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'Nothing new': responses to the introduction of antiretroviral drugs in South Africa

Mickey Chopra^{a,b}, Carl Kendall^c, Zelee Hill^c, Nikki Schaay^b, Lungiswa L. Nkonki^a and Tanya M. Doherty^{a,d}

Interviews conducted in South Africa found that awareness of antiretroviral therapy was generally poor. Antiretroviral drugs were not perceived as new, but one of many alternative therapies for HIV/AIDS. Respondents had more detailed knowledge of indications, effects and how to access alternative treatments, which is bolstered by the active promotion and legitimization of alternative treatments. Many expressed a lack of excitement about the introduction of antiretroviral therapy, and little change in their attitudes concerning the epidemic.

The introduction of HIV treatment in developed countries has been credited with reducing the denial, stigma and discrimination of HIV/AIDS as a disease [1]. In South Africa the introduction of antiretroviral drugs seems to have had a limited effect on high levels of stigma [2], risk behaviour [3] and HIV incidence [4]. We set out to examine the potential cause of this lack of a wider effect especially with respect to the influence of other treatment options.

We conducted a total of 197 interviews across three districts where antiretroviral therapy had been introduced for at least 6 months. Interviews were conducted in three populations: 76 individuals on antiretroviral therapy, 58 HIV-positive women not taking antiretroviral drugs, 45 community members, and 18 community gate-keepers such as leaders of non-governmental organizations, health workers and representatives from local government.

Respondents were interviewed using pretested semistructured guides. Transcripts were reviewed daily by site supervisors to identify emerging themes and ensure quality. Interviews were analysed through the systematic identification and coding of themes. The results were then discussed and consensus was reached in two analysis workshops by all authors. Ethical approval was granted by the University of the Western Cape and Tulane University.

Only seven of the 33 community respondents who had heard of antiretroviral agents reported them as a factor influencing the lifespan of a person with HIV, and only 10 reported antiretroviral treatment as a benefit of having an HIV test. Some explicitly expressed a lack of

excitement about antiretroviral therapy: 'When I asked if he had heard of the new HIV medication he looked excited and said "no"... As soon as I mentioned the word ARVs he said "oh those" and seemed to lose interest... I asked if people were excited about the provision of ARVs, he said "no they are nothing new, because they have said they are not curing, it's still the same"' (Community leader). A lack of excitement was also expressed by health workers: 'They (nurses in community clinics) just see it as another thing they have to do.' (Antiretroviral therapy nurse). Excitement was higher among the HIV-positive respondents: they reported that being on antiretroviral drugs had improved their health and given them hope, and nearly all of the 28 HIV-positive women who had heard of antiretroviral treatment reported that they would like to be on therapy at some point; however, only eight of the women had tried or were in the process of trying to access treatment.

All respondent groups reported that antiretroviral drugs were not a new class of treatment but, along with 21 other mass-produced 'immune boosters' also listed, were classified as boosting a person's 'body soldiers' (immune system); antiretroviral agents were, however, seen as particularly strong and powerful. Almost half (40%) of the antiretroviral therapy respondent group reported that they had used one or more of the alternative treatments before starting antiretroviral therapy.

Alternative treatments were popular despite their cost, which, for some respondents, consumed a large proportion of their income. We found several reasons why alternative treatment was more attractive. Many of the alternative treatments have been available for many years and knowledge of them is much greater than knowledge of antiretroviral drugs as regards details such as where to access them and how to take them.

Manufacturers and sellers of alternative treatments have adopted strong and successful marketing strategies, whereas antiretroviral therapy awareness activities are relatively low key and are focused on providing factual information rather than on a hard sell (Table 1). Alternative treatments have been legitimized through several routes (Table 1), and there is a strong belief that they are efficacious. Because many of the alternative treatments are not specifically targeted towards HIV they can be used without disclosure or stigmatization, and before formal testing.

Finally, alternative treatments are widely distributed, do not require a long wait or a prescription, and can be

Table 1. The marketing and legitimization of HIV medicines.

The marketing of HIV medicine		
	Antiretroviral drugs	Alternative medicines
Reported sources of information	Support groups, clinics, newspapers, radio, TV, word of mouth	Clinic, radio, newspapers, magazines, television, door to door selling, cars with loud speakers, word of mouth
Reported message	Improves health but doesn't cure It isn't for everyone Take it for life, don't miss doses and expect side effects	It works, buy it here, take it like this
Strength of marketing	Low It is somebody else's job 'They (church) are happy ARVs are available but are not very interested in them because they see them as a medical procedure.' (Local minister) 'They (community coordinators) don't see ARV provision as more than what goes on in the district hospital.' (Head of ARV clinic) There are concerns that 'marketing' could increase demand and overburden the health system	High Financial rewards can be great 'He knew a traditional healer that healed his friend. His friend paid R10,000 for the whole treatment. He was the richest traditional healer in X because he had a jet first time in the history of traditional healers.' (Community respondent)

The legitimization of alternative therapies

Health workers recommend:

'The Sister confirmed the results and started counseling. The nurse advised that the respondent should go on a healthy diet, buy Cell Food, which cost R200.00 a small bottle and to come back for more counseling whenever she feels like.'
(ARV respondent)

Health workers distribute:

'She mentioned that Tianshi Chinese boosters, which were used by her cousin who also passed away last year. They used to cost R500 a month and her cousin used to buy these medicines from the nurse working at Y.'
(Community respondent)

Treatments are medicalized:

'The respondent said Mr A is working with medical doctors from the University of X. Mr A praises himself and says he is curing HIV.'
(ARV respondent)

'She heard and saw on TV a traditional healer that was living at X. . . she visited this traditional healer. . . she was taken her saliva and told to come back after a week. After a week she went back and the traditional heather told her that she is HIV positive. She bought 2 of traditional medicines and each bottle (750 ml) cost R20.00.'
(ARV respondent)

