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Response to antiretroviral therapy in occult hepatitis B and HIV co-infection in West Africa

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This study evaluated the outcome of first-line antiretroviral therapy among 35 Ghanaians with occult HBV/HIV co-infection, comparing them over 2 years to 120 patients with HBsAg+ HBV/HIV co-infection and 230 patients without HBV co-infection. Increases in CD4 cell count and BMI were similar, whereas elevations of hepatic transaminases were more frequent in both the occult HBV and HBsAg+ patients. Occult HBV/HIV co-infection appears not to impact adversely on response to antiretroviral therapy in Ghana.

Up to 20% of HIV-infected people in sub-Saharan Africa are co-infected with hepatitis B virus (HBV) [1], as defined by positive HBsAg test, however, screening for chronic HBV infection is often not performed routinely in patients infected with HIV. The prevalence of HBsAg+ HBV co-infection is around 17% among HIV-infected patients in Ghana with predominantly HBV genotype E [2]. Progression of liver disease is more rapid in HIV/HBV co-infection [3], and there is also an increased risk of hepatotoxicity of antiretroviral drugs [4]. It is unclear whether HBV infection affects progression of HIV infection, or response to antiretroviral therapy (ART), particularly in Africa where only two large studies have been reported [5,6]. Occult HBV infection is characterized by a negative HBsAg test with positive HBV DNA, usually in patients with positive anti-HBc antibodies, and is common in Africa and Europe [7–9]. Occult HBV/HIV co-infection is poorly characterized and it is not clear whether such individuals are at increased risk of hepatotoxicity of ART, what is their long-term risk of developing liver disease or how they respond to ART. The aim of this observational study was to assess whether occult HBV infection influenced the outcome of first-line nonnucleoside reverse transcriptase inhibitor-based ART in a cohort attending a single government clinic in Kumasi, Ghana.

Between 2007 and 2008, a randomly selected subpopulation of adult patients attending the HIV clinic at Komfo Anokye Teaching Hospital in Kumasi, Ghana underwent screening for HBV infection by HBsAg (Determine-HBsAg; Inverness Medical, Stockport, UK), with HBsAg results confirmed by Murex-v3-enzyme-immunoassay (Abbott Diagnostics, Maidenhead, UK), and HBV-DNA using a quantitative real-time PCR assay with a lower limit of quantification of 14 IU/ml, as previously described [2]. Of 140 patients identified with a positive HBsAg result (16.7% of the population screened), 120 had started ART and had sufficient data for analysis. Of 83 patients identified with negative HBsAg and positive HBV DNA [median 76 IU/ml, interquartile range (IQR) 29–226], representing 9.9% of the population tested (and 25.8% of all ART-naive patients), 35 were found to have subsequently started ART. First-line ART in Kumasi consisted of zidovudine (or stavudine if anaemic), lamivudine and either efavirenz or nevirapine (if women). Both groups of co-infected patients were compared with 230 patients who had started ART with negative HBsAg and HBV DNA, in relation to demographic data and cumulative CD4 cell counts, BMI, alanine and aspartate transaminases (ALT/AST) and clinical events. Patients were followed every 3–6 months, in addition to an additional visit 1 month after starting ART, and outcome measures were compared for 2 years. Continuous variables were compared by either paired or unpaired *t*-tests or analysis of variance, and categorical variables by chi-squared tests. The study was approved by the Committee on Human Research Publications and Ethics at the Kwame Nkrumah University of Science and Technology, Ghana.

Baseline demographic characteristics and BMI at the start of ART were similar between the three groups, however, patients with both occult and HBsAg+ co-infection had higher median CD4 cell counts than non-co-infected patients: 160 and 163 versus 120 cells/μl ($P = 0.009$). More patients with HBsAg+ co-infection had hepatotoxicity (mostly ACTG Grade 1) than occult HBV co-infected or non-co-infected patients at baseline, and a higher proportion of non-co-infected patients started ART containing zidovudine rather than stavudine: 61 versus 45 and 46% ($P = 0.04$). HBV DNA levels were higher in HBsAg+ co-infected than in occult co-infected patients (mean 55 450 versus 994 IU/ml, $P < 0.001$). Response to ART, as defined by the surrogate markers of increase in CD4 cell count and BMI, is shown in Fig. 1a and b. There was no significant difference in increase in either marker at 1–2 years between the three groups. There was also no difference in the proportions of patients experiencing major clinical events or defaulting follow up between the three groups. A higher proportion of occult HBV co-infected patients had raised ALT/AST 1 month after starting ART compared with the other groups ($P < 0.001$), however, by 6 and 12 months the proportion

