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SAJHIVMED

Southern African Journal of HIV Medicine

- Guidelines for PrEP for MSM at risk of HIV infection
- Clinical case study resource for CME
- Analysis of CD4 count variability within and among laboratories
- Lung fibrosis, HIV and PcP
- Part 2: Blood and blood product use in HIV-infected patients

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From the Editor

SAJHIVMED has undergone a bit of a facelift since the previous issue. For more than a decade, the layout and formatting had been essentially unchanged, and a revamp was in order to provide a more contemporary profile suitable to a research-driven publication. The new look was shaped by the talented team at the Health & Medical Publishing Group (who produce the *SAMJ* and several other prominent journals, as well as the *South African Medicines Formulary*), particularly Siobhan Tillemans and Melissa Raemaekers. Thanks to both, and the entire HMPG team, for their assistance!

Along with the new aesthetic, we are pleased to announce a new editorial board to support the Journal's work. The names on the masthead will be known to many readers, as they represent some of South Africa's leading researchers and clinicians working in HIV/AIDS. In addition, there are some exciting developments planned to increase the international visibility and accessibility of *SAJHIVMED*, and I will keep you posted on news as it emerges.

This edition of the journal contains several notable items, with a particular focus on laboratory assessments. There is ongoing interest in the new markers that may be used to monitor HIV disease progression and response to antiretroviral therapy; Bipath and colleagues suggest that neopterin levels may be more strongly correlated with standard HIV disease markers (e.g. viral load or CD4 cell count) than either C-reactive protein or procalcitonin. In another interesting piece, Gounden *et al.* investigate a case of how host genetics – here, allelic variation in the genes that promote tumour necrosis factor-alpha – may influence HIV disease progression. Both these studies present intriguing findings that point to the need for further research with a particular view towards their clinical utility. A more practical laboratory assessment comes from Swaziland, where Mlawanda and colleagues examined the variability of CD4 enumeration both within and between labs – a real-world concern that is commonly raised by healthcare providers and patients alike. Although the sample sizes are small, the results are somewhat reassuring, with reasonable agreement in results between labs. Two case series explore common complications of advanced HIV disease, including CMV retinitis (Laher), suggesting reasonable outcomes despite the absence of systemic therapy, and *Pneumocystis* pneumonia (Shaddock), providing evidence for lung fibrosis in individuals with advanced disease requiring ventilation.

This edition also continues *SAJHIVMED*'s tradition of publishing important guidelines from the Southern African HIV Clinicians' Society that help to shape programmes and services across the region. Prevention strategies using antiretrovirals have demonstrated efficacy in reducing the sexual transmission of HIV, most notably in the realm of pre-exposure prophylaxis (PrEP). The evidence for PrEP's efficacy is strongest in research among men who have sex with men (MSM), yet there are currently no tools to guide service providers. Here, Bekker and colleagues present comprehensive guidelines on implementing PrEP among MSM, the first such document of its kind internationally.

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Message from the Executive

In late March 2012, all the elected members of the Board of the Southern African HIV Clinicians Society met for the first time under my leadership to discuss the way forward. We decided that our objectives would now include partnering with governments to implement optimal HIV programmes and policies. For South Africa, this means doing all we can to assist the government to achieve the goals of National Strategic Plan 2012 - 2016.

In line with this objective, we faced the following challenge in the ensuing weeks: We received reports from a number of sites that there were some medication stock-outs, including Tenofovir. Concerned healthcare workers wanted to know how they could provide for their patients. Knowing that treatment interruptions have long-term consequences in terms of resistance as well as other complications, many healthcarers were not sure how to handle drug shortages. Some patients can have treatment substitutions, so we convened a virtual ad hoc committee of the brains trust of the Society. Within a matter of days, we drafted a consensus statement that was sent to the Director-General of Health (and is included in this issue). As we went through this process, I was struck by the depth of experience we have in the Society. Our brains trust must be unequalled anywhere in the world.

To now assist the Department of Health, we are implementing an SMS stock-out line for healthcare workers to report drug shortages. All reports will be submitted to the Department of Health every second week of each month. We will monitor shortage trends and the progress towards resolution. Enclosed is a stock-out report form that includes details of applicable drugs and information on how to report shortages.

Another area we have agreed to focus on is the improvement of TB diagnosis, care and prevention within the context of the HIV epidemic. We have made many remarkable achievements in the diagnosis, care and treatment of HIV infection, and we need to repeat this for TB. The overlap between the two epidemics is very substantial. For the first time in my career, there have been some new and exciting developments. The GeneXpert technology has been adopted in a bold move for

the diagnosis of TB. The Department of Health has announced that ART should be provided for all HIV-infected individuals who get TB, irrespective of their CD4.

It seems to me that we are now at a stage in the TB epidemic where we were about 10 years ago in the HIV epidemic. We are on the brink of new interventions and there is renewed drive within the Department to tackle the problems. But the task ahead is huge. About 1% of South Africans contract TB every year. Will we achieve the NSP goal of a reduction of this incidence rate by half within 5 years? To my mind, we can only do so if we apply the same determination that we did in the HIV arena. TB is our next battle, and looming behind that is the ever-increasing burden of drug-resistant TB.

Finally, please don't forget that we have a conference coming up at the end of the year in Cape Town from 25 - 28 November. Entitled 'Striving for Clinical Excellence', this is a conference that I think all southern African HIV clinicians should attend. There is no better way to spend the last week in November than in the Mother City – rubbing shoulders with the best extant HIV healthcare workers.

Francesca Conradie
President
Southern African HIV Clinicians Society
Johannesburg

GUIDELINES

Southern African guidelines for the safe use of pre-exposure prophylaxis in men who have sex with men who are at risk for HIV infection

The Consensus Committee, Southern African HIV Clinicians Society, chaired by Linda-Gail Bekker and Kevin Rebe. MEMBERS: **Ben Brown, Peter Budnik, Glenn de Swardt, Zoe Duby, Nathan Geffen, Brian Kanyemba, James McIntyre, Landon Myer, Andrew Scheibe, Laurie Schowalter, Mark Sonderup, Wendy Spearman, Carlos Toledo, Tim Tucker, Reon van Dyk, Gert van Zyl**

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Background. The use of oral antiretrovirals to prevent HIV infection among HIV-negative men who have sex with men (MSM) has been shown to be safe and efficacious. A large, randomised, placebo-controlled trial showed a 44% reduction in the incidence of HIV infection among MSM receiving a daily oral fixed-dose combination of tenofovir disoproxil fumarate and emtricitabine (Truvada) in combination with an HIV prevention package. Improved protection was seen with higher levels of adherence.

Aim. The purpose of this guideline is to: (*i*) explain what pre-exposure prophylaxis (PrEP) is; (*ii*) outline current indications for its use; (*iii*) outline steps for appropriate client selection; and (*iv*) provide guidance for monitoring and maintaining clients on PrEP.

Method. PrEP is indicated for HIV-negative MSM who are assessed to be at high risk for HIV acquisition and who are willing and motivated to use PrEP as part of a package of HIV prevention services (including condoms, lubrication, sexually transmitted infection (STI) management and risk reduction counselling).

Recommendations. HIV testing, estimation of creatinine clearance and STI and hepatitis B screening are recommended as baseline investigations. Daily oral Truvada, along with adherence support, can then be prescribed for eligible MSM. PrEP should not be given to MSM with abnormal renal function, nor to clients who are unmotivated to use PrEP as part of an HIV prevention package; nor should it be commenced during an acute viral illness. Three-monthly follow-up visits to assess tolerance, renal function, adherence and ongoing eligibility is recommended. Six-monthly STI screens and annual creatinine levels to estimate creatinine clearance are recommended. Hepatitis B vaccination should be provided to susceptible clients. Gastro-intestinal symptoms and weight loss are common side-effects, mostly experienced for the first 4 - 8 weeks after initiating PrEP. There is a risk of the development of antiretroviral resistance among those with undiagnosed acute HIV infection during PrEP initiation and among those with sub-optimal adherence who become HIV infected while on PrEP. Risk compensation (increasing sexual behaviours that can result in exposure to HIV) while on PrEP may become a concern, and clinicians should continue to support MSM clients to continue to use condoms, condom-compatible lubrication and practice safer sex. Research is ongoing to assess optimum dosing regimens, potential long-term effects and alternative PrEP medications. Recommendations for the use of PrEP among other at-risk individuals, and the components of these recommendations, will be informed by future evidence.

S Afr J HIV Med 2012;13(2):40-55.