Tradition beliefs are optimized:

'Mr B gets all the ingredients from his ancestors who were also using muti (traditional medicine) when they were still alive. His knowledge of muti was further given power by talking to other nyanga (healers) like the late C from Y.'
(Community respondent)

accessed regardless of the stage of the illness. Antiretroviral drugs were available free of charge in all sites but there is a lengthy enrolment process. Antiretroviral drugs were perceived as being only for the critically ill, and the HIV-positive respondents reported using alternative treatments when they (or health staff) did not consider themselves sick enough for antiretroviral drugs or had difficulty accessing them: 'He asked the nurse about ARVs. . . The nurse said he is still strong and he can survive and advised him to seek traditional medicines. He went to a traditional healer that he had heard about in the community. . . He paid R500 and lived at the traditional healer's for some time so that he could take the medicines well.'
(Antiretroviral therapy respondent).

Antiretroviral therapies are being introduced into a complex HIV treatment environment where they are competing with a multitude of alternative treatments. The choices are overwhelming and range from age old indigenous 'cure-alls' to new herbal 'boosters'. Respondents across all three sites had more detailed knowledge of

the indications, effects and how to access these alternative treatments than they did of antiretroviral drugs. This is being bolstered by the promotion and legitimization of alternative treatments and the contrasting lack of similar activities for antiretroviral therapy.

There could be some selection bias in this study as neither the three sites nor the respondents were randomly selected. However, each of the three sites is representative of most of the different types of contexts seen across South Africa, and a relatively large number of respondents with a range of different characteristics were interviewed for this study.

The relatively poor acknowledgement, by senior politicians, state authorities, widely publicized 'experts' and members of the community, of the positive effects of antiretroviral drugs and the concomitant lack of excitement among those not already on treatment may impair both individual adherence to treatment and the possible HIV prevention benefits of antiretroviral treatment. A

more explicit communication strategy that promotes the benefits of antiretroviral therapy is required. If anti-retroviral agents are to compete more successfully in the therapeutic continuum, there needs to be explicit recognition of, and further strategies to counter, the attraction of alternative therapies for patients and the systematic promotion these treatments receive, including from professional health workers.

^aMedical Research Council, Tygerberg, South Africa; ^bSchool of Public Health, University of the Western Cape, Cape Town, South Africa; ^cTulane School of Public Health and Tropical Medicine, New Orleans, USA; and ^dHealth Systems Trust, Durban, South Africa.

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Unexpected drug–drug interaction between tipranavir/ritonavir and enfuvirtide

Daniel González de Requena, Andrea Calcagno, Stefano Bonora, Laura Ladetto, Antonio D'Avolio, Mauro Sciandra, Marco Siccardi, Olivia Bargiacchi, Alessandro Sinicco and Giovanni Di Perri

Fifty-five patients placed on tipranavir/ritonavir 500/200 mg twice a day (27 with enfuvirtide and 28 without) underwent tipranavir and ritonavir plasma concentration measurements by high-pressure liquid chromatography. Markedly higher tipranavir and ritonavir trough concentrations were observed in enfuvirtide recipients. The modelling of sparse plasma samples using a first order absorption and elimination monocompartmental model without time lag predicted higher tipranavir elimination half-life and volume of distribution in

enfuvirtide takers. This unexpected drug–drug interaction warrants further investigation.

The association of enfuvirtide and tipranavir/ritonavir (TPV/RTV) has been shown to be effective as a core of salvage HAART [1], and has become a primary option for multi-experienced patients. Even if no drug–drug interactions are expected between TPV/RTV and enfuvirtide, no data have yet been obtained. In our unit, where therapeutic drug monitoring is done on a regular basis, tipranavir, ritonavir and enfuvirtide plasma levels were measured in a cohort of patients enrolled in the Tipranavir Expanded Access Programme.

Patients administered with TPV/RTV (500/200 mg twice a day) plus two nucleoside reverse transcriptase inhibitors with or without enfuvirtide (90 mg subcutaneously twice a day) were considered. Subjects not taking concomitant interacting drugs and with self-reported compliance of more than 90% in the past week were evaluated. Plasma samples were obtained at scheduled follow-up visits, and tipranavir and ritonavir plasma concentrations were measured using a validated high-pressure liquid chromatography method with ultraviolet detection. Samples obtained between 11 and 13 h after the last TPV/RTV dose intake were considered as a trough concentration (C_{trough}), and comparison according to the concomitant administration of enfuvirtide was performed by using individual mean values. Modelling of sparse plasma samples was performed by using a first order absorption and elimination monocompartmental model without time lag. Time-averaged plasma tipranavir and ritonavir concentrations from each patient were modelled as naive pooled data according to enfuvirtide administration. Initial estimates of the volume of distribution ($V_d = 10\text{ l}$), constant of absorption ($K_a = 0.49/\text{h}$), and constant of elimination ($K_e = 0.1/\text{h}$) were obtained from a previous population pharmacokinetic study [2]. Parameter boundaries were allowed to be estimated by the software WinNonLin. This software was used for the modelling and estimation of pharmacokinetic parameters. Finally, tipranavir C_{trough} was sequentially measured in subjects in whom enfuvirtide was either discontinued or added to a tipranavir-based regimen. Student's t -test was used to study the differences between groups. Values were given as ng/ml.

A total of 463 samples from 55 subjects (27 with enfuvirtide, group A, and 28 without, group B) were considered. No differences in sex (male: 81.4% group A versus 82.1% group B, $P = 0.4$), weight (69 versus 70 kg, $P = 0.8$), height (175 versus 175 cm, $P = 0.73$), and hepatitis C virus co-infection status (18.5 versus 10.7%, $P = 0.54$), were observed between groups.

