**Highly Active Antiretroviral Therapy and adverse pregnancy outcome in Ouagadougou, Burkina Faso**

Francesca Cervi a M.D., Brunella Guerra a M.D., Jacques Simpore b Ph.D., Virginio Pietra e M.D., Halima Tougre b Pharm.D., Francesco Castelli d M.D. FCRF, Antonio Farina a M.D. Ph.D., Nicola Rizzo a M.D.

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**INTRODUCTION**

Integrated programmes for the prevention of Mother-To-Child-Transmission (MTCT), including administration of antiretroviral (ARV) drugs to women during pregnancy and labour and to the infant in the first weeks of life, elective caesarean delivery and complete avoidance of breast-feeding have reduced the risk of vertical transmission from 15-30% to under 2% (Cooper 2002; Coll 2002). These strategies are still not available in many resource-constrained settings: elective caesarean delivery is seldom feasible (Stanton 2006) and it is often neither acceptable nor safe for mothers to refrain from breast-feeding. Therefore ARV regimens, namely Highly Active Antiretroviral Therapy (HAART), are highly recommended by international Guidelines for the prevention of MTCT (WHO 2006). However the widespread use of HAART among HIV-infected women has raised the question of its safety for mother, foetus and infant. Apart from the known adverse effects, some reports from Europe suggested that use of antiretroviral therapy during pregnancy may be associated with substantially increased rates of prematurity and low birth weight (LBW) (Lorenzi 1998; ECS 2003; Thorne 2004). Few studies have addressed this problem in resource-constrained countries where an increasing number of women need HAART administration for their own health and to prevent MTCT.

In Burkina Faso, MTCT is a main transmission pathway of HIV, and contributes to >5000 new child infections per year (UNDP 2001). In 2002, a national programme to prevent mother-to-child transmission of HIV was launched by the Ministry of Health following WHO guidelines. Our aim was to explore the association between HAART administration and adverse pregnancy outcomes and LBW of newborns from women on HAART started after the first trimester.

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**ABSTRACT**

Objective: The administration of highly active antiretroviral therapy (HAART) to HIV-infected pregnant women raised the question of the association with adverse pregnancy outcomes. There is limited information regarding use of HAART during pregnancy in resource-constrained settings, where an increasing number of women need HAART administration. Our aim was to explore the association between HAART administration and adverse pregnancy outcomes and low birth weight (LBW) in a resource-limited setting. Methods: A retrospective cohort of HIV-infected pregnant women enrolled in the programme of Prevention of Mother-to-Child Transmission between 2003 and 2007 in Ouagadougou, Burkina Faso, was considered. Age, CD4+ T lymphocyte count, type and timing of antiretroviral drugs administration, pregnancy outcome, paediatric infection and birth weight were evaluated. Data were analysed using univariate analysis and binary logistic regression. Results: 678 HIV-infected were enrolled: 395 women received prophylactic regimen and 283 HAART regimen (115 started prior to conception, 168 started after the first trimester). Statistical analysis raised CD4+ T cell count < 200/mm3 as the only significant predictive factor for an adverse pregnancy outcome (A.O.R. = 3.9; P = 0.03). Women on HAART started later presented major incidence of LBW infants. This group contained the largest percentage (73.6%) of severe immunodepressed women, with unknown HIV status. Conclusions: Advanced immunocompromised status is a predictive factor for adverse pregnancy outcome. Unknown HIV-status and CD4+ T cell count lower than 200/ mm3 were significantly associated with LBW of newborns from women on HAART started after the first trimester. HAART is not a significant risk factor for adverse pregnancy outcome or LBW.
METHODS

Setting
This observational retrospective study included HIV-infected women followed at the Saint Camille Medical Centre (SCMC) in Ouagadougou, Burkina Faso between 2003 and 2007. The SCMC is a large mother-and-child health facility managed by the Camillian fathers, where >7000 deliveries take place every year (CMSC 2004). It has been the pilot centre of the national programme for prevention of MTCT, with the collaboration of the Institute for Infectious and Tropical Diseases and the Paediatric Department of the University of Brescia (Italy) and the NGO Medicus Mundi Italia, for the ESTHER Project (Ensemble pour une Solidarité Thérapeutique inter Hospitaliere En Réseau). The most important interventions to prevent MTCT consist in: a) voluntary counselling and testing offered to all pregnant women by an opt-in approach; b) administration of ARV drugs to mother and child in prophylaxis or HAART regimens following WHO recommendations (WHO 2006); c) informed choice between exclusive breast-feeding and early weaning at 4-6 months or formula feeding (available free of charge); d) follow-up of the mother and child, with the involvement of any other children and partner. Between 2004-2006 significant reduction of MTCT was achieved by these strategies, obtaining a rate very low rate of MTCT (Simpore 2007).