Men who have sex with men (MSM) is a term that describes men who have sex with men, regardless of social identity (gay, bisexual, heterosexual) or whether they also have sex with women.[1] MSM have been shown to be at disproportionately high risk of HIV acquisition and transmission.[2,3] Biological susceptibility (efficiency of rectal HIV transmission), behaviours (including unprotected anal intercourse and multiple partners) as well as structural and social factors (including homophobia and discrimination) have been associated with increased vulnerability to HIV.[3] Unprotected receptive anal intercourse is the main risk factor for sexual transmission of HIV among MSM.[4] The high concentration of rectal cells vulnerable to HIV-1 infection (macrophages, T-cells and dendritic cells) and the single-cell layer of rectal mucosa, results in a per-act risk for HIV transmission that is 10 - 20 times greater than unprotected vaginal intercourse.[4-6]

MSM and HIV in southern Africa

There is emerging and consistent evidence about the high HIV burden among MSM in southern Africa.[7] HIV prevalence among MSM sampled in cross-sectional surveys in South Africa has ranged from 10 - 50%.[8-11] However, owing to the lack of accurate population size estimates, it is hard to assess attributable risk.[12] A 2009 modelling study on the modes of

HIV transmission in South Africa estimated that 8% of all new HIV infections in South Africa occur among MSM.[13] High-risk sexual practices (including unprotected anal intercourse, multiple and concurrent partnerships, and sex work) and limited knowledge about HIV and substance use (alcohol, methamphetamines and heroin) have been associated with increased risk for HIV infection among MSM in South Africa.[2,9-11,14-16] Many MSM also have female sexual partners. Almost half (49%) of the participants in a Soweto-based MSM study reported recent female sexual partners.[10] Homophobia, stigma and discrimination (including criminalisation of same-sex behaviours in some southern African countries), health care worker ignorance (about MSM vulnerability to HIV and appropriate management of MSM clients) and the heterosexual focus of the HIV response have been contributing factors to the failure of southern African public health services to address the health needs of MSM.[2,12,17-24]

The purpose of the MSM pre-exposure prophylaxis guideline is to:

- explain what pre-exposure prophylaxis (PrEP) is
- outline current indications for its use
- outline steps for appropriate client selection
- provide guidance to monitor and maintain clients using PrEP.

Pre-exposure prophylaxis

Pre-exposure prophylaxis (PrEP) is the taking of a pharmaceutical agent prior to an exposure to prevent an outcome (e.g. infection by a microbe). PrEP for HIV utilises antiretroviral medications to prevent HIV infection. Research into the use of existing and novel PrEP agents, topical (microbicide) and oral (tablet) formulations is ongoing. In the Global iPrEx trial, PrEP was shown to decrease HIV incidence among at-risk MSM (see text box).[25] The results of this randomised placebo-controlled trial offer a new opportunity for HIV prevention. Truvada, the oral antiretroviral agent used in the iPrEx trial, is available for off-label use for PrEP in South Africa.

Development of PrEP

Truvada (tenofovir disoproxil fumarate (TDF) in combination with emtricitabine (FTC)) was chosen for the evaluation of pre-exposure prophylaxis because of its high level of activity in inhibiting HIV replication; its acceptable safety profile; its high barrier to generating resistant virus; and its low levels of side-effects.[26] The protective activity of TDF and FTC has been shown in animal models, with best efficacy when both agents were used together.[27,28] Several trials of daily oral TDF or TDF/FTC among heterosexual men and women have recently been completed. Additional trials with heterosexual women and injecting drug users are ongoing (http://www.avac.org/ht/a/GetDocumentAction/i/3113). The findings of the PrEP trials among heterosexual men and women have yielded differing efficacy results, with some showing efficacy among heterosexual sero-discordant couples receiving either TDF or TDF/FTC (Partners-PrEP) and among young men and women (TDF2) receiving TDF/FTC. One PrEP trial assessing the efficacy of daily oral TDF/FTC among women (FEM PrEP) was stopped for reasons of futility (the inability to determine efficacy), and the oral and topical tenofovir arms in the VOICE trial with women were stopped for futility while assesment of efficacy of daily oral TDF/FTC in the VOICE trial is continuing.[29-31] Research is under way to assess reasons for these differing results.

The global iPrEx trial

The global iPrEx trial was a double-blinded, randmised placebo-controlled trial to assess the safety and efficacy of daily oral Truvada for the prevention of HIV among MSM and transgender women. The subjects were 2 499 HIV-seronegative MSM or transgender women who have sex with men enrolled from 11 sites in 6 countries. The Cape Town site was initiated later than other sites, and only 88 MSM from South Africa were enrolled (3.5% of total cohort) before the study was fully enrolled. All subjects received monthly HIV testing, risk-reduction counselling, condoms and management of STIs. The study subjects were followed for 3 324 person-years (median 1.2 years, maximum 2.8 years)(until 1 May 2010). Of the subjects, 10 were infected with HIV at enrollment (in their 'window' period), and 100 became infected during follow-up (36 in the Truvada group and 64 in the placebo group). In the modified intent-to-treat analysis (excluding those who were infected at enrolment and those with no follow-up HIV test results), an overall 44% reduction in the incidence of HIV infection (95% confidence interval 15 - 63%; p=0.005) among those randomised to Truvada use was seen. An as-treated analysis showed that participants who reported taking the study drug at least 50% of the time, experienced 50% fewer infections. Participants who reported taking 90% or more of their daily doses, experienced an efficacy of 73%.[25]

Drug levels were assessed in a case-control analysis of a subset of trial participants. Each MSM who acquired HIV infection during the trial was matched with two MSM who remained uninfected. No drug was detected in participants in the placebo arm. Among participants in the Truvada arm, drug was detected in 22 of 43 participants without HIV infection (51%) and in 3 of 34 HIV-infected participants (9%) (p<0.001).[25]

Nausea and unintentional weight loss were reported more frequently during the first 4 weeks in the group receiving Truvada than in the placebo group (p<0.001). The two groups had similar rates of serious adverse events (p=0.57).[25]

Motivation for a MSM PrEP guideline

The iPrEx trial results contributed to the development of interim guidance on the use of PrEP among MSM by the United States Centers for Disease Control and Prevention.[32] Based on the results of the iPrEx and Partners PrEP trials, a submission to the United States' Food and Drug Administration is under consideration for expanding the indications for the use of Truvada to include the prevention of sexual acquisition of HIV among MSM and heterosexual adults. Truvada is not currently licensed for use as PrEP in South Africa. Southern African guidelines will assist practitioners who may be considering, or are already, prescribing PrEP to at-risk MSM clients. This guideline is based on current evidence, and future data will inform its revision and the potential extension of indications to other population groups.

Initiation of PrEP

Steps for the screening, initiation and maintenance of PrEP for MSM are shown in Fig. 1.

1. Identification of potential PrEP users

Providers should educate and counsel MSM clients about PrEP and conduct an individualised risk-benefit assessment to assess eligibility.

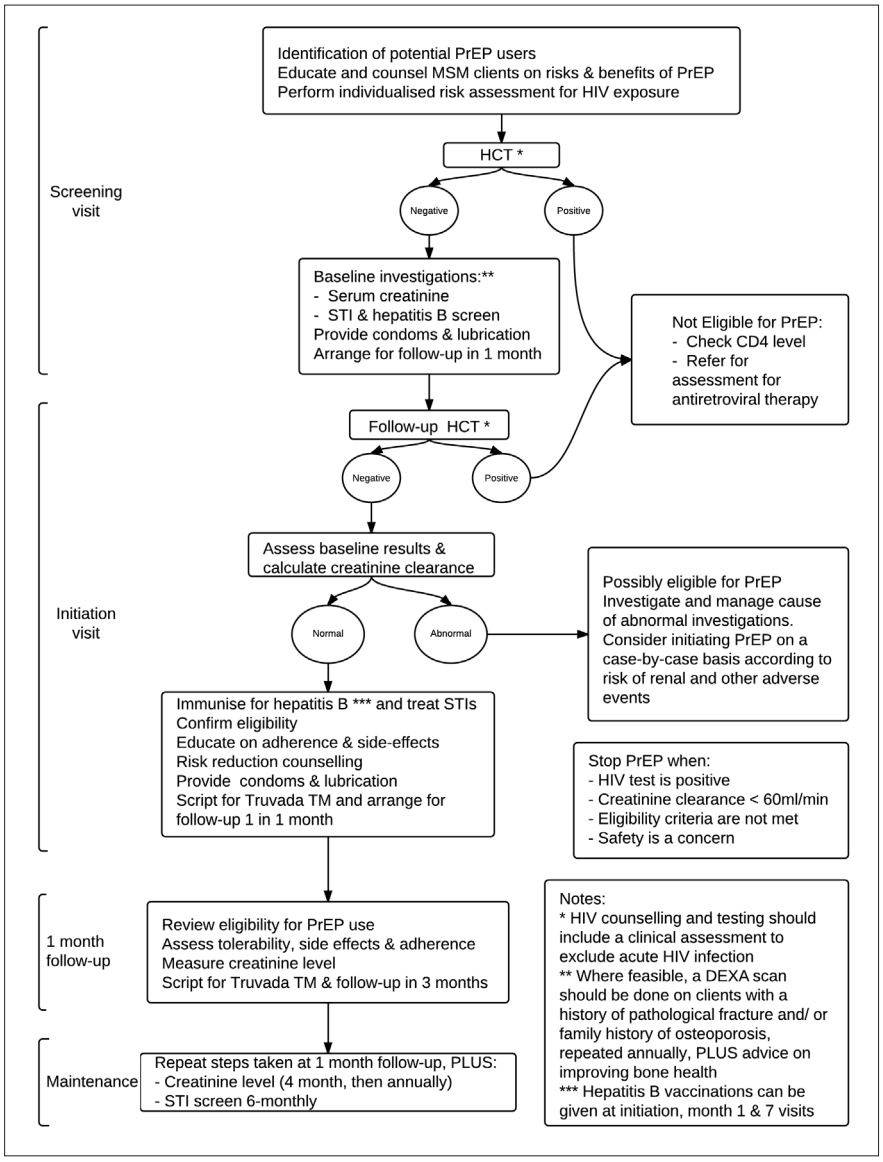

Fig. 1. Flowchart for the screening, initiation and maintenance of PrEP among MSM.