A total of 194 C_{trough} samples were considered (105 from group A). The mean (\pm SD) tipranavir and ritonavir C_{trough} concentrations were 34 431 ng/ml ($\pm 20\,010$) and

279 ng/ml (± 269), respectively. A higher mean tipranavir C_{trough} was observed in group A ($41\,069 \pm 20\,174$ ng/ml versus $27\,261 \pm 17\,516$ ng/ml, $P=0.011$), as well as a higher mean ritonavir C_{trough} (371 ± 333 ng/ml versus 188 ± 139 ng/ml, $P=0.017$). The mean (\pm SD) enfuvirtide C_{trough} concentration in subjects in group A was $3610 (\pm 1399)$. No correlation between enfuvirtide and either tipranavir or ritonavir C_{trough} values was detected.

The modelling of individual averaged tipranavir plasma concentrations gave a correlation coefficient (R) of 0.49 for group A and 0.615 for group B. A higher volume of distribution (V_d/F) (9.85 versus 6.5 l) and lower constant of elimination (K_e) value (0.07 versus 0.13/h) were observed in group A compared with group B, whereas the constant of absorption (K_a) was similar in both groups (0.66 versus 0.65/h). Half-life elimination ($t_{1/2}$) was 9.69 and 5.36 h in groups A and B, respectively. A higher minimum concentration was predicted in group A (41 812 versus 26 668 ng/ml), without marked differences in the maximum concentration (71 955 versus 67 779 ng/ml) and area under the concentration time curve (709 481 versus 595 490 ng/h per millilitre). The modelling of ritonavir concentrations gave $R=0.35$ for group A, and $R=0.47$ for group B. Results similar to those for tipranavir were observed for ritonavir, the differences being more pronounced in V_d/F (115.8 versus 95.5 l) and K_e (0.21 versus 0.34/h). Differences were also observed in the half-life (3.17 h in A versus 2 h in B) and in the predicted minimum concentration (385 ng/ml in A versus 189 ng/ml in B), whereas no appreciable differences were predicted either for the maximum concentration (849 versus 859 ng/ml), or for the area under the concentration time curve (7970 versus 6630 ng/h per millilitre).

In two subjects who discontinued enfuvirtide, tipranavir C_{trough} concentrations decreased by 50.8 and 25.6% (28 726 to 14 133 and 55 979 to 41 650 ng/ml), respectively. On the other hand, in the only subject who added enfuvirtide to a tipranavir-based regimen, the tipranavir C_{trough} increased from 29 045 to 50 051 ng/ml (72.3%).

To the best of our knowledge this is the first report showing significantly higher tipranavir and ritonavir C_{trough} in patients administered with enfuvirtide compared with values observed in individuals with no concomitant enfuvirtide intake (median values are reported in Fig. 1). This difference of tipranavir exposure was not attributable to an imbalance of weight, sex or liver disease between the two groups. Moreover, this trend was confirmed in two TPV/RTV takers who discontinued enfuvirtide and one who added enfuvirtide to an ongoing regimen with TPV/RTV.

The putative mechanism of such an unexpected interaction is unknown. In-vitro studies with hepatic microsomes showed that CYP 3A4 is the predominant

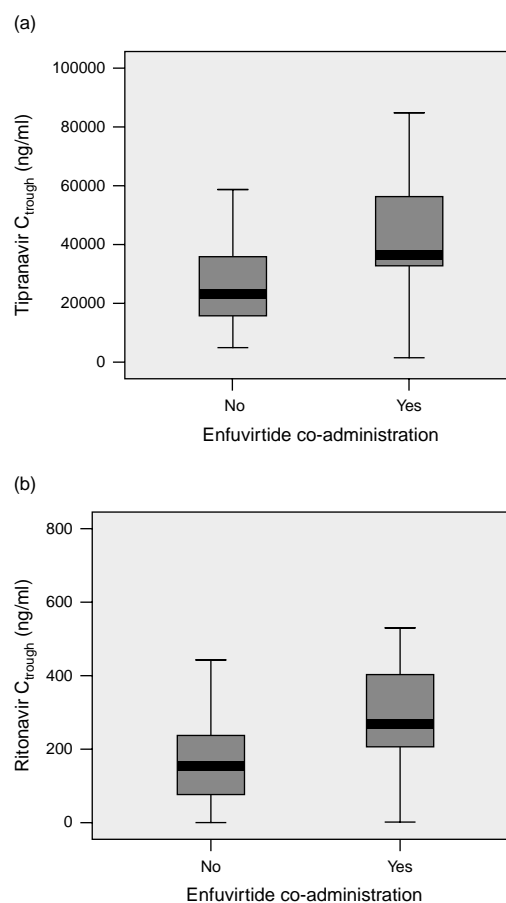


Fig. 1. Boxplot of tipranavir (a) and ritonavir (b) trough concentrations according to enfuvirtide co-administration.

Box represents the interquartile range (25–75th percentile), which contains 50% of the values. The vertical lines represent the 5th and 95th percentiles. The line across the box indicates the median value. Both median tipranavir (a) and ritonavir (b) trough concentrations (C_{trough}) are significantly different ($P=0.01$ and $P=.008$, respectively).

cytochrome P450 isoenzyme involved in tipranavir metabolism, whereas enfuvirtide is not a substrate for this metabolic pathway [3]. No clinically significant interaction was found between the latter and drugs with a high affinity for CYP3A4 [4,5]. On the other hand, enfuvirtide was found to have no significant inhibitory effect on the metabolism of probe drugs mediated by CYP3A4, CYP2D6 or *N*-acetyltransferase ($<20\%$), whereas a minimal inhibitory effect on CYP1A2, CYP2E1 or CYP2C19 was observed ($\leq 30\%$) [6]. The latter, however, have not been reported to be significantly involved in TPV/RTV metabolism.