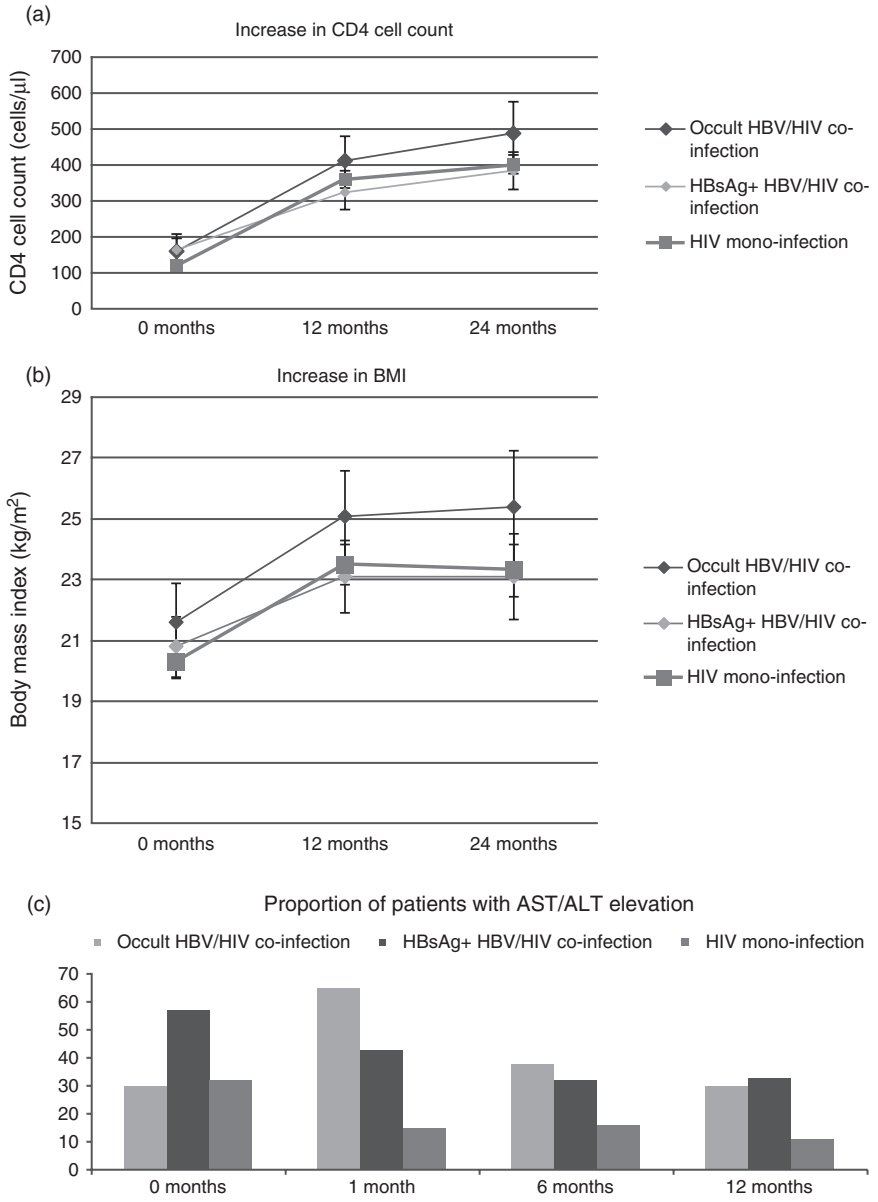

Fig. 1. Response to antiretroviral therapy by (a) CD4 cell count and (b) BMI after initiation of antiretroviral therapy, (c) Proportion (%) of patients with elevated AST/ALT after initiation of antiretroviral therapy. Error bars denote 95% confidence intervals. Median values shown.

with hepatotoxicity had reduced to levels similar to HBsAg+ co-infected patients, which were slightly higher than non-co-infected patients (Fig. 1c). There were no clear instances of patients stopping or switching antiretroviral medications due to hepatotoxicity, and no correlation of hepatotoxicity with any particular antiretroviral drug was apparent.

Our previous study identified nearly half of all patients with occult HBV infection having mutations in the S-gene (coding for HBsAg), indicating a high level of diagnostic escape mutants, hence a substantial underdiagnosis of HBV co-infection in this population using standard HBsAg assays [2]. A substantial proportion (26%) of all ART-naive patients had occult HBV/HIV co-infection, however, this may still have been an underestimate as previous longitudinal studies have suggested HBV DNA detection is often intermittent [9]. This is to our knowledge the first comparative study to describe the response of patients with occult HBV/HIV co-infection to ART. Our study is limited by relatively small numbers of patients with occult HBV/HIV co-infection, the absence of HIV-1 viral load outcome data and by both HBV co-infection groups starting ART at slightly higher CD4 cell counts than the non co-infected group making comparisons of CD4 responses problematic. It is possible that this was due to more co-infected patients starting ART after 2008, when guidelines for CD4 criteria for