Eligibility criteria for PrEP use include:

- men who have sex with men (MSM) (including those who also have sex with women) who are identified by the provider and client as being at high risk for HIV exposure (see text box on Indications for the use of PrEP)
- no contra-indications to Truvada (FTC/TDF)
- HIV-negative by routine rapid antibody test
- absence of symptoms of acute HIV infection (recent acute viral illness) and, if symptoms reported, HIV-negative by 4th-generation HIV test or other HIV antigen test if available (this reduces, but doesn't eliminate, the window period)
- motivated to follow PrEP prescribing guidelines
- willing and able to adhere to daily oral dosing†
- willing and able to attend 3-monthly PrEP maintenance visits, inclusive of HIV counselling and testing, clinical review and safety monitoring procedures
- client understanding that the protection provided by PrEP is not complete, and of the need for PrEP to be used as part of a package of HIV prevention services (inclusive of condoms, lubrication, risk reduction counselling and STI management)

2. Baseline investigations

After documenting eligibility and motivation for PrEP use, mandatory baseline investigations should be completed (Table 1). If resources permit, a DEXA scan to measure bone mineral density among individuals who report a history of pathologic fracture or a family history of osteoporosis should be considered. Unavailability or inability to cover the costs of a DEXA scan should not preclude PrEP use. Condoms and condom-compatible lubrication should be provided, and arrangements for follow-up made.

Indications for the use of PrEP

PrEP may be suitable for MSM who:

- engage in anal sex and are HIV uninfected
- are at high risk for HIV acquisition
  - MSM with multiple partners
  - MSM engaging in transactional sex, including sex workers
  - MSM who use or abuse drugs
  - MSM who drink alcohol heavily
  - More than 1 episode of a STI in the last year
  - Couples‡
    - HIV-negative partner in a discordant relationship, especially if the positive partner is not on antiretroviral therapy (ART)
    - Both partners HIV negative in a non-monogamous concordant relationship
  - MSM who are unable or unwilling to achieve consistent use of male condoms
- are motivated, able and willing to adhere to daily oral dosing.

Contraindications for PrEP:

HIV-1 infected or evidence of possible acute HIV infection

- allergy to tenofovir disoproxil fumarate and/or emtricitabine
- poor renal function (estimated creatinine clearance <60ml/min)
- unwilling or unable to return for 3-monthly HIV testing, counselling and safety monitoring visits.

3. Implementing PrEP

At the follow-up visit, repeat the rapid HIV test and do a review for acute viral symptoms. Review results from baseline investigations and confirm that estimated creatinine clearance >60 ml/min. Commence hepatitis B vaccination if susceptible and provide STI treatment as required (Table 2). Educate

†Therapeutic drug monitoring is currently not routine, although methods that require less invasive procedures, such as measuring drug levels in hair, are being validated.
‡Couples in this instance refers to men who have had sex with each other more than once.

Table 1. Mandatory baseline investigations for PrEP initiation among MSM

HIV infection	Rapid HIV antibody test
Renal function	Estimated creatinine clearance (ml/min) (formula for males) $\frac{(140 - \text{age in years}) \times \text{weight (kg)}}{0.82 \times \text{plasma creatinine (µmol/l)}}$
Hepatitis B screen	Surface antigen (HBsAg) Antibody to surface antigen (HBsAb)
STI screen	Symptomatic screen Examination if indicated Urine dipstix for urethritis Serological screening for syphilis (rapid or laboratory)

the client about potential PrEP side-effects and their management, as well as signs and symptoms of acute HIV infection (and need to return for 'urgent' HIV testing). Initiate a medication adherence plan and provide a 1-month Truvada prescription (1 tablet orally, daily) together with a 1-month follow-up date (Table 3).

Risk-reduction counselling

Risk-reduction counselling is a behavioural intervention that attempts to decrease an individual's chances of acquiring HIV and other STIs,[33] and should be implemented together with adherence counselling at follow-up visits for clients using PrEP. The main objective of risk-reduction counselling is for clients to set a realistic goal for behaviour change that could reduce their risk of contracting HIV. This is most effective when it is non-prejudicial and client-centred. Risk reduction counselling can be provided by any trained healthcare provider and should address the following points:

1. Explore the context of the user's specific sexual practices, and assist client to recognise which of their behaviours are associated with higher risks for HIV infection. Clinicians should also be aware that clients may not always perceive their own risk, or be in denial about it.
2. Identify the sexual health protection needs of the user and reflect on what their main concerns appear to be.
3. Strategise with the user on how they can manage these concerns or needs.
4. Agree on which strategies the user is willing to explore and guide the user to decide on how to implement the strategy.

Adherence support

Adherence to daily PrEP medication, as shown in the iPrEx study and other PrEP trials, is a challenge. Adherence counselling should be implemented at each visit where PrEP prescriptions or distributions are made. In iPrEx, MSM who took PrEP more consistently and had evidence of drug detection in their blood, had higher levels of protection than those who did not.[25]

Clients will need to be made aware of the fact that drugs only work if present at adequate levels in tissues and, preferably, drug levels should be adequate before and after exposure to HIV has occurred.

The use of cell phone reminders, pill boxes, and linking pill taking with a daily routine activity are currently being evaluated for their impact on improving PrEP adherence. Clinicians and clients could use any of these or other strategies to assist in maximising adherence (see text box on Tips to Support Adherence). Any trained healthcare worker can implement adherence counselling. A client-centred approach is recommended. Drug level testing for tenofovir levels in plasma is available, but is expensive. Drug level testing may be useful to assess adherence in the future.

Tips to support adherence

Include patient-focused adherence counselling at each contact. Provide a clear explanation of the benefits of adherence. In a neutral manner, ask if the client has any challenges that may make adherence difficult. Also explore possible facilitators to pill taking. Include identified facilitators when developing strategies to improve adherence.[34]

Options to improve daily pill taking:

- use reminders (cell phone, alarm clock, diary, partner reminder)
- link with daily activity (breakfast, brushing teeth)
- use of a pill box.

Strategies to reduce likelihood of antiretroviral resistance

Feasibly exclude acute HIV infection before initiating PrEP by:

- conducting antibody HIV testing before commencing or represcribing PrEP
- among persons with a negative antibody HIV test, conduct a clinical screen to detect signs and symptoms of acute HIV infection – history of fever, sore throat, rash, joint pain, cough in the past month and a targeted examination (temperature, ENT and skin exam)(see Acute HIV infection text box)
- If symptoms or signs of acute HIV infection found:
  - at screening: postpone PrEP until symptoms subside and rapid antibody test remains negative
  - at screening: do not initiate PrEP until confirmatory HIV antigen/antibody testing complete*
  - at follow-up: may elect to continue PrEP while awaiting results of confirmatory HIV antigen/antibody testing or may decide to withhold PrEP until confirmatory tests available
- support client to maximise adherence and include adherence counselling at each visit
- stop PrEP should requirements for PrEP eligibility not be fulfilled.

*Use 4th-generation HIV rapid (antigen+antibody) tests where available to confirm HIV infection status.

Table 2. Syndromic treatment of STIs among MSM

Urethritis	Cefixime 400 mg PO stat, **plus** doxycycline 100 mg PO 12-hourly for 7 days. If symptoms persist after 7 days and repeat exposure and poor adherence are excluded, give metronidazole 2g PO stat. If still symptomatic after a further 7 days, refer.
Genital ulcers	Benzathine penicillin 2.4 million units IM stat for primary syphilis (repeat benzathine penicillin x 2, at weekly intervals for late syphilis), **plus** erythromycin 500 mg PO 6-hourly for 7 days **and** acyclovir 400 mg PO 8-hourly for 7 days
Rectal discharge/proctitis	Cefixime 400 mg PO stat (or ceftriaxone 250mg IMI stat) **plus** doxycycline 100 mg 12-hourly for 7 days (also screen for syphilis and consider acyclovir if any suggestion of ulcerative anal disease).