Pharmacokinetic modelling suggested that V_d/F and half-life of both tipranavir and ritonavir could be the parameters principally affected. Therefore, an elucidation of the mechanism of this interaction needs further evaluation.

The increase of approximately 50% in tipranavir exposure associated with enfuvirtide co-administration is not negligible and could theoretically be important from a clinical viewpoint. The efficacy of a tipranavir-containing salvage regimen has been shown to depend on the drug plasma concentration, as witnessed by the predictive role of the inhibitory quotient on the virological response in RESIST trials [2]. On the other hand, hepatotoxicity related to the administration of TPV/RTV has also been reported to be concentration dependent. In the same trials, grade 3 and 4 of hepatic toxicity occurred mostly in subjects with very high plasma concentrations [7]. Therefore, the clinical impact of such a drug–drug interaction warrants further evaluation.

Department of Infectious Diseases, University of Turin, Turin, Italy.

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Cardiac isoform of alpha 2 macroglobulin, an early diagnostic marker for cardiac manifestations in AIDS patients

Subbiah Ramasamy^a, Reghunath Omnath^d, Andiappan Rathinavel^{a,c}, Perumal Kannan^e, Perundurai S. Dhandapani^a, Ponnambalam Annapoorani^a, Pitchai Balakumar^f, Manjeet Singh^f, Rajagopalan Ganesh^b and Govindan Sadasivam Selvam^a

This study investigated the possible role of the cardiac isoform of alpha 2-macroglobulin (CA2M)

as an early diagnostic marker for HIV-associated cardiovascular manifestations. A total of 349 samples were analysed by Western blot and quantified by sandwich enzyme-linked immunosorbent assay. The levels of CA2M present in sera of HIV-associated cardiac diseases were significantly higher than those of HIV without cardiac involvement and healthy sera. CA2M may act as a novel diagnostic marker to identify cardiac manifestations in HIV/AIDS patients.

HIV infection has emerged globally as a leading cause of acquired heart disease, culminating in symptomatic heart failure and a spectrum of manifestations from subclinical cardiac dysfunction to severe pericardial effusion, dilated cardiomyopathy (DCM) and pulmonary hypertension. Autopsy studies have revealed that cardiac involvement occurs in 25–40% of patients with AIDS [1,2]. With the advent of effective antiretroviral therapy, it is now possible to prolong the life expectancy of the diseased, but this has inadvertently led to an accelerated cardiovascular risk and atherosclerotic disease [2–5]. Nevertheless, HIV-associated heart diseases are not yet adequately emphasized. Viral myocarditis and direct HIV-1-mediated myocyte infection are the major causes of DCM in HIV/AIDS [1,6]. HIV-1 virions appear to infect myocytes in patchy distributions [4], but the exact underlying mechanism of HIV-1-mediated cardiac dysfunction remains unclear to date. An inflammatory process induced by HIV infection is believed to be a major pathogenic mechanism involved in myocardial dysfunction [7]. The HIV-mediated increase in TNF- α production has been shown to cause myocardial dysfunction [8]. The CD4 T-lymphocyte count and HIV-RNA levels have been the principal biological markers utilized hitherto for the clinical evaluation of HIV/AIDS patients [6,9]. However, no potential molecular marker for the timely detection of HIV-associated cardiac manifestations has yet been described. Earlier studies in our laboratory showed that the cardiac isoform of alpha 2-macroglobulin (CA2M) is a high molecular mass (182 000 M_r) serum protein, which is involved in the development of cardiac hypertrophy [10–12]. Furthermore, our studies confirmed that CA2M could be used as a diagnostic marker for cardiac diseases [13]. The present study has been undertaken to determine the correlation of CA2M levels with HIV-associated cardiovascular manifestations and the plausibility of its clinical utility as a diagnostic marker for the management of HIV infected patients.

A total of 349 samples from 90 healthy volunteers, 83 patients with different cardiac diseases without HIV infection, 127 HIV patients without cardiovascular involvement and 49 HIV patients with cardiac diseases were subjected in the present study after obtaining informed consent. This combined study has been carried out at Madurai Kamaraj University and Government

Rajaji Hospital, Madurai, India, in collaboration with Punjabi University, Patiala, India. Protocols used in this study have been approved by an institutional ethical committee. Patients were selected on the basis of a clinical evaluation, HIV status by standard tests, electrocardiogram and echocardiographic findings. Exclusion criteria were severe chronic heart failure (New York Heart Association class III/IV) and acute cardiovascular events (within 90 days before inclusion in the study). Furthermore, the sera drawn were subjected to an estimation of CA2M levels.

Young Wistar albino rats were used in the present study. CA2M protein from the sera of aorta-constricted rats was purified and antirat CA2M antisera were raised as previously described [11,14]. The immunocrossreactivity between human serum CA2M and antirat CA2M antibody was tested by Western blot analysis, and the quantification of CA2M levels was carried out by sandwich enzyme-linked immunosorbent assay [11,15].

Results were statistically analysed using repeated measures of analysis of variance, Kruskal–Wallis one-way analysis, and a post hoc test with pairwise multiple comparison using Dunn's method.

