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# Presence of occult HBV, but near absence of active HBV and HCV infections in people infected with HIV in rural South Africa

**Running head:** Occult hepatitis in rural South Africa

Roos E Barth*[1], Quirine Huijgen[2], Hugo A Tempelman[3], Tania Mudrikova[1], Annemarie MJ Wensing[4], Andy IM Hoepelman[1]

[1]: University Medical Centre Utrecht, Department of Internal Medicine and Infectious Diseases. Utrecht, the Netherlands

[2]: Medical student at the University Medical Centre Utrecht. Utrecht, the Netherlands

[3]: Ndlovu Medical Centre, Elandsdoorn, Limpopo, South Africa

[4]: University Medical Centre Utrecht, Department of Virology and Microbiology. Utrecht, the Netherlands

This work was performed both at the Ndlovu Medical Centre in South Africa and at the University Medical Centre Utrecht in the Netherlands

**Correspondence to*:**

Roos E Barth

For Peer Review

University Medical Centre Utrecht

F02.126

Postbus 85500

3508 GA Utrecht

The Netherlands

E-mail: r.e.barth@University Medical Centre Utrechttrecht.nl

Tel no: 0031-621224407

Fax no: 0031-302523741

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**Abstract:**

Human immunodeficiency (HIV), hepatitis B (HBV) and hepatitis C (HCV) viruses are endemic in Sub-Saharan Africa, but data regarding the prevalence of hepatitis co-infections in HIV-positive individuals residing there are limited. The aim of the study was to determine the prevalence of HBV, HCV and occult HBV (presence of HBV-DNA in the absence of HBsAg) in a rural, South African cohort. The results were compared to various ethnic groups in a Dutch cohort of people infected with HIV. Antiretroviral-naïve individuals with HIV from both a rural South African clinic (n=258), and a Dutch University hospital (n=782), were included. Both serological (HBV and HCV) and molecular (occult HBV) assays were performed. Logistic regression analysis was used to define independent predictors of a hepatitis co-infection.

HBV and HCV prevalence rates in the South African cohort were exceptionally low (0.4%, 1/242 and 0.8%, 2/242 respectively), compared to those observed in Caucasians (HBV 4.4%, HCV 10.9%) and African immigrants (HBV 8.9%, HCV 4.8%). Conversely, occult HBV was observed in a considerable proportion (10%, 6/60) of South African patients who were anti-HBc-positive but HBsAg-negative. Occult infections were less frequent in Caucasians and Africans in the Dutch cohort (3.2% and 1.4% respectively). Independent predictors for occult HBV were not identified, but a trend towards more occult HBV at lower CD4 counts was observed.

Local HBV/HCV prevalence data are needed to optimise vaccination and antiretroviral treatment strategies. Occult HBV in patients with HIV may be missed regularly when molecular analyses are not available.

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**Keywords:**

Hepatitis B

Hepatitis C

Human immunodeficiency virus

Occult infection

HBV-DNA

Sub-Saharan Africa

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**Introduction:**

Millions of people are infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) globally. Sub-Saharan Africa is affected severely by all three viruses; two-third of the world's 33 million people infected with HIV reside there, as well as an estimated 50 million chronic HBV carriers [Modi and Feld, 2007; WHO, 2002; WHO, 2007]. In addition, three percent of the African population is thought to be infected with HCV [Madhava et al., 2002].

Chronic hepatitis can lead to cirrhosis and hepatocellular carcinoma. Due to shared routes of transmission, the prevalence of HBV and HCV is believed to be high amongst persons infected with HIV [Burnett et al., 2005; Thomson and Main, 2008]. Life expectancy of people living with HIV in Sub-Saharan Africa has improved dramatically since the increased availability of antiretroviral treatment (ART). The long-term effects of HBV and HCV co-infections will therefore become more evident, causing a potential increase in morbidity and mortality [Hoffmann and Thio, 2007; Modi and Feld, 2007]. This is stressed by the observation that the progression of HBV- and HCV-related liver disease is more rapid in patients with HIV [Chen et al., 2009; Nikolopoulos et al., 2009].