starting ART changed. Nonetheless, these preliminary findings suggest patients with occult HBV/HIV co-infection respond equally well to ART as those patients without HBV co-infection or patients with HBsAg+ co-infection. Although a higher (and substantial) proportion of occult HBV/HIV co-infected patients had raised ALT/AST after 1 month of ART, most elevations were low-grade and were of limited clinical relevance as no patients stopped or switched ART subsequently. It is not clear whether these elevations were mostly due to drug-induced hepatotoxicity or immune reconstitution inflammatory syndrome, both of which may occur within the first few weeks of ART. Two further questions arise in relation to patients with occult HBV/HIV co-infection: are they at increased risk of developing liver disease, and if so is ART containing lamivudine, as the sole agent active against HBV, adequate to reduce that risk? Larger longitudinal studies of longer duration are needed to answer these questions.

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Conflicts of interest

A.M.G.: consultancy and speakers' bureau: Abbott, Bristol Meyers Squibb, Gilead, Glaxo Smith Klein, Merck, Monogram Biosciences, Pfizer, Tibotec, Roche, ViiV Healthcare, Virco. Research support: Monogram Biosciences, Pfizer, Merk, Tibotec, Roche, ViiV Healthcare, Virco. There are no conflicts of interest for the other authors.

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Is etravirine and two nucleosides an option for HIV with an isolated K103N mutation?

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We report long-term virologic response to etravirine and tenofovir/emtricitabine in four HIV-1-infected patients who had prior standard genotypic resistance testing showing an isolated K103N mutation (three acquired, one transmitted). In three patients tested, the K103N mutation was detected in cellular HIV-1 DNA whereas remaining suppressed on etravirine plus tenofovir/emtricitabine.

Efavirenz is a nonnucleoside reverse transcriptase inhibitor (NNRTI) that in combination with two

nucleoside/nucleotide [N(t)RTI] reverse transcriptase inhibitors is recommended as initial treatment of HIV-1 infection [1]. Failure of efavirenz-containing regimens is often associated with the development of the K103N resistance mutation. The K103N mutation confers cross-resistance to nevirapine, but does not decrease by itself etravirine susceptibility [2].

Etravirine has been evaluated as part of a salvage regimen after multiple-antiretroviral failures. In this context, etravirine has demonstrated efficacy in the DUET and TRIO clinical trials, combined with other active drugs such as darunavir/ritonavir and raltegravir [3,4]. However efficacy data about the combination of two N(t)RTIs and etravirine in patients with prior NNRTI failures are scarce with one trial suggesting poor activity (TMC125-C227) [5]. However, it should be noted that the 227 trial included patients with multiple N(t)RTI and NNRTI mutations and not only the K103N mutation. The objective of our study was to retrospectively report the efficacy of the etravirine and two N(t)RTIs in patients harboring HIV-1 isolates with only the K103N mutation.

In this study, we included HIV-1-infected patients, who showed an isolated K103N mutation in historical standard population genotypes and whose clinicians had decided to use an antiretroviral regimen consisting of two N(t)RTI and etravirine (without any other antiretroviral). For all patients, information on clinical variables, antirretroviral treatment, drug resistance mutations and laboratory parameters were obtained from clinical and laboratory databases. For patients, who at the time of inclusion remained virologically suppressed while receiving etravirine, we wished to demonstrate the persistence of archived K103N. To do so, total DNA was collected from whole blood, and the K103N mutation on cellular HIV-1 DNA was analyzed by single-genome pyrosequencing, a method based on the single-genome sequencing method [6], in which single genomes were obtained by serial dilutions and PCR detection, and sequencing was directed to codon 103 by a specific pyrosequencing assay [7]. The local ethic committee approved the study.

Of the 1876 patients followed in our unit, we found six patients in whom standard genotypic resistance testing revealed K103N and who received treatment with two N(t)RTI along with etravirine. Two patients had K103N and other N(t)RTI mutations (M184V in both and H208Y, P236L in one and 69N, 70R in the other) and were excluded from this analysis. Four patients (two men) had K103N but no other major reverse transcriptase inhibitor resistance mutations. In all cases, the N(t)RTI combination was tenofovir/emtricitabine. Three patients (1, 2 and 4) had acquired K103N after virologic failure of an efavirenz-containing regimen. In the other patient, (3) the K103N mutation was transmitted. According to the Stanford HIV Database algorithm the tenofovir/emtricitabine combination was judged to be completely active in all four patients based on prior standard resistance genotyping (Table 1).