In the study participants, the presence of CA2M was identified using Western blot and was quantified by sandwich enzyme-linked immunosorbent assay. The CA2M levels of the various study groups are given in Fig. 1. The levels of CA2M present in the sera of HIV-associated cardiac diseases (143.4 ± 7.84) were significantly ($P < 0.05$) higher than those of healthy human sera (42.1 ± 4.8) and the sera of HIV/AIDS patients without cardiovascular manifestations (74.7 ± 4.43). Furthermore, it was observed that CA2M levels in the sera of HIV patients without cardiac manifestations were moderately elevated compared with the sera of healthy

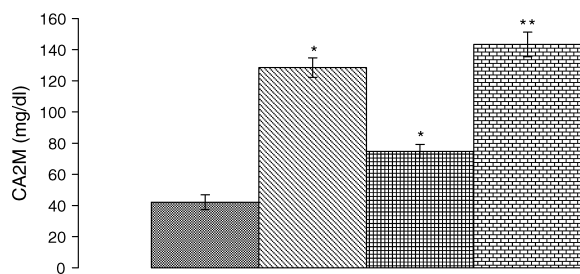


Fig. 1. Bar diagram shows levels of the cardiac isoform of alpha 2-macroglobulin in various groups. Results were expressed as mean \pm SEM. CA2M, Cardiac isoform of alpha 2-macroglobulin. * $P < 0.05$ versus healthy volunteers; ** $P < 0.05$ versus HIV patients without cardiac problems. ■ Healthy volunteers; ▨ cardiac patients without HIV; ▩ HIV patients without cardiac problems; ▪ HIV patients with cardiac problems.

volunteers. Similarly, there was a moderate elevation in CA2M levels in cardiac patients with HIV infection (143.4 ± 7.84) when compared with cardiac patients without HIV infection (128 ± 6.27).

Cardiovascular involvement in determining the morbidity and mortality in patients with HIV/AIDS has been widely reported [16]. Therefore, the earlier detection of cardiovascular involvement could assist in the risk stratification and institution of appropriate therapy for this specific subset of patients with HIV/AIDS [17]. The available modalities for assessing cardiovascular manifestations currently include non-invasive techniques such as echocardiography and invasive measures such as angiography. The available cardiac markers such as myocardial-type creatine kinase, lactate dehydrogenase and troponin I/T are ineffectual in assessing the degree of cardiac dysfunction [18,19]. No specific molecular marker has hitherto been identified to assess cardiac dysfunction in HIV/AIDS patients. Therefore, the identification of a novel serum molecular marker correlating with cardiac involvement is expected to help in the management of this subset of patients. In the present study, we found that CA2M can be used as a novel diagnostic serum marker for the early detection of cardiac involvement in patients with HIV/AIDS. Earlier, our laboratory had shown that CA2M could be an early molecular marker for identifying cardiac dysfunction in diseases such as ventricular septal defect, atrial septal defect, aortic regurgitation, aortic stenosis etc., resulting from abnormal left ventricular hypertrophy [11]. Furthermore, our laboratory showed that the in-vivo administration of purified protein of CA2M or complementary DNA of CA2M could induce cardiac hypertrophy in rats [20]. We recently reported CA2M as a potential biomarker for myocardial infarcted diabetic patients [21]. In the present study, we have shown that the level of CA2M increases significantly in HIV/AIDS patients with cardiac involvement. The noted HIV-associated cardiac diseases in our study included pericardial effusion, DCM, pulmonary hypertension and idiopathic left ventricular dysfunction. A moderate elevation of CA2M levels in HIV patients without cardiac abnormalities has also been observed. No influence of sex or age differences was observed in this study. This elucidates the role of CA2M as a novel diagnostic maker for the early identification of cardiac abnormalities in HIV/AIDS patients. Further studies correlating CD4 cell counts with CA2M levels and the monitoring of CA2M levels with antiretroviral therapy are underway. In conclusion, CA2M may play a pivotal role in the management of HIV-infected patients with cardiac manifestations.

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^aDepartment of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamilnadu, India; and Departments of ^bSexually Transmitted Diseases, ^cCardio-Thoracic Surgery, ^dMedicine and ^eCardiology, Madurai Medical College and Government Rajaji Hospital, Madurai, India; and ^fDepartment of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India.

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Effects of infant sex on mother-to-child transmission of HIV-1 according to timing of infection in Zimbabwe

Ellen G. Piwoz^a, Jean H. Humphrey^b, Edmore T. Marinda^{c,d}, Kuda Mutasa^c, Lawrence H. Moulton^b, Peter J. Iliff^c and the ZVITAMBO Study Group

We examined the relationship between sex and the risk of intrauterine, intrapartum and postnatal HIV transmission among 4495 infants born to HIV-infected mothers in Harare, Zimbabwe. Intrauterine transmission was 8.6%, and consistent with other studies was higher among girl than boy infants (AOR 1.53; 95% CI 1.23–1.91). Unlike previous studies, we observed no independent effect of infant sex on intrapartum or breastfeeding-associated HIV transmission. Sex-specific postnatal prevention strategies are not warranted in this population.

In recent reports from vertical HIV transmission studies in Europe [1] and Africa [2,3], intrauterine transmission was 1.4 to twofold higher among live-born girl compared with boy infants. In all studies, intrauterine transmission was defined as testing positive by DNA or RNA HIV polymerase chain reaction (PCR) at or near birth, intrapartum transmission as testing PCR negative at birth and positive at 4–6 weeks, and postnatal transmission as testing PCR negative at 4–6 weeks and then subsequently testing positive during breastfeeding exposure. In these analyses, the reported sex differences remained

after adjusting for a large number of established risk factors for mother-to-child transmission of HIV. Genetic, hormonal, and immunological factors have been proposed as reasons as to why HIV may either preferentially infect female infants or protect boys [3], although higher male fetal mortality cannot be ruled out as a possible explanation [4].

The picture with respect to intrapartum and postnatal transmission is less clear. In a combined analysis of data from two studies in Malawi, intrapartum transmission was higher for girls among twin-birth but not singleton-birth infants and the postnatal infection risk was similar ($P = 0.52$), but surprisingly low, for girls (3.2%) and boys (2.6%) at 18 months [3]. The Breastfeeding and HIV International Transmission Study Group (BHITS) meta-analysis of 2030 breastfed infants enrolled in nine African trials reported no sex differences for early (intrauterine plus intrapartum) HIV transmission, but a 40% lower rate of postnatal transmission among girl compared with boy infants [adjusted hazard ratio (AHR) 0.6; 95% confidence interval (CI) 0.4–0.9] [5].