Prior to starting ART, it is important to know whether a co-infection with hepatitis is present. An increased hepatotoxicity of antiretrovirals (ARVs) and other drugs is observed in patients with both HIV and hepatitis, compared to those with HIV infection alone [den Brinker et al., 2000; Hoffmann et al., 2007; Sulkowski et al., 2002]. Although immune reconstitution soon after initiating ART may cause further liver damage, ART

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may be beneficial for patients who are co-infected with HIV and HBV, as it can be used to treat both infections simultaneously.

Screening for co-infection with HBV or HCV in people infected with HIV is incorporated in western clinical guidelines [BHIVA, 2010; EACS, 2009; Soriano et al., 2008; Soriano et al., 2007]. However, consequent serological screening for hepatitis is generally not feasible in low-income countries (LICs). As a result, limited HBV and HCV prevalence data are available regarding African patients infected with HIV, and the data that are available indicate wide geographical variation [Cooper et al., 2009; Hoffmann and Thio, 2007; Marcellin, 2009].

While some people have an active, HBV surface antigen (HBsAg) positive infection, others may harbour an occult HBV infection. Occult hepatitis B is defined by the presence of plasma HBV-DNA in individuals who are HBsAg negative. Even if serological testing for HBV is available, such infections will be missed.

The aim of the current study is to assess active HBV, occult HBV and HCV prevalence rates in a cohort of people infected with HIV in rural South Africa. The results were compared to co-infection rates with HBV and HCV among Caucasian and African individuals with HIV in the Netherlands. Furthermore, predictors for the presence of these hepatitis viruses were determined.

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**Methods:**

***Setting and study population***

Ndlovu Medical Centre is a non-governmental organisation in Elandsdoorn (www.ndlovucaregroup.com). Elandsdoorn is situated in a rural area in Limpopo, a province in the north east of the Republic of South Africa. The centre provides primary health care, including an ART program for people with HIV that has been active since 2003. Cohort details have been described elsewhere [Barth et al., 2008]. For this study, all ART-naïve adults (≥15 years) with HIV who enrolled in the Ndlovu ART program from September till November 2008, were included in this study.

The University Medical Centre Utrecht is an academic hospital in the city of Utrecht, situated in the centre of the Netherlands. All adults (≥18 years) with HIV, who came into care at the University Medical Centre Utrecht from January 1985 till January 2008, were included. Patients were ART-naïve at time of serological HBV and HCV testing.

For comparisons, analyses were limited to African and Caucasian people. Asians and people originating from the Americas who were in care at the University Medical Centre Utrecht were excluded.

***Data collection and definitions***

Patient characteristics were extracted from medical charts and clinical databases. Data included in the study were the following: age, gender, ethnicity, country of birth, probable route of HIV transmission and baseline laboratory variables (CD4+ T-cell count, ASAT, ALAT). Venous blood was collected from all patients, and standard methods for

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blood sample processing were used. Plasma was locally stored at -80$^{o}$ C and shipped to the Netherlands on dry ice.

HBV and HCV analyses were performed at the accredited (according to Dutch guidelines) diagnostic laboratory of the virology department at the University Medical Centre Utrecht. Serological tests for HBV (anti-HBc, HBsAg and anti-HBs) and HCV (anti-HCV) were performed using the AxSYM® automated analyzer (Abbott Laboratories, Abbott Park, IL, USA). Anti-HBc was determined using the CORE$^{tm®}$ kit (Abbott Laboratories, Abbott Park, IL, USA). Anti-HBc was considered positive if the ratio was above 1. All anti-HBc-positive blood samples were tested for HBsAg and anti-HBs using HBsAg(V2) and AUSAB® kits (Abbott Laboratories, Abbott Park, IL, USA). Ratios above 4 and 2 were used to define positive anti-HBs and positive HBsAg respectively. In the case of an indeterminate HBsAg result (ratio between 2 and 5) samples were centrifuged using an Eppendorf centrifuge and retested.

