblastic tissue is usually directly attached to the cervical tissue with marked vascularization. A better definition of the uterine cervical invasion by trophoblast is possible with MRI. In fact, the use of MRI improves the evaluation of the tissue's invasion because it allows the visualization of proliferating chorionic villi into the fibromuscular layer.[6]

Cervical pregnancy is frequently associated with extensive hemorrhage, which, in severe cases, may be stopped only by hysterectomy. However, current treatment options permit effective conservative management in women who want to preserve their fertility. In recent years, several fertility-sparing techniques, such as cervical cerclage after curettage,[4] curettage and packing, local excision and repair, ligation of the cervical branch of the uterine arteries,[8] systemic or local methotrexate application,[9–11] local intra-sac KCl injections,[12] use of a Foley catheter balloon for compression,[13,14] and BUAE[15–17] or combination of these,[18] have been proposed.

Since 1982, methotrexate administration represents the most conservative approach. Furthermore, its effectiveness is reduced in particular conditions, such as serum β-hCG concentration greater than 10 000 IU/L or a gestational age greater than 9 weeks, presence of fetal activity, and CRL greater than 10 mm.[19] Methotrexate may be also administered as a medical pretreatment to a planned hysterectomy or a very cautious dilation and curettage, because it decreases vascularization of ectopic mass.[20]

Another method to decrease the mass vascularization is BUAE, which stops nutritional support to the trophoblast. The removal of gestational sac and placental tissue is usually followed by bleeding from the vessels supplying the placenta. By selective embolization of 1 or both uterine arteries, the bleeding is usually controlled. This procedure can be performed alone or in association with methotrexate administration. Table 3 shows the main case or series of cases of cervical pregnancy treated with BUAE.

Table 3 Main case reports of cervical pregnancy

Authors	No. of reported cases	Type of intervention	Outcomes
Nappi et al[16]	1	Methotrexate administration + BUAE + vacuum evacuation and curettage + tamponade with Foley catheter within the cervical canal	Successful hemostasis Preservation of uterus
Takano et al[17]	1	Selective BUAE	Stopping of vaginal bleeding Immediate decrease of β-hCG Preservation of uterus
Trambert et al[21]	5	One selective pelvic embolization	Successful hemostasis
	2	Two selective pelvic embolization	Preservation of uterus
	1	One selective pelvic embolization + methotrexate administration	One blood transfusion required
Has et al[22]	1	BUAE + methotrexate administration + vacuum evacuation and curettage	Minimal hemorrhage Preservation of uterus Long-term amenorrhea after the procedure
Yitzhak et al[23]	1	Methotrexate intra-arterial administration + selective bilateral hypogastric artery embolization	Successful hemostasis Preservation of uterus
Su et al[24]	1	Selective BUAE	Rapid decrease of β-hCG Preservation of uterus
Gui et al[25]	1	BUAE + methotrexate administration + BUAE + curettage	Preservation of uterus
	1	BUAE + methotrexate administration + BUAE + curettage	Preservation of uterus
Sherer et al[26]	1	Methotrexate administration + BUAE + tamponade with Foley catheter within the cervical canal	Complete resolution Preservation of uterus
Vilos et al[27]	1	Transfemoral BUAE + resectoscopic evacuation of the gestational product	Minimal maternal morbidity Preservation of uterus
Einarsson et al[28]	1	Systemic methotrexate + selective uterine embolization	Spontaneous expulsion Vaginal bleeding requiring a tamponade with a Foley catheter Preservation of uterus

β-hCG = β-human chorionic growth hormone; BUAE = bilateral uterine artery embolization.

Bilateral uterine artery embolization for the management of cervical pregnancy was first described in 1990.[15] It was useful to greatly reduce bleeding caused by ectopic pregnancy. With patients under general anesthesia or conscious sedation, a special catheter is introduced through the femoral artery. Catheterization is extended to the internal iliac artery and then to the uterine arteries and is followed by injection of a thrombotic substance (polyvinyl alcohol, gelatin sponge). Bilateral uterine artery embolization was never performed at a gestational age later than the seventh week until 1999, when it was first successfully performed at 12 weeks.[16]

Although BUAE could be useful in emergency conditions after heavy vaginal bleeding,[29] we performed BUAE before dilation and curettage to prevent hemorrhage as showed by other authors.[16,30] With this prophylactic procedure we decreased the risks of hemorrhagic complications, reducing blood flow from the descending cervical arteries and allowing the evacuation of the products of conception with low blood loss. In this series, the immediate success rate for controlling the bleeding was 100%. This result is in agreement with a series of 8 cervical pregnancies treated by means of selective embolotherapy either as emergency or nonemergency procedure.[21] In that report, BUAE was performed with or without methotrexate administration, no case required the hysterectomy, and the control of the hemostasis was obtained in all cases.[21]

The clinical course and follow-up was uneventful for 2 patients who resumed normal menstruation 1 month after BUAE. The patient with the uterine myoma was treated with hysterectomy after 2 weeks because of a febrile status

resistant to antibiotic therapy because of ischemic degeneration of the myoma.

A comprehensive Medline search from 1975 revealed that this appeared to be the second case of cervical pregnancy with coexisting leiomyomata. In the first case reported,[22] the management of the cervical pregnancy consisted of BUAE followed by intraamniotic injection of methotrexate 70 mg. No remarkable bleeding was observed, but the patient complained of amenorrhea until 11 months after the intervention. [22]

Although a long-term outcome study on uterine embolization of leiomyomata reported the failure of the procedure, with failure defined as subsequent hysterectomy, myomectomy, or repeated embolization in 13.7%, 4.4%, and 1.6% of cases, respectively,[31] when the cervical pregnancy is associated with a uterine leiomyomata, the prognosis for future fertility could be poor, as noted in our third case. In this regard, with BUAE we intended to reduce blood loss and decrease the size of myoma. In fact, Has et al[22] showed a reduction in myoma size after BUAE of about 70%. Furthermore, myoma degeneration may be a potential complication because of the deep and acute reduction in uterine vascularization.

Conclusion

Even if modern diagnostic and treatment options provide an opportunity for conservative treatment of cervical pregnancy, during counseling it is critical to explain carefully that potential complications can make extirpative treatment necessary.

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