To obtain a better understanding of the role of infant sex in mother-to-child HIV transmission, we examined the relationship between sex and rates of intrauterine, intrapartum, and postnatal transmission among 4495 infants born to HIV-infected mothers in Harare, Zimbabwe, participating in the ZVITAMBO postpartum vitamin A supplementation trial.

Full methods have been described elsewhere [6–9]. Mothers and their neonates were enrolled within 96 h of delivery and followed at 6 weeks, 3 months, and 3-monthly intervals up to 24 months. Mother–baby pairs were eligible if neither had an acutely life-threatening condition, if the baby was a singleton with a birth weight of 1500 g or greater, and if the mother planned to stay in Harare after the delivery. Enrollment took place from November 1997 to January 2000, before the availability of antiretroviral prophylaxis.

Intrauterine, intrapartum, and postnatal transmission were defined as in previous studies. Baseline characteristics were compared by infant sex using proportions and exact tests for binary variables and means and t -tests for continuous variables. Intrauterine and intrapartum transmissions were calculated as simple proportions. Cumulative, early (intrauterine plus intrapartum) and postnatal transmission rates were estimated by the Turnbull method [10]. The independent contribution of sex to intrauterine and intrapartum transmission was assessed using logistic regression with sex forced into the models and other variables retained at the $\alpha = 0.05$ level. Cox proportional hazards methods were used to assess the effect of sex on postnatal transmission after controlling for other risk factors [8]. Data analysis was conducted

using SAS version 8.2 (SAS Institute, Inc., Cary, North Carolina, USA).

At baseline, the maternal mean age (SD) was 25.6 years (5.0), the median plasma viral load was 4.17 \log_{10} copies/ml [interquartile range (IQR) 3.61–4.77], CD4 cell counts were 400 cells/ μ l (IQR 258–568), hemoglobin was 111.1 g/l (19.5), and mid-upper arm circumference was 25.7 cm (2.9). A total of 4070 infants (91.2%) were born via vaginal delivery, and 261 (5.9%) and 131 (2.9%) were born via emergency and elective caesarian section, respectively. The mean (SD) gestational age was 39.1 weeks (1.5), and 1690 (39.2%) of deliveries involved membrane rupture for greater than 4 h. All baseline maternal health and delivery-related characteristics were similar for male and female infants, except mean (SD) birth weight was lower for girls [2849 g (451)] than boys [2981 g (474); $P < 0.0001$].

Of the 4495 HIV-exposed infants, 2282 (50.8%) were male, slightly lower, but not significantly different to that of HIV-negative women in ZVITAMBO (51.7%) [9]. Overall intrauterine transmission was 8.6% (383/4443; 95% CI 7.2–10.0%; 27 girls and 25 boys were missing baseline PCR). Intrauterine infection was significantly greater for female (229/2186; 10.5%) compared with male infants (154/2257; 6.8%; $P < 0.0001$). After adjusting for significant baseline co-variables, girls were 53% more likely to have been infected intrauterinally (95% CI 1.23–1.91; Table 1).

Six-week PCR results were available for 3375 infants (83.1%) who were PCR-negative at baseline. The median age at the time of the 6-week visit was 41.4 days (IQR 40.4–47.3). Among the 685 intrauterine-negative infants missing 6 week data, 72 had died (46 boys and 26 girls; $P < 0.15$) and 613 did not provide a sample [337 (55.0%) boys and 276 (45.0%) girls; $P < 0.02$]. The intrapartum transmission rate was 15.0% (505/3375; 95% CI 14.1–16.5%), and was not different between the sexes (AHR 1.10; Table 1). Findings were unchanged when all infants who died before 6 weeks or who were missing 6-week data were assumed to be HIV infected (data not presented). Cumulative early (intrauterine plus intrapartum) transmission at 6 weeks was 23.6% (95% CI 22.3–24.9%), with girls having significantly higher early transmission compared with boys (25.6 versus 21.6%; $P = 0.004$) because of their increased risk of intrauterine infection.

Of the 2870 infants who were PCR negative at 6 weeks, 262 became infected during the postnatal period, and an additional 74 infants died with a last negative PCR test. Accounting for censoring caused by maternal death and weaning [10], cumulative postnatal transmission to 24 months was 15.8% (95% CI 10.5–23.1%). Infant sex was not a significant predictor of either postnatal transmission alone (AHR 1.00; 95% CI 0.77–1.31) or the

Table 1. Risk factors for intrauterine, intrapartum, and postnatal mother-to-child HIV transmission.