At the time of switching to tenofovir/emtricitabine and etravirine median age was 37 years, CD4 cell count was 547 (IQR: 537–597) cells/μl and duration of antiretroviral treatment was 5 years (IQR: 2–9). Two patients with acquired K103N (patients 1 and 2) and the patient with transmitted K103N (patient 3) were virologically suppressed before the switch to etravirine while receiving tenofovir/emtricitabine and lopinavir/ritonavir. The median period of suppressed viraemia was 8 months (IQR: 7–9). In these virologically suppressed patients, the main reason to change to etravirine was the presence of diarrhea due to lopinavir/ritonavir (patients 1 and 2). Substitution of lopinavir/ritonavir for etravirine resolved the diarrhea in both cases. One patient (patient 4) was experiencing virological failure when he switched to tenofovir/emtricitabine and etravirine.

After the switch to tenofovir/emtricitabine and etravirine all patients remained virologically suppressed for at least 2 years with a median exposure time to etravirine of 28 months (IQR: 27–31). No adverse event occurred during the etravirine treatment. By clonal analysis the K103N mutation was detected in cellular HIV-1 DNA in all the three patients (2, 3 and 4), who remained virologically suppressed while receiving etravirine at the time of entering the study (Table 1). One patient (patient 1) failed after 27 months of virological suppression because of an episode of very poor adherence and developed new N(t)RTI resistance mutations: K65R, K103N, Y181C, K219E (patient 1).

A prior study using ultradeep pyrosequencing [8] did not detect additional major NNRTI-resistance mutations in the plasma of patients with transmitted K103N but did so in NNRTI-experienced patients, suggesting that etravirine may not be fully active in patients with acquired K103N. In our study, the combination of etravirine and tenofovir/emtricitabine was efficacious despite infection with HIV-1 isolates showing acquired or transmitted K103N mutation in plasma and in cellular HIV-1 DNA. It is interesting that the patient with primary NNRTI resistance who had K103N detected in cellular HIV-1 DNA in 35/36 clones tested had remained suppressed during 35 months while receiving etravirine and tenofovir/emtricitabine.

In the TMC125-C227 clinical trial [5], etravirine in combination with two nucleoside reverse transcriptase inhibitors (NRTIs) was less effective than the control arm consisting of two NRTIs and a protease inhibitor (mainly boosted). The C227 study included treatment-experienced patients with high levels of NNRTIs and NRTIs

Table 1. Summary of patients' characteristics.

Patient	ART: Failed regimen	Before ETR switch	After ETR switch	Cause of switch	Historical standard genotype RT mutations (ETR Weighted score)	HIV RNA at ETR switch	Last HIV RNA	Months suppressed on ETR	Cellular HIV-1 DNA[a] % with K103N (clones with K103N/ clones tested)
1	TDF–FTC–EFV	TDF–FTC–LPV/r	TDF–FTC–ETR	Diarrhea	K103N (0)	<50	1230	27	NA[c]
2	d4T–3TC–EFV	TDF–FTC–LPV/r	TDF–FTC–ETR	Diarrhea	K103N, A98S (0)	<50	<20	35	67 (6/9)
3	None[b]	TDF–FTC–LPV/r	TDF–FTC–ETR	Patient request	K103N (0)	<50	<20	35	97 (35/36)
4	TDF–FTC–EFV	TDF–FTC–EFV	TDF–FTC–ETR	Failure	K103N (0)	1900	<20	24	5 (4/86)

3TC, lamivudine; ABC, abacavir; ART, antirretroviral treatment; d4T, stavudine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; NA, non-applicable; TDF, tenofovir.
[a]Performed only in patients who remained virologically suppressed while receiving ETR.
[b]Transmitted NNRTI resistance.
[c]Standard genotype showed K65R, K103N, Y181C, K219E.

resistance. In contrast, our four patients with isolated K103N received fully active etravirine and fully active tenofovir/emtricitabine.

There are no clinical trial data to support a preferred regimen in patients failing an efavirenz-containing regimen with isolated K103N. Conducting such a trial in the developed world has not been possible due to the low frequency of failure and because failure is due in general to poor adherence. Although more data are needed our small case series suggest that in selected patients in whom standard genotype detects only the K103N mutation, etravirine and two fully active N(t)RTIs might be efficacious.

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Conflicts of interest

F.X.Z. has received advisory fees and speaker fees from BMS, Gilead, Abbot and ViiV. J.I.B. has received consulting and speaking fees from BMS, Gilead, Abbott, Janssen. I.P.-V. has received speaking fees from BMS, Gilead and Abbott and travel grants from Janssen. J.R.A. has received advisory fees, speaker fees and grant support from Viiv, Tibotec, Janssen, Abbott, BMS, Gilead, MSD.

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