HBV-DNA load was assessed in samples that tested anti-HBc positive, but did not have detectable HBsAg levels. Quantitative HBV-DNA was determined using the Taqman® fast real-time nested PCR, as described previously [Cohen Stuart et al., 2009; Pas et al., 2000]. HBV-DNA was isolated using an automated nucleic acid extraction system (MagNA Pure LC®; Roche Diagnostics, Penzberg, Germany). Lower limit of quantification is 200 IU/mL; lower limit of detection is 10 IU/mL. Amplification was performed in duplicate and a three-tiered control was executed (internal, no template and whole procedure control). Outcomes were averaged and in the case of a 3-point difference in

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cycle threshold values (Ct values) or a positive/negative difference, the procedures were repeated.

A positive HBsAg result was used to define an HBV infection. Patients with detectable HBV-DNA levels were considered to have HBV viremia. Patients with detectable HBV-DNA levels with the absence of HBV surface antigens were considered to have occult HBV infections.

The HCV version 3.0 kit® (Abbott Laboratories, Abbott Park, IL, USA) was used to determine the presence of anti-HCV. A ratio above 5 was considered positive. Blood samples with indeterminate results (ratio between 1 and 5), were centrifuged and retested. Subsequently, a western blot was performed on all blood samples with a ratio above 1, using the Autoblot 3000® (Innogenetics NV, Gent, Belgium). A positive western blot in anti-HCV positive individuals was used to define an HCV infection.

***Statistical analysis:***

Frequencies were compared using the $\chi 2$ and Fischer's exact tests. Continuous data were compared with the independent-sample t-test or Mann Whitney U test for non-parametric data. Determinants that were associated with the outcome in univariate analysis (defined as a p-value <0.1) were included in multivariate analysis. Subsequently, logistic regression analysis was done to determine independent predictors of the outcome. A P-value ≤0,05 was used to define statistical significance. Data were processed and statistical analyses were done using SPSS version 15.

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**Results:**

***Patient characteristics:***

Two hundred forty-eight ART naïve adults with HIV from Elandsdoorn, South Africa were included in this study. The majority of included patients were female (73.8%). The Dutch cohort consisted of 846 people infected with HIV. Caucasian Europeans (614, 73%) and African immigrants (168, 20%) predominated. In contrast to the South African cohort, more males were included in the Dutch cohort; 535 (87%) of Caucasians and 93 (55%) of African immigrants were male. Heterosexual contact was the main route of HIV transmission in African immigrants and was assumed to be the HIV-transmission route in all South African residents. With Caucasian Europeans, HIV was transmitted frequently via homosexual contact. Baseline characteristics are summarised in Table 1.

***Serological evidence of HBV and HCV co-infections:***

Anti-HBc test results were available for 98% (242/248) of South African residents. Of the 242 patients, 68 (28.1 %) tested positive. Most anti-HBc positive patients were anti-HBs positive as well (62.7%). Only one individual tested HBV surface-antigen positive. This patient did not have anti-HBs. Overall, the prevalence of active HBV was only 0.4% (1/242). Serological evidence of an HBV infection was predominantly present in men; HBsAg and anti-HBc prevalence rates were 5.4% and 45.9% in men, compared to 2.3% and 34.1% in women ($p=0.2$ and $p<0.01$ respectively). No gender difference was seen with regard to the anti-HBs prevalence.

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Compared to the South African cohort, anti-HBc prevalence rates were significantly higher in both Caucasians and African immigrants (41.4% and 57.6% respectively, p<0.01). Furthermore, a larger proportion of patients was HBsAg-positive, resulting in an active HBV prevalence of 4.4% in Caucasians and 8.9% in African immigrants p<0.01). The prevalence of active HBV remained significantly lower in the South African cohort, as compared to the other groups, when patients infected via heterosexual contact only were included.

Serological evidence of an HCV infection was also infrequent in the South African cohort. The anti-HCV prevalence rate was 0.8% for African residents, compared to 10.9% and 4.8% for Caucasians and African immigrants respectively (p<0.01).

All serological outcome data are presented in Table 2.