	Adjusted OR (95% CI) or HR ^{a,b}		
	IU (N = 4443) 383 infections	IP (N = 3375) 505 infections	PN 6 weeks–24 months (N = 2870) 262 infections
Maternal age	0.96 (0.94–0.98) P = 0.0002	–	1.04 (1.01–1.07) P = 0.007
Maternal viral load (log ₁₀ copies/ml)			
< 3.61	1.00	1.00	1.00
3.61 < 4.17	2.89 (1.52–5.50) P = 0.001	1.65 (0.91–3.00) P = 0.10	1.83 (0.86–3.87) P = 0.12
4.17 < 4.77	3.10 (1.65–5.85) P = 0.0005	1.86 (1.04–3.32) P = 0.04	2.35 (1.15–4.82) P = 0.02
≥ 4.77	6.42 (3.50–11.77) P < 0.0001	2.71 (1.52–4.84) P = 0.0008	3.73 (1.83–7.61) P = 0.0003
Maternal hemoglobin (g/l)	–		
< 70	–	2.40 (1.16–4.97) P = 0.02	2.66 (1.06–6.68) P = 0.04
70–< 90	–	1.53 (0.99–2.37) P = 0.05	1.02 (0.54–1.96) P = 0.94
90–< 110	–	1.25 (0.93–1.69) P = 0.15	1.03 (0.67–1.59) P = 0.88
≥ 110	–	1.00	1.00
Maternal CD4 cell count (μl)	–		
< 200	–	3.37 (2.43–4.67) P < 0.0001	3.74 (2.42–5.79) P < 0.0001
200–349	–	1.75 (1.30–2.35) P = 0.0002	2.21 (1.46–3.34) P = 0.0002
350–499	–	1.44 (1.06–1.96) P = 0.02	1.35 (0.87–2.10) P = 0.18
≥ 500	–	1.00	1.00
Maternal MUAC (cm)	–	–	0.94 (0.89–0.98) P = 0.01
Maternal death during follow-up	–	–	
Yes	–	–	2.07 (1.29–3.32) P = 0.003
No	–	–	1.00
Gestational age (weeks)	–	0.92 (0.86–0.99) P = 0.03	–
Duration of membrane rupture (h)	–		
< 4	–	1.00	–
≥ 4	–	1.55 (1.27–1.89) P < 0.0001	–
Birth weight (kg)	0.61 (0.48–0.77) P < 0.0001	0.78 (0.61–1.00) P = 0.05	–
Early feeding pattern	–	–	
EBF	–	–	1.00
PBF	–	–	1.39 (0.65–3.00) P = 0.40
MBF	–	–	2.23 (1.09–4.54) P = 0.03
Sex			
Male	1.00	1.00	1.00
Female	1.53 (1.23–1.91) P = 0.0001	1.10 (0.90–1.35) P = 0.36	1.00 (0.77–1.31) P = 0.98

CI, Confidence interval; EBF, exclusively breastfed (breastmilk only to at least 3 months of age); HR, hazard ratio; IP, intrapartum; IU, intrauterine; MBF, mixed solid foods (breastmilk and/or breastfed plus animal milk < 3 months before/of age); MUAC, mid-upper arm circumference; OR, odds ratio; PBF, predominantly breastfed (breastmilk plus non-milk liquids before 3 months of age); PN, postnatal.

^aRisk estimates for IU and IP infection are OR based on multiple logistic regression models and estimates for PN transmission are HR from Cox proportional hazard models as described in the text.

^bNeither maternal nor infant postpartum vitamin A supplementation was associated with increased risk of IU, IP, or PN HIV transmission [6].

combined outcome of postnatal transmission or death (AHR 0.94; 95% CI 0.75–1.19; P = 0.25).

As a result of higher intrauterine infection, cumulative HIV transmission was 41.0% (95% CI 34.1–48.3%) for girls and 35.8% (95% CI 26.2–46.8%) for boys at 24 months. There was no sex-related difference in HIV-free survival, however, caused by higher mortality in uninfected boys throughout this period (Kaplan–Meier estimates of 10.8 versus 8.8%; P = 0.03).

These data support previous observations that girls are either at increased risk of intrauterine HIV infection or that intrauterine-infected boys are at increased risk of fetal death [1–4]. Unlike the BHITS meta-analysis [4], we observed no sex effect on postnatal transmission. Our findings may vary because we were able to adjust for the breastfeeding pattern, suggested as a possible confounding variable in the meta-analysis. Elucidating the mechanisms of sex differences in intrauterine trans-

mission or fetal survival may inform new prevention strategies. Increased access to prevention of mother-to-child transmission services is urgently needed. Sex-specific postnatal prevention strategies are not warranted in this population.

^aAcademy for Educational Development, Washington DC, USA; ^bJohns Hopkins Bloomberg School of Public Health, Department of International Health, Baltimore, MD, USA; ^cZVITAMBO Project, Harare, Zimbabwe; and ^dSchool of Public Health, Wits Medical School, Johannesburg, South Africa.

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APOBEC3G genetic variants and their association with risk of HIV infection in highly exposed Caucasians

Han Sang Valcke^{a,b}, Nicole F. Bernard^c, Julie Bruneau^d, Michel Alary^e, Christos M. Tsoukas^c and Michel Roger^{a,b}

The cytosine deaminase APOBEC3G has been identified as a host factor that inhibits HIV-1 replication. We investigated whether genetic variants of APOBEC3G that could potentially affect the

protein's expression or function were associated with the risk of infection in 122 Caucasians highly exposed to HIV-1. A novel C40693T variant was significantly associated with an increased risk of infection, suggesting that there might be a role for APOBEC3G in susceptibility to HIV-1 infection that warrants further investigation.

APOBEC3G, in the absence of the HIV-1 accessory Vif gene, is packaged into HIV-1 particles during assembly, apparently through the formation of a complex with the RNA-recruiting nucleocapsid region of the Gag viral protein [1,2]. APOBEC3G subsequently associates with the viral reverse transcription complex, where it deaminates cytidine residues to uridine in the nascent minus-strand viral DNA. These deoxyuridine-rich transcripts are then either degraded or serve as templates for the synthesis of plus-strand DNA, thus yielding proviruses that are largely non-functional as a result of G→A hypermutation [3–5]. The HIV-1 Vif protein counteracts the antiretroviral activity of APOBEC3G by inducing its ubiquitination and degradation [6].