Materials
Data were collected from registers of the SCMC (Obstetric Service, Paediatric Service, Delivery Room, Neonatal Pathology Service, and the Counselling Service for HIV-infected adults). Pregnant women enrolled in the MTCT prevention program between 1st January 2003 and 31st December 2007 were evaluated. Women lost to follow-up prior to the end of pregnancy and women pregnant at the end of the study period were excluded.

Measures of interest for each woman with a diagnosis of pregnancy included: a) age (years); b) serological diagnosis of HIV 1 or HIV 2 infection performed sequentially using the two rapid Determine® and Genie-II® tests (discordant results were confirmed by an ELISA test); c) CD4+ T lymphocyte count (cells/mm³) closest to conception by FACS Count (last 6 months prior to conception or the first month of pregnancy were evaluated); d) type and timing of ARV drugs administration; e) pregnancy outcome; f) result of qualitative RT-PCR for diagnosis of paediatric HIV 1 infection undertaken at one and six months of age; g) birth weights of live-born infants.

Women were divided into three groups on the basis of the type and timing of ARV drugs administration: a) PROPHYLAXIS group: HIV-infected pregnant women receiving ARV prophylaxis; b) HAART 1 group: women receiving a HAART regimen started prior to conception; c) HAART 2 group: women starting a HAART regimen after the first trimester. The HAART 2 group included women receiving serological HIV-infection diagnosis during pregnancy, when late access to antenatal care prevented ARV administration in the first trimester. Indications for HAART started during pregnancy met the WHO 2006 criteria: WHO clinical stage 4 irrespective of CD4+ T cell count; WHO stage 2 or 3 and CD4+ T cell count <350 cells/mm³; or CD4 T cell count <200 cells/mm³.

Abortion, stillbirth and maternal death were defined as adverse pregnancy outcomes. LBW was defined as a value lower than 2500 grams. The exact gestational age of pregnancies enrolled could not be determined because of the uncertain reported date of last menstrual period by women and the unavailability of ultrasound dating. CD4+ cell counts were classified into the four categories of immunodepression: severe (<200 cells/mm³), advanced (200–349 cells/mm³), moderate (350–499 cells/mm³) and absent (≥500 cells/mm³).

Statistical analysis
Quantitative variables were analysed using descriptive statistics. To examine the association between categories and variables Chi Square Test and Student’s “t” test were used. Binary logistic regression was used to calculate the odds ratio of the predictive variables on outcomes of interest. Different regression models were implemented with the following study topics: possible interactions, multicollinearity and contrast analysis to assess and maximize the odds ratio associated with each predictor. The level of significance was set at a p-value of <0.05. Epi Info 6 (CDC, U.S.A.) and SPSS for Windows (Inch, Chicago) statistical software packages were used.

RESULTS

911 pregnant HIV-infected women registered at the MTCT Prevention Service (Figure 1) during the study period: 107 were lost to follow-up prior to delivery and 110 were excluded due to unavailable or incomplete data. Sixteen non-compliant women not taking any ARV treatment, in spite of clear indications, were excluded. The final sample consisted in 678 HIV-seropositive pregnant women aged between 17 and 45 years (mean = 29.1 yrs ± SD 4.9) with 662/678 (97.6%) HIV 1 mono-infection, 8/378 (2.1%) HIV 2 mono-infection and 8/678 (1.2%) HIV 1/HIV 2 co-infection. Women were divided into three groups as shown in figure 1.

Different protocols were used in the PROPHYLAXIS group: the majority received Nevirapine (NVP) single-dose during labour; HIV-2 infected women received two daily Zidovudine (AZT) doses starting from approximately the 16th week of gestation and one dose during labour; a restricted number of pregnant women enrolled after WHO guidelines revision (2006) had two daily AZT doses from the 28th week of gestation, a single dose of NVP+AZT and Lamivudine (3TC) intrapartum and AZT+3TC in the first week of post-partum. Women on HAART received the standard combination therapy recommended by international guidelines (WHO 2006): two nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTIs) and one non-nucleoside analogue reverse transcriptase inhibitor (NNRTI). In particular we used AZT or Stavudine (if anaemic) and 3TC as NRTIs, and NVP as NNRTI. Twenty women received Efavirenz as NNRTI, assigned FDA pregnancy category D, based on teratogenic effects observed in animals and on neural tube defects reported in humans (Fundaró 2002), that was not changed because diagnosis of pregnancy occurred when EFV has probably exhausted its teratogenic effects. Women who were HIV-2 infected or experienced unfavourable Nevirapine reactions had NVP replaced with a protease inhibitor (PI) (Indinavir or Lopinavir), known for controversial association with an increased risk of preterm delivery (ECS 2003; Cotter 2006).