***Occult HBV infections:***

The presence of HBV-DNA was assessed in 93% (62/67) of South African patients who were anti-HBc positive but tested HBsAg negative. For the remaining 5 individuals, an insufficient amount of plasma was stored to perform molecular analyses. An occult HBV infection was established in 9.7% (6/62) of tested individuals. HBV-DNA levels were low in patients with an occult HBV infection. In 3 patients, viral loads were below the level of quantification (between 10 and 200 IU/mL), twice just above this threshold (224 and 259 IU/mL respectively), and only once clearly higher (5222 IU/mL).

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For 259 Dutch patients who were anti-HBc positive and HBsAg negative, HBV-DNA levels were also determined. Occult HBV was present in 2.7% (7/259) of these patients. The prevalence rate was higher in Caucasians (6/187, 3.2%) compared to African immigrants (1/72, 1.4%), but significantly lower compared to the South African cohort (p=0.04). Nearly half of the patients with occult HBV also had anti-HBs (6/13, 46%).

***Predictors for co-infections with HBV or HCV:***

Age, gender, ethnic origin, HIV transmission route, baseline log HIV-RNA and baseline CD4+ T-cell count were tested as possible determinants for having a HBV or HCV co-infection. In univariate analysis, all but the CD4+ T cell count were associated with the presence of HBsAg and with the presence of anti-HCV. In multivariate analysis, only lower age and male gender were independently predictive of an HBV infection. The determinants that remained associated with a positive anti-HCV test were Caucasian ethnicity, HIV transmission route (IVDU or blood products) and a lower baseline log HIV-RNA.

Among anti-HBc positive individuals, the above mentioned determinants and the presence of anti-HCV and anti-HBs were tested as possible predictors for an occult HBV infection. None remained associated significantly with this outcome in the multivariate analysis, but a trend towards a higher occult HBV prevalence in case of a lower baseline CD4+ T-cell count was observed (Table 3).

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**Discussion:**

In this study an exceptionally low prevalence of HBs antigenemia (0.4%) and HCV antibodies (0.8%) was found in a rural South African cohort of people infected with HIV. Occult HBV on the other hand was observed in a considerable proportion (10%) of patients who tested anti-HBc-positive and HBsAg-negative.

In Western countries, HBV is clearly more prevalent in people with HIV compared to people without HIV [Burnett et al., 2005; Kellerman et al., 2003; Thio, 2009]. This relationship is attributed to common routes of transmission. In Sub-Saharan Africa the association between HBV and HIV infections is less apparent, as people generally come into contact with HBV long before the acquisition of HIV. Without making a distinction between people with and without HIV, HBsAg prevalence in Africa is reported to be well over 8% [Barth RE, 2010; Burnett et al., 2005; CDC, 2006; WHO, 2002]. A similar rate was observed among African immigrants in the current study. Published HBsAg prevalence data among South African patients infected with HIV vary (5-23%, [Burnett et al., 2007; Firnhaber et al., 2009; Hoffmann et al., 2008; Lukhwareni et al., 2009; Mphahlele et al., 2006]), but all are well above that observed in the cohort presented here. This is remarkable, as HBV prevalence rates are generally assumed to be high in rural African areas [Abdool Karim et al., 1989; Kew, 1996]. It is unclear why the observed HBV prevalence in this cohort is so low. Contact with high-endemic areas was possibly limited due to geographical and ethnic factors that may have reduced the amount of migration and number of interracial marriages.

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The paradigm is that the vast majority of people in Sub-Saharan Africa come into contact with HBV early in life. This would result in the presence of anti-HBc in 70-95% of individuals [Burnett et al., 2005; Modi and Feld, 2007]. However, the majority (73%) of patients infected with HIV in this South African cohort lacked anti-HBc. These results are in line with other studies in South Africa that reported rather low anti-HBc prevalence rates (35-38%, [Burnett et al., 2007; Firnhaber et al., 2009; Lukhwareni et al., 2009]). People without anti-HBc are still at risk for acquiring HBV. Therefore, providing them with an HBV vaccine may be worth considering. Such vaccination is common practice in western countries. Future studies will have to establish the risk of acquiring HBV at an older age in African settings and determine whether routine vaccination of patients with HIV who test anti-HBc negative is cost-effective there.