As APOBEC3G is an important host factor that may confer an intrinsic block to HIV-1, the APOBEC3G gene was screened in American [7] and European [8] cohorts to identify genetic variants that might influence the progression of AIDS. Twenty-nine single nucleotide polymorphisms (SNP) with frequencies of 1% or greater were identified in the APOBEC3G gene. Of these, three mutations are located in the 5' putative regulatory region and three SNP are located in exons. The T→C variant at codon 119 (exon 3) leads to a synonymous change and does not modify the amino acid composition of the protein. The A→G at codon 186 (exon 4) and the C→G at codon 275 (exon 6) substitutions result in amino acid changes from histidine to arginine (H186R) and glutamine to glutamic acid (Q275E), respectively. The H186R variant was strongly associated with a decline in CD4 T cells and accelerated progression to AIDS-defining conditions in African Americans, but not in American and European Caucasians [7,8].

As APOBEC3G could possibly confer resistance to HIV-1 infection, we investigated the effects of both APOBEC3G regulatory and coding region variants that may modify the protein's expression or function in a cohort of highly exposed Caucasians to HIV-1 for their influence on the susceptibility to infection.

The study population consisted of 122 Caucasian individuals exposed to HIV enrolled in prospective cohort studies in Montreal. We analysed the DNA samples of 69 individuals infected with HIV-1 by homosexual contact or injection drug use recruited during primary infection. We identified 53 exposed seronegative (ESN) individuals matched for age and sex,

Table 1. Allelic distribution of APOBEC3G single nucleotide polymorphisms among HIV-positive and exposed seronegative (HIV-negative) study subjects.

APOBEC3G		Allelic distribution (%)		
Base change ^a	Amino acid change	HIV-positive (n = 138)	HIV-negative (n = 106)	P value
Promoter region				
G-802C		3 (2.2)	7 (6.6)	0.10
C-321G		48 (34.8)	47 (44.3)	0.15
Exon 3				
T40128C	F119F	55 (39.9)	36 (34.0)	0.35
Exon 4				
A40601G	H186R	4 (2.9)	3 (2.8)	1.00
Intron 4 ^c				
C40693T		6 (4.4)	0 (0.0)	0.03
Exon 6				
C45406G	Q275E	13 (9.4)	8 (7.6)	0.65

E, Glutamic acid; F, phenylalanine; H, histidine; Q, glutamine; R, arginine.

^aNucleotide position according to APOBEC3G sequence, GenBank accession no. NC_000022.

^bFisher's exact test. Bonferroni corrections were not performed.

^cNovel variant.

from whom DNA samples were available from prospective studies on the basis of their continued seronegative status for at least 5 years, despite high-risk sexual behaviours or documented exposure to contaminated blood products [9]. The DNA samples of these ESN individuals were analysed as the control group (HIV negative). APOBEC3G genotyping was carried out by DNA direct sequencing procedures. The polymerase chain reaction primers were designed to cover the entire putative promoter region and exons 3, 4 and 6, as well as their exon-intron boundaries that are important for messenger RNA splicing. Primer sequences and conditions are available upon request. Control subjects were also genotyped for the CCR5 gene, and none were found to be homozygous for the 32-base pair deletion allele. The study was approved by ethics committees, and all participants gave written informed consent.

Table 1 shows the allelic distribution of the APOBEC3G polymorphisms among HIV-infected and ESN subjects. The overall APOBEC3G allelic distribution was similar to that observed in other populations, with two notable differences. The F119F variant was observed more frequently in our sample population (37%) than in African Americans (8%), whereas the H186R was less prevalent ($\cong 3\%$) in our cohort than in African Americans (37%) [7,8]. There was no difference in allelic frequencies for each APOBEC3G promoter and coding region SNP among HIV-infected and ESN subjects. However, a novel C40693T variant in intron 4 of APOBEC3G was significantly associated with an increased risk of HIV-1 infection ($P = 0.03$).

The C40693T nucleotide variant is located 68 bp downstream of the exon 4 natural 3' splice site and could potentially affect mRNA splicing by creating an alternative acceptor splice site. This situation could lead to the production of aberrant mRNA and the absence of functional APOBEC3G protein. There are over a hundred different examples of point mutations that lie in

the vicinity of mRNA splice junctions and that have been held responsible for human disease by altering the efficacy of mRNA splicing [10]. Nevertheless, further studies are needed to investigate whether this mutation could indeed cause aberrant APOBEC3G splicing at the mRNA level.

We cannot rule out the possibility that the observed association between APOBEC3G intron 4 polymorphism and HIV infection might be caused by strong linkage disequilibrium to other sequence variations in the APOBEC3G gene or other linked genes. The APOBEC3 gene complex is a cluster of eight genes (APOBEC3A to 3H) arrayed in tandem on human chromosome 22 [11]. Additional members of the human APOBEC family are endowed with activity against HIV-1. For example, APOBEC3B and APOBEC3F, which is largely co-expressed with APOBEC3G, exhibit moderate levels of activity against HIV-1 [12,13], and might both play a role in susceptibility to HIV infection. Moreover, extensive screening of HIV exposed-uninfected and infected cohorts revealed that homozygosity for the 32-bp deletion at the CCR5 locus conferred significant protection against infection in homosexual individuals. This was not the case in our study because none of the high-risk exposed uninfected individuals were homozygotes for this allele.

Our study focused on a well-characterized group of individuals that were carefully assessed for exposure, and demonstrated that a novel genetic variant in intron 4 of APOBEC3G is significantly associated with an increased risk of acquiring HIV-1 through homosexual contacts or injection drug use. Further studies are needed to define the net impact of this mutation on the susceptibility to HIV-1 infection.

^aLaboratoire d'immunogénétique, Centre de Recherche du Centre Hospitalier and ^bDépartement

de Microbiologie et Immunologie, Université de Montréal, Montreal, Canada; ^cImmunodeficiency Treatment Center, McGill University Health Center, Montreal, Canada; ^dCentre de Recherche Clinique André Viallet, Hôpital St Luc du Centre Hospitalier de l'Université de Montréal, Montreal, Canada; and ^eDépartement de Médecine Sociale et Préventive, Université Laval, Québec, Canada.

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