Table 3. Summary of PrEP visits and procedures

Visit	Recommended procedures
Screening visit	Educate about the risks and benefits of PrEP Assess eligibility and motivation Conduct HIV counselling and testing, serum creatinine level and STI and hepatitis screen Arrange follow-up
PrEP initiation visit	Conduct HIV counselling and testing Confirm eligibility (including investigation results and a calculation of creatinine clearance) Commence hepatitis B immunisation (if indicated) Provide STI treatment (if indicated) Educate client about PrEP side effects and their management Educate client about signs and symptoms of acute HIV infection Discuss behaviours that promote bone health, such as weight-bearing exercise, maintaining adequate calcium and vitamin D intake, and avoiding alcohol, tobacco and recreational drugs Initiate a medication adherence plan Provide condoms and lubricant Provide 1-month Truvada prescription and 1-month follow-up date
1-month follow-up	Same as PrEP initiation visit, plus: assess tolerability, side-effects and adherence measure serum creatinine and calculate creatinine clearance provide 3-month Truvada prescription and follow-up date
4-month follow-up and maintenance	Repeat procedures done at 1-month follow-up Measure serum creatinine and calculate creatinine clearance at 4-month follow-up, and annually thereafter Conduct 6-monthly STI screen for urethritis, genital ulcers and proctitis, including urine dipstix and rapid syphilis test Complete hepatitis B immunisation

Managing abnormal screening results

Clients with abnormal renal function (estimated creatinine clearance <60 ml/min) should not be placed on PrEP. An abnormal estimated creatinine clearance result could be rechecked after 2 weeks and, if renal function returns to normal and other PrEP criteria are met, PrEP may be initiated. MSM who are susceptible to hepatitis B should be immunised.* Clients with a history of pathological bone fracture, a family history of osteoporosis, or decreased bone mineral density on DEXA scanning, should be educated on ways to improve bone health, such as weight-bearing exercise, maintaining adequate calcium and vitamin D intake, and avoiding alcohol, tobacco and recreational drugs.[35] MSM who are ineligible for PrEP require support to assess other prevention options (see HIV Prevention for MSM text box). Treat STIs syndromically as per national guidelines (Table 2).[36] MSM who test HIV positive should be clinically staged, have a CD4 count taken and be managed in line with HIV treatment guidelines (http://www.sahivsoc.org/practise-guidelines/national-dept-of-health-guidelines).

Safety monitoring and maintenance

MSM using PrEP require an initial 1-month follow-up to assess ongoing eligibility, tolerance, safety and adherence. Hepatitis B vaccination and STI treatment (as appropriate), condoms and condom-compatible lubricant, risk reduction counselling, adherence support, a 3-month prescription for Truvada and a follow-up date should be provided. Thereafter, 3-monthly visits are recommended (Table 3). Details on recommended monitoring of bone mineral density is provided under **Other notes for PrEP prescribers** below.

Managing abnormal follow-up visit results

PrEP should be stopped if estimated creatinine clearance <60 ml/min. Repeat creatinine clearance should be rechecked after 2 weeks; if renal function returns to normal and other PrEP criteria are met, PrEP may be restarted.

*Hepatitis B immunisations could be provided at PrEP initiation and at 1-month and 7-month follow-up visits. This schedule differs from standard vaccination at months 0, 1 and 6, but would minimise additional visits.

STIs should be treated syndromically (Table 2).

By mutual agreement, PrEP should be stopped if: HIV test is positive; the client no longer meets eligibility criteria; the client and provider feel that adherence to PrEP is too onerous; or it is perceived by the clinician that the risks of PrEP outweigh potential benefits.

MSM who are ineligible for PrEP require support to access other prevention options (see **HIV prevention for MSM** text box below).

Risks and side-effects

Antiretroviral resistance

The only HIV resistance documented to date among PrEP users has been among clients who started using PrEP when they were already HIV-infected (during acute HIV infection). Predictably, FTC resistance mutations were the first to occur.[25] To prevent the risks of ARV resistance, clinicians must focus on not providing PrEP during acute HIV infection.

HIV testing should be done 3-monthly, and should be accompanied by a symptom screen and a targeted examination to exclude acute HIV infection (see text box on **Acute HIV infection**). HIV testing should also be repeated whenever symptoms of a viral illness are present. Clinicians should advise clients on the need for an HIV test before resuming PrEP if it was stopped, particularly if they have potentially been exposed to HIV during this period.

Side-effects

Most available Truvada safety data are derived from studies of HIV positive individuals receiving ART.[26] Safety data of Truvada use in HIV-negative individuals are emerging from PrEP trials and are reassuring.[25]

Gastro-intestinal side-effects

The side-effects related to Truvada use in PrEP trials (nausea, weight loss) were mostly self-limiting start-up symptoms (first month), but these may adversely affect PrEP adherence. Supportive counseling and symptomatic treatment (anti-emetics) of these symptoms are often sufficient. Rates of other GIT symptoms (bloating, abdominal tenderness, flatulence) among PrEP trial participants who took Truvada were not significantly different from those who took placebo.[25]

Acute HIV infection

Severity of the syndrome ranges from mild non-specific 'viral' or 'flu-like' symptoms to a severe infectious mononucleosis like illness with immune dysregulation and transient profound CD4 depletion.[37,38]

Symptom	Sign
Malaise	Fever, sweating
Anorexia	Generalised lymphadenopathy
Myalgias	Hepatospenomegaly
Headache	Non-exudative pharyngitis
Sore throat	Aphthous ulceration
Sore glands	Truncal rash (maculopapular or urticarial)
	Viral meningitis
	Guillian-Barre syndrome
	Pneumocystis pneumonia
	Cryptococcal meningitis
	Oesophageal candidiasis

Potential predictable side-effects

Major side-effects: renal toxicity and metabolic complications (decreased bone mineral density)

Minor side-effects: gastrointestinal symptoms (diarrhoea, nausea, vomiting and flatulence), unintentional weight loss and a small risk of lactic acidosis and hepatic steatosis or steatohepatitis

Less predictable side-effects: may include hypersensitivity reactions and flares of hepatitis B in clients who are chronic carriers who receive and then stop tenofovir, lamivudine or emtricitabine

Renal toxicity

Modest, transient increases in serum creatinine have been noted in completed PrEP studies, but these did not persist after stopping PrEP nor recur on rechallenge. Proteinuria, decreasing glomerular filtration rate (GFR) and Fanconi's syndrome* have been described in the setting of ART, and decreased GFR has been described in the setting of PrEP but has either been statistically or clinically insignificant.[25]

Renal function needs to be measured prior to commencement and monitored in clients using PrEP by measuring serum creatinine and calculating the estimated creatinine clearance. These parameters should be measured at baseline, at month 1, month 4 and then annually thereafter. Hypertensives, diabetics, and those with existing glomerulonephropathies (if the benefit of PrEP is still deemed to outweigh clinical risk) should have monthly renal function checks. Truvada-based PrEP should be avoided in patients who require the use of other nephrotoxic drugs such as aminoglycosides for the treatment of drug-resistant tuberculosis (TB). Clients with creatinine clearance <60 ml/min should **not** be placed on PrEP and, if found during maintenance, PrEP should be discontinued.

Decreased bone mineral density

Decreases in bone mineral density associated with TDF and FTC/TDF have been observed in completed PrEP trials. Decreases were less than those observed in HIV-infected individuals treated with the same drugs, and appeared to stabilise over time.[39,40] No difference in fracture rates were seen. Recreational drugs (amphetamines and inhalant use) were associated with reductions in bone mineral density in HIV-negative MSM taking TDF while enrolled in a PrEP study.[39]

Hepatitis B management

Tenofovir and emtricitabine both have hepatitis B antiviral activity. The risk exists that exposure to these antivirals may treat unidentified chronic hepatitis B infection with a consequent viral flare (rebound) upon drug withdrawal that can result in a severe liver injury.[41] It is recommended that screening for hepatitis B surface antigen and antibodies occurs prior to PrEP commencement. It is recommended that, if hepatitis B surface antigen (HBsAg) is positive, the client be referred for assessment prior to commencement of – in particular – short-term PrEP (Table 4). A possible approach to those with chronic hepatitis B infection may be to prescribe long-term tenofovir/emtricitabine. Liver function tests should be checked after stopping PrEP in those with chronic hepatitis B infection. Clients who are negative for both HBsAg and hepatitis B surface antibody (HBsAb) should commence a hepatitis B vaccine schedule. Clients with a history of injecting drug use should be

*Fanconi's syndrome consists of renal tubular acidosis, hypophosphataemia, hypouricaemia together with urinary losses of glucose, amino acids and protein sometimes coupled with a reduced glomerular filtration rate.