Mean ages in the three groups were 22.8 (± SD 5.0)
Pregnant women delivered 645 live-born infants, but only 374 children were born by women who remained in our center. At the first and the sixth months of their life the RT-PCR test detected 21/374 (5.6%) children infected with HIV and 353/374 (94.4%) children were found negative for HIV infection. All infected children were belonged to PROPHYLAXIS group, none of them were delivered from women on HAART (HAART 1 and HAART 2 groups) (p<0.001).

Irrespective of the HIV neonatal status, 33 adverse pregnancy outcomes were recorded, as shown in table 1: six abortions, one maternal death and six stillbirths in the PROPHYLAXIS group, eight abortions and six stillbirths in the HAART 1 group and two abortions, four maternal deaths and three stillbirths in the HAART 2 group. The different distribution of adverse outcomes in the three groups did not reach statistical significance. Multivariate analysis showed a positive trend of association of adverse outcome in the HAART 1 group, but HAART was not a significant predictive factor for adverse outcome (Table 1).

Distribution of adverse outcomes observed in relation to immunodeficiency categories showed a significant association with lowest CD4+ T cell count level (<200/mm³) (Table 1). The risk stratified by multivariate analysis in the four immunological categories showed a progressive odds increase with decreasing CD4+ T cells levels, with a significant prediction of adverse outcome for the severe immunocompromised category (CD4+ T cells <200/mm³; A.O.R. =3.9, C.I. = 1.08-14.00).

Pregnant women delivered 645 live-born infants, with a birth weight between 750 and 4540 grams (means value= 2799 ± SD 500 grams): 153 were low birth weight infants (<2500 grams) distributed in the three groups of comparison as shown in table 2. Univariate analysis reported a significant major incidence of LBW newborns in the HAART 2 group (34%) compared with the PROPHYLAXIS and HAART 1 groups. Multivariate analysis found a negative association trend with LBW in the HAART 1 group and a positive trend of association in the HAART 2 group, not statistically significant. None of the studied variables showed a significant odds increase for low birth weight (Table 2).

DISCUSSION

HAART was not a significant risk factor for adverse pregnancy outcome like abortion, stillbirth or maternal death in a population of HIV-infected pregnant women living in a resource-limited setting like Burkina Faso. Adverse outcomes distribution in the three groups of women did not show any significant associa-
tion with the ARV regimen.
Severe laboratory documented immunodepression was the most important predictive factor of adverse outcome in the multivariate analysis: the highest incidence was observed in the most severely immunocompromised women, with about a fourfold increase in risk compared with women belonging to higher CD4+ T cell count categories. This finding confirms data of the pre-HAART era, wherein adverse pregnancy outcomes were associated with low maternal CD4 percentage (Stratton 1999).

Although immunological conditions cannot be severed from therapeutic intervention, our results indicate that HAART is not related to an unfavourable pregnancy outcome. Tuomala et al. (2002) reported a large retrospective study on 2123 HIV-infected women who received some form of ARV treatment and 1143 women who did not receive any. Both groups had similar rates of low Apgar scores, preterm births, and low-birth-weight infants, and also after adjustment for multiple risk factors, combination ARV treatment was not associated with an increased risk in comparison with monotherapy. Cotter et al. (2006) confirmed this observation in a cohort of 999 women, finding no differences in rates of LBW and stillbirth, regardless of therapy, adjusted for CD4+ count, CDC disease stage, and multiple risk factors of adverse outcomes.

LBW has long been recognized as a major risk factor for mortality in the neonatal period, particularly in a resource-limited setting where maternal health is already influenced by nutritional privation in the pre- and post-natal periods (McCormick 1985). In 2006,

Table 1_ Adverse outcomes: distribution and predictive factors (n°=678).
*A.O.R.s=Adjusted Odds ratios, 95%C.I.=confidence interval

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<th>Multivariate analysis</th>
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Table 2_ Low birth weight infants: distribution and predictive factors (n°=645).
*A.O.R.s=Adjusted Odds ratios, 95%C.I.=confidence interval

