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# DIAGNOSIS OF DUAL HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 INFECTIONS IN A RESOURCE-LIMITED SETTING

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## ABSTRACT

***Background:*** **The presence of dual HlV-l/HIV-2 infection in Ghana and the different drug requirements for the treatment of HlV-1 and HIV-2 presents difficulties for the treatment of dual infections with both viruses.**
***Objectives:*** **To determine the prevalence of the dual sero-positive profile in treatment naive patients at a principal ART Clinic in Accra, Ghana and to investigate if rapid screening assays could be useful for diagnosis.**
***Design:*** **A cross-sectional study.**
***Setting:*** **A principal antiretroviral treatment centre in Accra, Ghana.**
***Subjects:*** **Three hundred and twenty eight antiretroviral treatment naive patients.**
***Results:*** **A total of 12 (3.7%) of patients seen were dual seropositive. There was a slight tendency of dual seropositive females being older than their HIV-l counterparts (p=0.088, CI=-l 0.833 to 0.753). Eight of the 12 of the dual seropositives were reactive for Genie II and were considered as possibly infected with both HIV-I and HIV-2. Seven (87.5%) of Genie II dual seropositives had strong intensities (> 1+) on both HIV-2 specific bands (sgp105 and gp36) on Innolia. CD4 counts were not significantly different in dual seropositives as compared to HIV-1 infected patients.**
***Conclusions:*** **Dual HIV-l/HIV-2 seropositives (and possibly infections) may be common especially in older women. The Genie II will be useful as a supplemental rapid test for rapid and accurate differentiation of HIV-l and HIV-2 antibodies at treatment centres.**

## INTRODUCTION

Due to the presence of individuals with both HlV-2 and dual HIV-l/HIV-2 seropositive (and infection) profiles in West Africa, there are challenges in the antiretroviral treatment programmes in this setting. Highly active antiretroviral regimens (HAART) which efficiently suppress HIV-l may not be useful for HIV-2 (1), and this may adversely affect the treatment of dual infected patients (2).

Although the highest absolute and relative proportion of dual seropositivity has been seen in female commercial sex workers (CSW) elsewhere (3), significant proportions have been seen in other populations in Ghana (4, 5). In these studies, HIV-l was shown to be predominant, with dual seropositivity being more common than HlV-2 alone.

HIV-l but not HIV-2 DNA is mostly detected in peripheral blood lymphocytes of dual seropositive individuals (4,6-8), the converse is also possible (9). The diagnostic problem has therefore been the accurate detection of HIV-2 antibodies. With appropriate screening algorithms, a strong correlation between dual seropositivity and infections is possible (10). Very few rapid tests have been evaluated for their differential ability to distinguish between HIV-l and HIV-2 infections. However, one of the previously cited studies has shown that patients with dual seropositive profiles on Genie II HIV-l/HIV-2 (BioRad Laboratories, France) are more likely to be infected

with HIV-2 (9). Although rapid tests are likely to give some unclear results for differentiating between HIV-l and HIV-2 infections, a stringent screening algorithm that includes Genie II HIV-I / HIV-2 may be useful in predicting dual infections.

With the scale-up of antiretroviral therapy in Ghana, there is the need to understand the dynamics of patients with the dual seropositive profile in order to establish optimal sero-screening procedures for the effective treatment of all HIV infected patients. This study therefore determined the prevalence of the dual seropositive profile in treatment naive patients at a principal ART Clinic in Accra, Ghana, and investigated if the Genie II HIV-l HIV-2, BioRad, Marnes La Coquette, France (Genie II), could be used as a second screening test to correctly diagnose dual seropositivity.

## MATERIALS AND METHODS

*HIV/AIDS patients:* A randomly selected set of 328 HIV / AIDS treatment naive individuals attending the Fevers Unit (a principal ART treatment centre in Ghana), Korle-Bu Teaching Hospital, being a large subset of patients used in a previous study (11), were included in this study. These patients were obtained by convenient sampling between August 2003 and September 2004. Ethical approval was granted by the University of Ghana Medical School Ethics Committee (MS-Et / M.04-P02 / 2003-04) and written consent was obtained from all patients before enrolment. Demographic data and CD4 counts were also obtained from patient files.