Table 4. Hepatitis B immune status and eligbility for PrEP

Hepatitis B surface antigen (HBsAg)	Hepatitis B surface antibody (HBsAb)	Action
negative (-)	negative (-)	Start PrEP, vaccinate concurrently
negative (-)	positive (+)	Start PrEP, no vaccine needed
positive (+)	N/A	Refer for evaluation

screened for hepatitis C and, if positive, referred for further care.

Risk compensation

This is the theoretical risk that individuals commencing PrEP will neglect other safer-sex measures, and put themselves at increased risk of HIV exposure. To date, no PrEP trials have borne out evidence in support of this risk. Providers should gauge this during risk reduction and adherence counselling opportunities.

HIV prevention package for MSM

The prevention of HIV acquisition requires a comprehensive approach, inclusive of a combination of biomedical and behavioural/psychosocial interventions tailored to individual needs. Where feasible, condoms and condom-compatible lubrication are key components of all HIV prevention packages, supported by STI detection and treatment, appropriate use of ART (post-exposure prophylaxis), and counselling around the identification of high-risk practices and ways to circumvent or reduce risk.

Stopping PrEP

PrEP should be stopped: whenever an HIV test is positive; at client request; for safety concerns (particularly if creatinine clearance <60 ml/min); and if the risks of PrEP outweigh the potential benefits. Linkage to appropriate HIV services should be arranged, and use of other HIV prevention strategies used, as needed.

The duration of PrEP use may vary and individuals are likely to start and stop PrEP depending on their risk assessment at different periods in their lives – including changes in relationship status, behaviours and ability to adhere to a PrEP maintenance programme. Clients should be advised that an HIV test should be done before PrEP is recommenced. Clinicians may want to discuss the options of when to discontinue PrEP with their clients.

Other notes for PrEP prescribers

PrEP will not suit all users. PrEP should be considered for MSM clients who are most likely to benefit from this specific prevention strategy as part of a package of HIV prevention services.

PrEP usage requires commitment. Usage will require commitment from both the provider and the user to ensure success. A paradox is that MSM clients who are most likely to benefit from PrEP because they are at the highest risk of exposure to HIV may find adherence to a programme particularly challenging. Providers may need to be innovative in providing support to these users.

HIV prevention for MSM

- accessibility of condoms and compatible water-based lubricant should be addressed
- no single HIV-risk reduction intervention is likely to suit all MSM
- combinations of prevention options, tailored to address specific risks, should be offered ('menu of prevention choices'), inclusive of biomedical and psychosocial/behaviour change interventions
- prevention options are likely to increase as new evidence becomes available.

Biomedical	Psychosocial
Male condoms and compatible lubrication	Regular HIV counselling and screening
Early access to ART	Reducing number of sex partners
Post-exposure prophylaxis (PEP)	Reducing alcohol and substance abuse
Pre-exposure prophylaxis (PrEP)	Addressing mental health needs
STI screening and treatment	Couples counselling and programming
Needle syringe exchange and opioid substitution therapy for MSM who inject drugs	Harm reduction counselling and support for drug using MSM

Drug-drug interactions

Tenofovir should not be co-administered with adefovir. Other drugs listed below can be co-administered but may require close monitoring, alteration of dosage or timing of administration.

Common drugs which may interact with emtricitabine (FTC) or tenofovir (TDF)

Drug name	FTC	TDF
Adefovir		X – do not co-administer
Cimetidine		X
Digoxin	X	X
Furosemide	X	X
Metformin	X	X
Naproxen	X	X
Ofloxacin	X	X
Streptomycin	X	X
Sulfadoxine/pyrimethamine	X	X

Source: University of Liverpool. Interactions with NRTIs, October 2011 (http://www.hiv-druginteractions.org/data/PrintableCharts/NRTI_col.pdf).

Table 5. Monitoring bone mineral density (DEXA scan) among MSM using PrEP

HIV acquisition risk	Osteopaenia risk	Resources	Intervention
High	High	High	PrEP + DEXA scan (baseline and 12-monthly)
Moderate	High	High	PrEP + DEXA scan (baseline and 12-monthly)
High	High	Low	PrEP + advise and observe
Moderate	Low	Low	PrEP + advise and observe
High	Low	High	PrEP + DEXA scan (baseline, repeat if indicated)
High	Low	Low	PrEP + observe

Monitoring of bone mineral density. Based on current evidence and expert opinion, and where feasible, baseline DEXA scans should be done in clients with a family history of osteoporosis and/or a pathological fracture. Importantly, the unavailability of DEXA should not preclude PrEP use. Annual follow-up DEXA scanning is suggested (Table 5). Ongoing research on the role of DEXA scanning will inform future recommendations.

PrEP: What we don't yet know

- What is the long-term efficacy of PrEP for MSM?
- What is the effect of PrEP on sexual behaviour and HIV risk?
- What are the long-term effects of tenofovir/emtricitabine on renal function, bone mineral density, chronic viral hepatitis B and other effects in HIV-negative MSM?
- Will resistance be a common event among those infected while using PrEP?
- What is the ideal PrEP regimen and dosing interval?
- What are the predictors of adherence for MSM who use PrEP?
- Which MSM are most likely to benefit from PrEP?
- What will be the role of PrEP among sero-discordant MSM couples?
- What will be the long-term effect on treatment programmes that share ART medications with PrEP programs?

The future of PrEP

Many questions surrounding the safe and effective use of PrEP exist; ongoing research aims to address these knowledge gaps (**PrEP: What we don't yet know** text box above).

The iPrEx open-label extension study, and other similar studies, are trying to increase our understanding around long-term PrEP usage (http://iPrExole.com/) specifically for MSM. Health facilities and health workers may be able to help answer these questions by keeping careful records of side-effects, patient adherence reports and HIV and hepatitis infections in their clients taking PrEP. Adverse events can be reported to the National Adverse Drug Event Monitoring Centre which is housed in the Division of Pharmacology at the University of Cape Town. The reporting guideline is available at: http://www.mccza.com/genericDocuments/2.11_ADR_reporting_Jun11_v2.doc.

EXTERNAL REVIEWERS: Stefan Baral, Chris Beyrer, Robert Grant, Al Liu, Kathy Muligan, Tim Lane, Dawn Smith.

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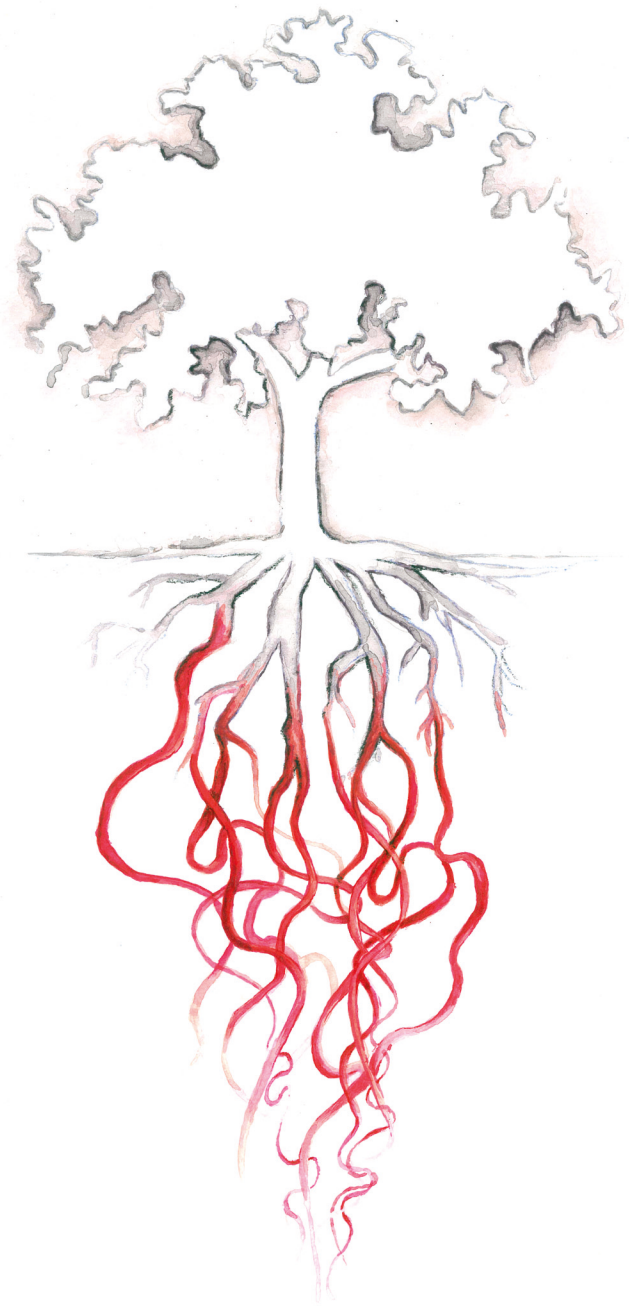

FORUM

Approaches to tenofovir and abacavir drug shortages in South Africa: A guide for clinicians

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Shortages of the nucleoside reverse transcriptase inhibitors (NRTI) abacavir and tenofovir have been reported recently at health facilities across South Africa.The Society issued the following clinical advice to healthcare providers experiencing shortages on 29 March 2012. These recommendations are intended only as a guide to clinical therapy, based on expert consensus and best available evidence. Treatment decisions for patients should be made by their responsible clinicians, with due consideration for individual circumstances.