Reported anti-HCV prevalence rates for peoples infected with HIV in Africa vary; for South Africa 1 to 13% of people with HIV are believed to be co-infected with HCV [Lodenyo et al., 2000; Mphahlele et al., 2006; Parboosing et al., 2008]. In LICs, the clinical consequences of being infected with HCV are limited. Treating HCV is generally not feasible due to high costs and frequent adverse events associated with currently available therapies. Moreover, a HCV vaccine is not yet available.

Although active HBV was detected rarely in the current study, a considerable proportion of anti-HBc positive patients in South Africa harboured an occult HBV co-infection. Previous South African studies reported approximately 23% of people with HIV - who tested anti-HBc positive and HBsAg negative - to have detectable HBV-DNA levels [Lukhwareni et al., 2009; Mphahlele et al., 2006]. In one study that included severely

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immunocompromised patients infected with HIV (mean CD4+ t-cell count 49 cells/mm$^3$), an even higher rate of occult HBV (88%) was found [Firnhaber et al., 2009]. These observations contribute to the idea that occult HBV represents opportunistic viral reactivation [Cohen Stuart et al., 2009; Lo Re et al., 2007; Shire et al., 2007]. In the current study, a significant association between the level of immunosuppression and the presence of occult HBV was not found, but a trend towards more occult infections in the case of a lower CD4 count was observed. In accordance with other African cohorts [Harania et al., 2008; Nagu et al., 2008], no correlation between the CD4+ T-cell count and the presence of an active HBV or HCV infection was found. The idea that HCV-infected individuals show the highest occult HBV prevalence [Raimondo et al., 2007] could not be confirmed by the data presented here.

In patients having an occult HBV infection only, occult HBV is linked to the transmission of HBV and the development of liver disease [Raimondo et al., 2007]. However, in the absence of prospective studies, it is unclear whether occult infections are associated with higher liver-related morbidity and mortality rates. Moreover, the clinical relevance of occult HBV is debatable as HBV-DNA loads are generally low and are likely to become undetectable with lamivudine, the only drug with anti-HBV activity included frequently in African ART regimens [Cohen Stuart et al., 2009].

There are potential limitations to this study. First, molecular analyses were limited to anti-HBc positive individuals. Although an occult HBV infection is associated with the presence of anti-HBV antibodies, a minority of occult carriers are negative for all HBV

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serum markers [Torbenson and Thomas, 2002]. Such cases would not have been identified. Second, due to the low HBV-DNA levels (all but one <1000 copies/mL) and the limited amount of plasma available, in-depth virological characterisation of HBV-DNA positive specimens was not performed. The possibility of a false positive result can therefore not be ruled out completely, but is unlikely considering the extensive control procedure that was applied. Third, the cohorts under study vary with regard to gender distribution, nadir CD4 counts and routes of transmission. This may have contributed to the observed difference in co-infection rates. Nonetheless, the results remain of interest as the differences in baseline characteristics represent how the epidemics vary between different geographical areas.

In conclusion, an exceptionally low frequency of HBV and HCV was observed in a cohort of individuals infected with HIV in rural South Africa. Rates were lower compared to both Caucasians and African immigrants who are receiving care in the Netherlands. Occult HBV on the other hand was observed more often in the South African cohort. This study highlights the need for local HBV/HCV prevalence data in order to guide decisions on future vaccination and treatment strategies.

**Conflicts of Interest:**

The authors have no conflicts of interest to declare.

**Ethics Statement:**

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This study was performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

Due to the retrospective nature of this study, no experiments were performed; only data used for clinical practice were included.