*Diagnosis of HIV-I and HIV-2 infections:* Serological differentiation for HIV-l and HIV-2 antibodies using plasma obtained from EDTA tubes was done with the Rapitest (Morwell Diagnostics GmbH, Gewerbestr, Switzerland), and all dual HIV -l / HIV-2 seropositivity confirmed with a line-immunoassay, lnnolia N.V lnnoGenietics, Antwerp, Belgium, according to manufacturer's instructions using the l6 hour incubation as previously described (12). Bands on test strips were rated as indeterminate (±) or reactive (1 +, > 1+ or 3+) in relation to the intensity of the internal control bands on strips. If both HIV-l specific bands (sgpl20 and gp4l) were present (≥1+) and the maximum banding intensity for one HIV-2 specific band (sgpl05 or gp36) was 1+, the patient had HIV-1 antibodies. However, if only one HIV-1 specific band was present (≥1 +), the maximum rating of ± was allowed on one HIV-2 specific band. A similar but converse interpretation was used for the diagnosis of HlV-2 seropositivity. Plasma sample that exceeded these maximum ratings had both HIV-1 and HIV-2 antibodies (dual seropositive).

HIV-2 infection in dual seropositivity has been shown to be high with Genie II (9). All dual seropositives on Innolia with strong intensities (>1+) on all envelope bands for HIV-l and HIV-2 (sgp120, gp4l, sgp105 and gp36) and dual seropositive on Genie II, were considered as likely HIV-l and HIV-2 infections. A cross-section of 13 HIV-1 seropositives by lnnolia were also screened with Genie II as controls, but plasma was not available for lnnolia and Genie II testing for the only HIV-2 patient identified during the study. This patient was subsequently excluded from all analyses. All 13 HIV -1 seropositives screened were concordant with lnnolia results, and had strong intensities (>1 +) for all HIV -1 envelope specific bands (sgp120 and gp41).

*Statistical analysis:* The Independent T-test was used to compare the age differences, gender proportions and CD4+ counts of dual and HIV-1 seropositives and chi-squared test used in comparing the proportion of women who were dual and HIV-l infected. The SPSS v14.0 for Windows (SPSS Inc., Chicago, USA), was used for all statistical analysis.

## RESULTS

A total of 114 males and 214 females participated in the study. The ages of the male ranged from 18 to 68 years (n=110), while that of the female was from 21 to 64 years (n=211). Twelve (3.7%) of the patients were dual seropositive. In the absence of DNA for HIV-1 and HIV-2 PCR, the stringent criterion described earlier (see methods) was used in classifying dual infections. Only eight (2.7%) of the 12 dual seropositives were therefore considered as infected with both HIV-1 and HIV-2 (Table 1), with six (75%) being women. Genierally, Genie II dual seropositives mostly had strong intensities (>1+) on both HIV -2 specific bands (sgp105 and gp36) of Innolia. The only exception was CAL 187 which had a background reactivity for sgp105 (Table 1).

There was a slight difference, though not statistically significant, between the ages of HIV-1 and dual seropositive patients (P=0.130, CI=-9.364 to 1.203). Although there was a tendency of dual seropositive females being older than their HIV-l counterparts (p=0.088, CI=-10.833 to 0.753), this reduced when only dual infected females were considered (p=0.233, CI=-10.249 to 2.506). There was no difference in the proportion of females who were HlV-1 infected as compared to those who were dual seropositive (p=0.228, OR=2.756, CI=0.594 to 12.881), and those who were dual infected (p ≥0.717, OR=1.655, CI=0.329 to 8.337).

**Table 1**
*Comparative reactivity of dual HIV-1 /HIV-2 seropositive plasma using Innolia and Genie II*

| Sample ID | Serologic profile | Innolia results *Innolia band reactivities sgpl20 | gp41 | sgpl05 | gp36 | Genie II results |
|---|---|---|---|---|---|---|
| CALl0 | Dual HIV 1/2 | 3+ | 3+ | neg | > 1+ | HIV-1 |
| CAL33 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CAL88 | Dual HIV 1/2 | 3+ | 3+ | neg | 3+ | HIV-1 |
| CAL90 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CAL105 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CALl56 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CALl79 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CALl87 | Dual HIV 1/2 | 3+ | 3+ | >± (<1+) | 3+ | Dual |
| CAL244 | Dual HIV 1/2 | 3+ | 3+ | neg | 3+ | HIV-1 |
| CAL246 | Dual HIV 1/2 | 3+ | 3+ | neg | 3+ | HIV-1 |
| CAL298 | Dual HIV 1/2 | 3+ | 3+ | 3+ | 3+ | Dual |
| CAL364 | Dual HIV 1/2 | 3+ | 3+ | >1+ | 3+ | Dual |

All plasma samples were screened Rapitest, Morwell Diagnostics GmbH, Gewerbestr, Switzerland, and dual seropositives confirmed. *The line immunoassay, Innolia, Innogentics, Antwerp, Belgium, was used for confirmatory testing and interpretation done according to manufacturers' instruction. sgpl20/gp4l and sgpl05/gp36 are HIV-l and HIV-2 specific bands on Innolia respectively. Genie II HIV-1/HIV-2 (BioRad, Marnes La Coquette, France) results have been shown for all Innolia dual seropositives.