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Tenofovir

If rationing of tenofovir (TDF) is required at a facility, the following patients should be prioritised to receive remaining TDF stocks:

- patients with chronic hepatitis B, as indicated by positive Hep B surface antigen. Interrupting TDF can cause life-threatening rebound hepatitis in these patients.
- patients who have experienced severe side-effects from d4T or AZT previously.
- If the patient developed symptomatic hyperlactataemia previously, d4T should not be used as this may result in life-threatening lactic acidosis.

In the event of TDF shortages, and if a patient on TDF is **virologically controlled:**

- The patient can in the short term be safely switched to d4T 30 mg bd or AZT 300 mg bd.
  - d4T is well tolerated in the short term, but prolonged use (>6 months) results in high rates of mitochondrial toxicity, causing peripheral neuropathy, lipoatrophy and hyperlactataemia. In any patient on d4T >4 months who complains of nausea, vomiting and/or weight loss, the diagnosis of symptomatic hyperlactataemia should be excluded with a measure of blood lactate. Peripheral neuropathy can be caused by d4T, so avoid in patients with pre-existing peripheral neuropathy.
  - Short-term side-effects of AZT include nausea, vomiting, headache, dizziness, fatigue, weakness and muscle pain. In addition, AZT can cause bone marrow suppression and may result in severe anaemia or neutropaenia. This drug should not be started in patients with haemoglobin <8 g/dl. Even if the patient has had AZT previously, Hb should be monitored after 4, 8 and 12 weeks after switching to AZT.
- It is very important to explain to the patient that both d4T and AZT are given twice daily, not once daily as with TDF.

If a patient is currently on TDF, and **NOT virologically controlled:**

- Changing a single drug in these patients may fuel development of resistance.
- We recommend continuing TDF for 3 months – with step-up adherence counselling – and repeat the viral load after 3 months. If the patient becomes virally suppressed, and TDF stocks are still limited, switch TDF as described above. However, if the viral load remains detectable, switch to regimen 2.

In ART-naïve patients, do not delay ART initiation. Instead of TDF, use d4T 30 mg bd or AZT 300 mg bd. Counselling as to side-effects should be provided and monitoring performed as per guidelines.

When TDF stocks are adequate, patients can transition immediately back to TDF from d4T or AZT if they are virologically controlled and have normal creatinine levels. Poor adherence during this disrupted period might have resulted in the emergence of drug-resistance.

Abacavir

Older children and adults on abacavir (ABC) have faced disruption owing to stock-outs of the tablet formulation. The response in this situation is to dispense the paediatric syrup to replace the tablets. However, the syrup is not very palatable, particularly in the large quantities required for older children and adults. Many of these patients cannot tolerate the syrup as it causes vomiting owing to its taste. As this threatens adherence, it may be preferable to switch these patients to an alternative NRTI for the short term and reserve the syrup for the younger children who require smaller, more manageable volumes. The same principles as described for TDF above should be followed.

If rationing of ABC is required at a facility, children with previous lactic acidosis or peripheral neuropathy owing to d4T or AZT should be prioritised to receive remaining ABC stocks.

In the event of ABC shortages, and if a patient on ABC is **virologically controlled:**

- The patient can in the short term be safely switched to d4T 1 mg/kg twice daily (with counselling on side-effects).
- Patients with current or previous lipodystrophy owing to d4T may benefit from switching to AZT 240 mg/m^2 (with counselling on side-effects).

If a patient is currently on ABC, and **NOT virologically controlled:**

- Changing a single drug in these patients may fuel development of resistance. We recommend continue ABC for 3 months – with step-up adherence counselling – and repeat the viral load after 3 months. If the patient becomes virally suppressed, and ABC stocks are still limited, switch ABC as described above. However, if the viral load remains detectable, switch to regimen 2.
- Children on an NNRTI-based regimen should switch to a second-line PI-based regimen as per guidelines.
- Children on a PI-based regimen should be discussed with an expert before switching to a second-line regimen.
- In adults, be alert as to why the patient is on ABC. Is it due to previous severe side-effects such that the patient should not be re-challenged with certain other NRTIs?

In ART-naïve patients, do not delay ART initiation. Instead of ABC, use d4T 1 mg/kg twice daily or AZT 240 mg/m^2 twice daily, with counselling on side-effects.

When ABC stocks are adequate, patients can transition immediately back to ABC from d4T or AZT when they are virologically controlled.

FORUM

Clinical case study programme

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The Southern African HIV Clinicians Society's online clinical cases are geared at providing excellent continuing medical education for members of the Society. This activity has been created to offer clinicians working in the HIV area access to online education. Cases are written by experienced HIV specialists and can range from general adult HIV/TB through specialist paediatric cases to other related infectious diseases encountered when managing patients with HIV.

Cases can be marked with one, two or three stars. One-star cases are basic cases directed towards clinicians who are new to the practice of HIV or who intend to start providing HIV care. They are also appropriate for clinicians who have more experience but who do not see HIV-positive patients on a routine basis and would like to refresh their knowledge around the management of key HIV-related conditions.

Cases marked with two stars are meant for clinicians who work daily with HIV-positive patients but would not consider themselves experts in the field. Cases marked with three stars are meant for clinicians who currently provide comprehensive HIV care and/or are specialists in the field of HIV and infectious disease. Each activity is accredited for four CPD points through the Health Professions Council of South Africa. While the activity is open to all users of the website, only members of the Society can redeem CPD points for successfully completing the activity.

Box 1 provides an abbreviated example of a case from the case library. This particular case illustrates the complex interaction between viral hepatitis B and HIV in mode of transmission, diagnosis and treatment. It also explores reasons why an HIV-1 viral load could be below the level of detection in this patient, or in an untreated patient in general. Other case studies focus on ART complications, paediatric HIV treatment and ethical issues. Case studies will be added on a regular basis to form a library of resources available to all Society members.

For more information, please visit our website case library at http://www. sahivsoc.org/case-studies/overview.

Regina Osih is a public health consultant in private practice in Johannesburg.

Box 1. Summary of clinical case study: Hepatitis B

A 24-year-old man presents to the clinic with a history of hepatitis B, treated at another hospital a year go. He had presented with acute hepatitis a few years ago, and on follow-up at 6 months was found to be hepatitis BsAg positive and treated for 1 year with 3TC (lamivudine). He presents to a new clinic after 1 year of 3TC treatment (which has just been discontinued) for further management. This patient was not tested for HIV at the time of his first presentation. An HIV test is done at this presentation, and it is positive – but with an undetectable HIV-1 viral load.

ORIGINAL ARTICLE

Inter- and intra-laboratory variability of CD4 cell counts in Swaziland

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Background. Analytical variability in CD4 enumeration is well known, but few studies from southern Africa have quantified the inter- and intra-laboratory variability in CD4 count measurements. In addition, the possible impact of time lapse after sample collection on CD4 reliability is not well understood.

Methods. A cross-sectional study was conducted at Royal Swaziland Sugar Corporation Hospital and three laboratories, Lab A (comparator), Lab B (national reference) and Lab C (rural hospital). Blood from HIV-infected individuals was collected using routine venepuncture into separate specimens for each of the three laboratories. The samples were further subdivided at each laboratory: one was run at 12 hours and the second at 24 hours after venepuncture. The results of absolute CD4 count and CD4 percentage testing were compared within (intra-laboratory) and between (inter-laboratory) laboratories.

Results. Among 53 participants, the mean CD4 count at 12 hours was 373 cells/µl, 396 cells/µl and 439 cells/µl, and at 24 hours 359 cells/µl, 389 cells/ µl and 431 cells/µl, for laboratories A, B and C, respectively. The coefficient of intra-laboratory variation was 4%, 8% and 20% for CD4 count for laboratories A, B and C, respectively. Comparing 12- and 24-hour measurements, the mean difference (bias) within the laboratories between the two time points (and limits of agreement, LOAs) was 14 (-46 to 73), 8 (-161 to 177) and 7 (20 to 33) cells/µl for labs A, B and C, respectively. Comparing Lab A versus Lab B, lab A versus Lab C and Lab B versus Lab C, the inter-laboratory bias for the CD4 count at 12 hours was -32, -64 and -38 cells/µl, respectively. The corresponding LOAs were -213 to 150, -183 to 55, and -300 to 224, respectively. At 24 hours, the biases and LOAs were similar to those at 12 hours.