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the Health Minister of Burkina Faso recorded a 21.2% incidence of LBW infants among the general population of our study region (Centre) (MoH/BF 2006). A study from South Africa demonstrated that LBW is a substantial factor of increased mortality (AOR = 8.3; P < 0.001) and HIV status by eight weeks of age (Rollins et al. 2007). LBW infants accounted for 23.7% of liveborns in our sample, with a significantly lower incidence (17.8%) in newborns from women on HAART started prior to conception, followed by women on prophylaxis and women on HAART started after the first trimester, with the highest incidence (34.0%). A recent African study (Ekouevi 2008) found association between LBW and HAART in women with advanced HIV disease, after adjustment on maternal CD4+ cell count, WHO stage, age and maternal BMI, HAART started prior to conception and during pregnancy and maternal body mass index at delivery. Also a study from Ireland and the UK reported that HAART was slightly associated with lower birth weight and an increased risk of stillbirth in comparison with exposure to mono/dual therapy, but not so strongly in clinical terms (Townsend 2007). Recent findings from a large cohort in the US suggested that HAART is associated with improved obstetric outcomes, including LBW (Tuomalais 2005). Similarly, data from paediatric spectrum of HIV Disease (a retrospective study of 14464 infants) showed a decline of LBW from 35% to 21% coinciding with a period (1989–2004) when any maternal antiretroviral therapy use increased from 2% to 84%, and therapy for maternal treatment and perinatal HIV prevention evolved into highly potent HAART regimens (Schulte 2007).

The highest percentage of LBW infants found in our HAART 2 group is more probably due to the immunological conditions of these women than to the effect of ARV drugs on foetal development. Even if categories of CD4+ T cell count were not independent variables predictive for LBW, distribution of categories was highly significant among the three groups: 75% of women starting HAART after the first trimester had a CD4+ T cell count lower than 200/mm³. As Tuomalais et al. (2002) suggested, this difference may reflect an effect of the stage of maternal HIV disease on birth weight. In fact, the HAART 1 group comprised women followed by adult services for HIV-infected patients, pregnancy was most frequently programmed, allowing for clinical and immunologic disease progression and even when unpredicted, was promptly referred to the prevention programme. The HAART 2 group usually discovered seropositive status thanks to testing offered to all pregnant women at the first antenatal counselling. Unfortunately this usually occurs after the first trimester, late for a timely initiation of ARV treatment to replace immunologic conditions. Recently, Shulte et al. (2007) reported a significant association between LBW and unknown maternal HIV status, and the declines occurred during a period, beginning in 1995, of increased emphasis on routine HIV testing of all pregnant women and close follow-up and comprehensive pregnancy management (CDC 1995).

Preterm delivery is an important cause of LBW. The association of HAART with preterm delivery was reported for the first time by the Swiss Neonatal HIV Study (Lorennzi 1998) and is still a matter of controversy. Tuomalais et al (2002) in a meta-analysis of seven studies found that combination therapy was not associated with increased rates of premature delivery, LBW, low Apgar scores or stillbirth compared with no antiretroviral therapy or monotherapy. A significant association between shorter duration of pregnancy was found in pregnancy on combination with a protease inhibitor (ECS 2000; ECS 2003), more evident when it was started prior to conception (Thorne 2004). A recent meta-analysis of 14 studies failed to find an odds increase in preterm delivery among women on ARV treatment (Kortis 2007). Our data did not allow us to analyse the incidence of preterm delivery because information on gestational age at delivery was not available. This is not surprising in a resource-limited country, where it is often not possible to date gestation exactly because of frequently inaccurate reports of the last menstrual period and the unavailability of US monitoring. Other limitations of our study consist in the lack of control over other factors potentially influencing the rates of adverse pregnancy outcomes, no information on early pregnancy loss and an inability to measure the HIV RNA load. One clinical problem with our African cohort is significant loss to follow-up. During the time period 2003–2007, only 678/911 (74.4%) were followed until delivery, with complete data, that is not so unusual in a real-life contest in a resource limited-setting.

Our results support an active strategy of offering HIV testing offer to women, especially prior to conception. This enables seropositive women to enrol in the most appropriate care programme, prompt treatment will improve their health, with best results in reduction of MTCT, positive effects on pregnancy outcomes and improved child health. Unfortunately in a real-life setting this evidence is hampered by the difficulty of testing women in the pre-conception period, a problem also encountered in industrialized countries. At the SCMC voluntary counselling and HIV testing are organized as a two-step process: information on HIV infection is provided collectively during the group health education session offered to all pregnant women, then individual counselling and HIV test free of charge are offered to all consenting women. Data from a previous study in 2002–2004 showed a rate of test acceptance of 18.1% (Pignatelli 2006).

Our study again emphasizes the importance of HIV/AIDS prevention campaigns focused on the need for screening and the availability of effective care to prevent the MTCT. Campaigns should aim to reduce the social stigma and fear of not having effective treatment that still limit access to healthcare in these settings. Integrated health programmes of women of childbearing age and their children can provide improving health benefits for both mothers and their infants, and they should be increased.

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