Mean (range) CD4 counts were 245 (5 to 593) and 291 (1 to 1299) for dual (n=6) and HIV-l (n=110) seropositives respectively. There was no significant difference between CD4 counts for dual seropositives as compared to those with HIV-l infection (p=0.699, CI=-0.188.362 to 280.369). CD4 counts were Genierally unavailable at the time of the study because antiretroviral therapy had just begun.

## DISCUSSION

Although the proportion of dual seropositives seen was small (Table 1), their occurrence suggests that effective screening for HIV-l and HIV-2 antibodies for patients about to begin ART using appropriate tests is essential. This will enable appropriate regimens to be given to those infected with HIV-2 either as dual or in single infections. Previous studies have reported dual infections in dual seropositives in Ghana (4, 8), and the results of this study indicate that dual infections are definitely present, albeit with a low prevalence. Also, baseline information for understanding the trends of dual seropositivity in treatment naive patients is now available.

This study did not consider whether or not any of the participants were commercial sex workers. Therefore, our observation that women were not likely to have a higher proportion of dual seropositive or infection as described in the high prevalence seen in a previous study, (3), may be due to the small numbers of dual seropositives and infection seen. However, the data provides valuable information for future comparison with prevalence rates that may be seen later. Almost 29 (40%) of the CSW in Senegal in a particular study on dual infections were Ghanaians (13). This suggests that international migration may contribute to the current prevalence of dual infections attending treatment centres in Ghana. The need to understand the dynamics becomes increasingly important since international migration may affect prevalence rates and therefore policy. However, continuous screening with a confirmatory assay may overburden the already taxed health care budget for ART. Just as two different screening tests can be used to diagnose HIV infection (14), the possibility of using rapid screening assays to discriminate HIV-1 and HIV-2 antibodies is emphasised. This is especially so since prevalence rates are relatively low.

HIV-2 infections are common in Genie II dual seropositives (9), and the main problem of dual infections is related to the correct diagnosis of HIV-2 (4,6-8). Although Genie II does not have sgp105 peptides or recombinant proteins, the majority of Genie II dual seropositives had strong sgpl05 HIV-2 specific bands on the lnnolia (Table 1). Therefore, screening with Rapitest and confirming with the

Genie II provides a clear distinction between dual seropositives with two HIV-2 specific bands, and those with only gp36 bands on lnnolia. The reactivity on the Genie 11 HlV-2 spot may reflect high antibody titers that may reflect an ongoing or just subdued HIV-2 infection (15). This is confirmed by the fact that all monotypic HIV-2 infections on lnnolia we have seen in our routine screening turned out to be HIV-2 on Genie II and had both sgpl05 and gp36 bands on lnnolia (data not shown).

A possible explanation for the Genie II reactivity for CAL 187 could be an early infection with HIV-2. Super infection with either HIV-l or HIV-2 is also possible (13), so this patient may therefore have an active HIV-2 infection which is being established. Alternatively, HIV-1 may have outgrown HIV-2 *in vivo* resulting in a reduction in HIV-2 specific antibodies overtime. This suggestion is supported by the report that patients with advanced decline in CD4 counts may not have or may loose HlV-2 when co-infected with HIV-1 (15). CAL l87 had a very low CD4 count (85 cells/ml) (data not shown) and therefore reflects this picture. The possibility of the presence of a Genietically aberrant HIV-2 strain can also not be ruled out. Though very little work has been done on HIV-2 subtypes (16), there is a possibility that like HIV-1 subtype 0 (17), different assays may have different abilities for detecting HIV-2.

Our results confirm those of others (3), and show that dual seropositives may have similar immunological characteristics as compared to HIV -1 infected individuals. There is a need for this proposed algorithm of using a first rapid assay that can discriminate between HlV-l/HIV-2 antibodies and Genie II to be compared with PCR results to enable a decision to be made on its usefulness for routine screening of patients beginning HAART in Ghana. Just as two rapid assays can be used in diagnosing HIV infections (14), this study shows that it will be possible to use a similar approach in diagnosing dual seropositivity and possibly dual infections.

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