Conclusions. CD4 counts appeared reliable at all three laboratories. Lab B and Lab C were clinically interchangeable with the comparator laboratory, Lab A, but not between themselves. Time to measurement does not affect the inter-laboratory agreement within 12 and 24 hours.

S Afr J HIV Med 2012;13(2):59-63.

Thirty-five million people are infected by HIV globally, two-thirds of whom live in sub-Saharan Africa.[1] Antiretroviral therapy (ART) is a critical intervention for reducing HIV-related morbidity and mortality, but delivery of ART requires multiple laboratory investigations.[2] In particular, determination of eligibility for ART initiation relies heavily on CD4 enumeration, and CD4 results are monitored as the major indicator of response to treatment over time.

The gold standard technique for CD4 enumeration is flow cytometry.[3,4] Biological and analytical (laboratory) variations are known to affect CD4 enumeration; biological factors can that influence CD4 results include haemodilution in pregnancy, seasonal and diurnal variations (lowest at approximately 12:30 pm, highest at 8:30 pm), surgery, viral infections, tuberculosis, some intercurrent illnesses, corticosteroids, interferon and cancer chemotherapy.[3]

Laboratory variations are known to occur when enumeration techniques different from the gold standard, flow cytometry, are used.[3,4] In addition, variations are known to be subject to inter-observer differences as well as inter-laboratory differences.[5] The time to performing CD4 may also cause variation in final CD4 count; the World Health Organization (WHO) therefore recommends that all CD4 counts be done within 72 hours from the time of blood collection.[3,4] In Swaziland and many other parts of southern Africa, blood for CD4 testing is collected from various health centres and then sent to central laboratories where analysis is done. The time of arrival of samples differs greatly according to distance from the laboratory, but the impact of time differences on CD4 results is not well understood.

Clinicians rely on accurate CD4 values, despite this variability, to make decisions regarding ART initiation and management. Some previous studies of CD4 variability have produced worrying results. Sax and Boswell analysed the implication of between-laboratory variations and found that 58% of CD4 count results had enough variation to have led to conflicting treatment recommendations.[6] Pattanapanyasat and Chimma found CD4 variation between CD4 cell count results conducted using flow cytometers of different ages in service.[7] Various new CD4 enumeration techniques, for example the Guava Easy CD4 and capillary-based CD4, have been compared with gold-standard techniques and found to be comparable.[3,4,8]

Ensuring accurate CD4 counts has become more important recently, since ART is being initiated at higher CD4 counts, when clinical signs tend to be less sensitive in detecting immune suppression.[2] In Swaziland, there has been widespread suspicion among HIV clinicians regarding discrepancies in CD4 count results within and between laboratories, and concern that these discrepancies may potentially be large enough to affect decisions to start ART. In order to address this problem, this study sought to evaluate the intra- and inter-laboratory variability in CD4 cell enumeration.

Methods

This study was undertaken at HIV clinics at the Royal Swaziland Sugar Corporation Hospital in Swaziland and three laboratories, Lab A, Lab B and Lab C (identity of the laboratories deliberately not disclosed). Lab A was a reputable, internationally accredited South African laboratory commonly used as standard in clinical practice across southern Africa. Lab B was the Swazi national reference laboratory based in the capital city, 250 km away from the study setting, and had a turnover of 4 000 CD4 enumerations per

Table 1. Demographic, clinical and laboratory data of participants

Demographic characteristics				
Gender (*n*)				
Male	28			
Female	25			
Age (years) (mean (SD))	37.4 (9.5)			
Weight (kg) (mean (SD))	64.8 (12.2)			
Clinical characteristics				
WHO stage (%)				
I	32.1			
II	22.6			
III	13.2			
IV	32.1			
On TB treatment (%)	11.3			
On ART (%)	47.2			
Inpatients (%)	9.4			
Outpatients (%)	90.6			
Laboratory parameters				
Lab A (52 observations)	Mean	25th centile	50th centile	75th centile
CD4 count at 12 h (cells/µl)	373	181	336	539
CD4 count at 24 h (cells/µl)	359	177	323	518
CD4 % at 12 h	17	10	15	22
CD4 % at 24 h	17	10	15	21
Lab B (52 observations)	Mean	25th centile	50th centile	75th centile
CD4 count at 12 h (cells/µl)	396	185	359	568
CD4 count at 24 h (cells/µl)	389	183	346	535
CD4 % at 12 h	18	11	17	24
CD4 % at 24 h	18	10	17	23
Lab C (51 observations)	Mean	25th centile	50th centile	75th centile
CD4 count at 12 h (cells/µl)	439	249	397	611
CD4 count at 24 h (cells/µl)	431	233	396	594
CD4 % at 12 h	18	10	16	22
CD4 % at 24 h	18	10	16	22
Mean time to running CD4 tests (h)	First CD4	Second CD4		
Lab A	12.0	24.0		
Lab B	12.0	24.0		
Lab C	12.0	25.1		

month. Lab C was a rural mission hospital laboratory located 80 km from the study site and had a turnover of 1 700 samples per month. All the three laboratories used a flow cytometric CD4 enumeration method, and trained laboratory technicians performed the CD4 tests.

To be eligible, patients had to be adults (>18 years), give informed consent to the study, and be visiting the health facility for routine CD4 count. The study included patients regardless of whether they were on ART or not. After participants' consent had been obtained, blood was collected into EDTA tubes, using routine venepuncture technique, in three aliquots, one each for Lab A, Lab C and Lab B. The samples were further split into two aliquots at each respective laboratory, one of which was run at 12 hours and the second at 24 hours after venepuncture. A reliable transport vehicle ensured that specimens reached all laboratories within stipulated time.

A sample size of 53 was used. For this type of study, Altman and Bland recommend a sample size of 30 as 'minimum acceptable' and 50 as 'good' as it gives a 95% confidence interval (CI) about ±0.34 *s*, where *s* is the standard deviation (SD) of the differences between measurements by the two methods.[9]

Data were analysed using STATA version 10. For intra-laboratory variability, the coefficient of variation (CV) and Bland-Altman (BA) method were used. The BA method was the predominant technique for inter-laboratory variability. Bland-Altman plots were generated in Excel Analyze-it. In both cases, for repeatability and agreement, comparison was based on clinically significant reference ranges used previously in most studies: 0 - 10% for CV,

Table 2. Intra-laboratory bias and limits of agreement for CD4 count and CD4 percentage at 12 and 24 hours

		Limits of agreement		Interpretation
	Bias (95% CI)	Lower (95% CI)	Upper (95% CI)	Clinically repeatable?
Absolute CD4 count				
Lab A	13.5 (5.0 to 21.9)	-46.0 (-60.6 to -31.5)	73.0 (58.5 to 87.6)	Yes
Lab C	8.2 (-16.0 to 32.4)	-160.5 (-202.2 to -118.9)	176.9 (135.3 to 218.6)	Yes
Lab B	7.0 (3.2 to 10.7)	-19.5 (-25.9 to -13.0)	33.4 (26.9 to 39.9)	Yes
CD4 %				
Lab A	0.1 (-0.2 to 0.3)	-1.7 (-2.2 to -1.3)	1.9 (1.5 to 2.4)	Yes
Lab C	-0.3 (-0.7 to 0.1)	-2.9 (-3.5 to -2.2)	2.3 (1.7 to 3.0)	Yes
Lab B	0.1 (-0.3 to 0.5)	-2.8 (-3.5 to -2.1)	3.0 (2.3 to 3.7)	Yes

*Interpretation based on comparison of limits of agreement with clinically significant range of CV <10%, and ranges for clinical significance: ±19.5% for CD4% ±250 cells/µl for CD4 count.[7,8,10,11]

Table 3. Inter-laboratory bias and limits of agreement for CD4 count and CD4 percentage at 12 and 24 hours