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**Table 1: Baseline characteristics of the different cohorts of persons who were infected with HIV, but antiretroviral naïve**

| | **Caucasians [a] (n=614)** | **African immigrants (n=168)** | Asian immigrants [b] (n=34) | South American immigrants [b] (n=30) | **South African residents (n=248)** |
|---|---|---|---|---|---|
| Age, years; mean (SD) | 40,1 (10,4) | 31,8 (8,6) | 34,0 (7,9) | 34,7 (8,2) | 40,5 (9,4) |
| Female gender; n (%) | 79 (13) | 93 (55) | 12 (35) | 8 (27) | 158 (74) |
| HIV-transmission route; n (%) | | | | | |
| • Heterosexual | 180 (29) | 156 (93) | 25 (74) | 17 (57) | 250 (100) [c] |
| • Homosexual | 371 (60) | 4 (2) | 9 (26) | 13 (43) | - |
| • Blood products | 20 (3) | 2 (1) | - | - | - |
| • IV drug use | 26 (4) | 1 (1) | - | - | - |
| • Unknown | 17 (3) | 5 (3) | - | - | - |
| CD4, cells/mm$^3$; median (IQR) | 337 (159-549) | 264 (155-420) | 232 (137-503) | 378 (115-625) | 273 (91-393) |
| ASAT, U/l; median (IQR) | 31 (24-43) | 35 (27-50) | 33 (24-43) | 36 (28-49) | 32 (20-52) [d] |
| ALAT, U/l; median (IQR) | 27 (20-44) | 22 (16-35) | 31 (17-47) | 26 (18-45) | 24 (17-37) [d] |

[a]: Mainly western Europeans. This group also includes 12 Eastern Europeans, 3 patients from the USA and 1 from Australia. [b]: Asian and South American immigrants were excluded from further analyses. [c]: HIV-transmission route was not clearly documented in Elandsdoorn, South Africa. Heterosexual contact was considered to be the most probable route of transmission. [d] : ASAT and ALAT in Elandsdoorn were only tested when patients started ART. Results are therefore based on a mere subset of patients.
n: number of patients, SD: standard deviation, %: percentage of patients, IV drug use: intravenous drug use, CD4: number of CD4+ T-cells, IQR: inter-quartile range

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## Table 2: Serological outcome data according to ethnicity

| | Caucasians (n=607) | African immigrants (n=168) | South African residents (n=242) |
|---|---|---|---|
| **HBsAg, n (%)** | 27 (4.4) | 15 (8.9) | 1 (0.4) |
| **Anti-HBc, n (%)** | 251 (41.4) | 97 (57.7) | 68 (28.1) |
| • *also anti-HBs positive, n (%)* [a] | *173/230 (75.2)* | *47/83 (56.6)* | *37/59 (62.7)* |
| **Anti-HCV, n (%)** | 66 (10.9) | 8 (4.8) | 2 (0.8) |

n: number of patients with a positive test result, [a]: proportion of patients who were anti-HBc positive and were tested for anti-HBs

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**Table 3:**
**Predictors of an occult HBV infection in anti-HBc positive individuals**

| | **Occult HBV** Yes | **Occult HBV** No | **Univariate** P value | **Multivariate** P value |
|---|---|---|---|---|
| CD4 (mean, cells/mm$^3$) | 214 | 363 | ***0.06*** | 0.06 |
| Presence of anti-HCV (%) | 7.1 | 11.8 | 0.94 | - |
| Presence of anti-HBs (%) | 63.6 | 67.9 | 0.77 | - |
| 10log HIV-RNA (mean) | 5.0 | 4.5 | 0.25 | - |
| Ethnic origin (%) | | | ***0.02*** | 0.18 |
| • Caucasians | 46.2 | 59.1 | | |
| • African immigrants | 7.7 | 24.4 | | |
| • South Africans | 46.2 | 16.5 | | |
| Male gender (%) | 64.3 | 72.4 | 0.51 | - |
| HIV transmission route (%) | | | 0.70 | - |
| • Heterosexual | 57.1 | 48.3 | | |
| • Homosexual | 35.7 | 46.1 | | |
| • IV drug use | 7.1 | 3.3 | | |
| • Blood transfusions | 0 | 2.2 | | |

CD4: number of CD4+ T-cells, %: percentage of patients, IV drug use: intravenous drug use

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