		Limits of agreement		Interpretation*
Laboratories	Bias (95% CI)	Lower (95% CI)	Upper (95% CI)	Clinically interchangeable?
CD4 count at 12 h				
Lab A/Lab B	-31.5 (-57.6 to -5.5)	-213.3 (-258.2 to -168.4)	150.2(105.3 to 195.1)	Yes
Lab A/Lab C	-64.3 (-81.6 to -47.0)	-183.8 (-213.6 to -154.0)	55.2 (25.4 to 85.0)	Yes
Lab B/Lab C	-38.2 (-75.6 to -0.6)	-300.2 (-364.8 to -235.5)	223.9 (159.2 to 288.5)	No
CD4 % at 12 h				
Lab A/Lab B	-1.2 (-2.7 to 0.3)	-11.7 (-14.3 to -9.1)	9.3 (6.7 to 11.9)	Yes
Lab A/Lab C	-0.7 (-1.1 to -0.4)	-3.1 (-3.7 to -2.5)	1.7 (1.1 to 2.2)	Yes
Lab B/Lab C	0.5 (-1.1 to 2.1)	-10.7 (-13.4 to -7.9)	11.6 (8.9 to 14.4))	Yes
CD4 count at 24 h				
Lab A/Lab B	-35.6 (-60.0 to -11.1)	-205.7 (-247.6 to -163.7)	134.5 (92.5 to 176.5)	Yes
Lab A/Lab C	8.2 (-16.0 to 32.4)	-195.0 (-227.6 to -162.5)	65.8 (33.3 to 98.3)	Yes
Lab B/Lab C	7.0 (3.2 to 10.7)	-265.0 (-321.3 to -208.7)	191.4 (135.0 to 247.7)	No
CD4 % at 24 h				
Lab A/Lab B	-1.2 (-2.5 to 0.2)	-10.5 (-12.8 to -8.2)	9.2 (5.7 to 10.5)	Yes
Lab A/Lab C	-1.1 (-1.6 to -0.5)	-4.9 (-5.8 to -3.9)	2.7 (1.8 to 3.6)	Yes
Lab B/Lab C	0.1 (-1.3 to 1.5)	-9.7 (-12.1 to -7.3)	9.9 (7.4 to 12.3)	Yes

*Interpretation based on comparison of limits of agreement with clinically significant range of CV <10%, and ranges for clinical significance: ±19.5% for CD4 % and ±250 cells/µl for CD4 count.[7,8,10,11]

±250 cells/µl for CD4 count and 19.5% for CD4 percentage.[7,8,10,11] Clinical impact on antiretroviral therapy (ART) initiation was assessed by Kappa coefficients with comparison to the standard reference scales.[12]

Results

Fifty-three participants consented to participate in the study. The mean CD4 count was 373 cells/µl, 396 cells/µl and 439 cells/µl at 12 hours, and 359 cells/µl, 389 cells/µl and 431 cells/µl at 24 hours, for Lab A, Lab B and Lab C, respectively. Subsequent Wilcoxon sign-rank test revealed some statistically significant differences in CD4 count between the laboratories. Table 1 summarises the demographic, clinical and laboratory characteristics of participants.

Intra-laboratory variability. The CV for CD4 count for Lab B was low (3.4%) compared with Lab A (8.5%). This was consistent with intra-laboratory repeatability based on clinically significant CV range of 0 - 10%. For Lab C the CV was 20.1%, a finding consistent with poor repeatability. For all three laboratories, the CV of CD4 percentage was even lower: 5.6%, 8.34% and 7.5% for Lab A, Lab B and Lab C, respectively. The results using the BA method showed that both CD4 count and CD4 percentage were repeatable, when compared with clinically significant ranges ±250 cells/µl and ±19.5%, for all the laboratories: for CD4 count, the limits of agreement were -46 cells/ µl to 73 cells/µl for Lab A, -20 cells/µl to 33 cells/µl for Lab B, and -161 cells/µl to 177 cells/µl for Lab C, as per Fig. 1 and Table 2. The BA plots for Lab A, Lab B and Lab C had no dispersion suggesting evidence of systematic error.

Inter-laboratory agreement at 12 hours. For CD4 count, at 12 hours, both Lab C and Lab B could be clinically interchanged with the comparator, Lab A, based on the limits of agreement which fell within the clinically significant range (defined as ±250 cells/µl): -184 cells/ µl to 55 cells/µl for Lab C, and -213 cells/µl to 150 cells/µl for Lab B, which was much wider than for Lab C. When Lab B was compared for agreement with Lab C, the limits of agreement were -300 cells/ µl to 224 cells/µl, which were out of the clinically significant range, and we therefore concluded that the two laboratories could not be clinically interchanged. For CD4 percentage all the laboratories could be clinically interchanged. Compared with the comparator, Lab A, the limits of agreement for Lab B were -12 cells/µl to 9 cells/µl and -3 cells/µl to 2 cells/µl for Lab C; between Lab B and Lab C the limits were -11 cells/µl to 12 cells/µl. Table 3 summarises the results for inter-laboratory variability based on BA results at 12 hours and at 24 hours.

Inter-laboratory agreement at 24 hours. Time to measurement had no significant impact on inter-laboratory agreement based on the limits of agreement and biases at 24 hours were similar to those at 12 hours for both CD4 count and CD4 percentage. When compared with Lab A, the limits of agreement at 24 hours were -205 cells/µl to 135 cells/µl for Lab B and -195 cells/µl to 66 cells/µl for Lab C. For Lab B/Lab C the limits of agreement were -265 cells/µl to 191 cells/µl. For CD4 percentage, all the laboratories were clinically interchangeable. The limits of agreement were -11% to 9% for Lab A/Lab B, -5% to 3% for Lab A/Lab C and -10% to 10% for Lab B/Lab C, which were within the reference range, ±19.5%.

Clinical impact on ART initiation. Compared with Lab A, the percentage agreement for ART eligibility was 81% (i.e. 19% of patients were misclassified) for Lab B and 89% (11% of patients misclassified) for Lab C. For Lab A/Lab B, 23% eligible patients would be misclassified and not initiated on ART, as shown in Table 4.

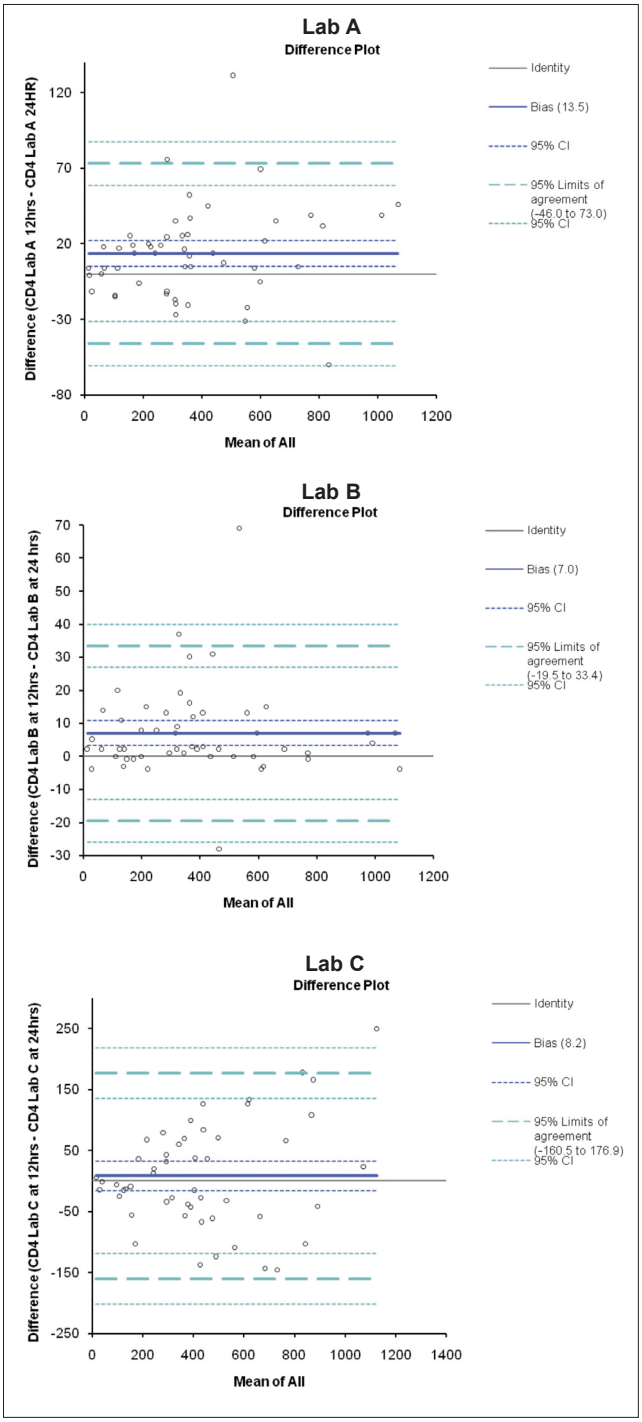

Fig. 1. Bland Altman plots for intra-laboratory variability of CD4 count for Lab A, Lab B and Lab C.

Discussion

In this study we looked at intra- and inter-laboratory variability, a topic that has been investigated previously but for which there are few data from southern Africa.[5-8,11,13] We also analysed the impact of time to measurement on the eventual CD4 result, both within the same laboratory and across participating laboratories. CD4 count had good repeatability for all the three laboratories, based on preset clinically significant ranges. Likewise, CD4 percentage had minimal variation for

all the laboratories and even lower CV, a sign of stronger repeatability than for CD4 count. These findings concurred with previous intra-laboratory studies.[7,8,10,11]

Inter-laboratory variability. Several studies on inter-laboratory and inter-method variability of CD4 count have been published and most show good agreement and interchangeability.[7,10,11] Two studies, however, found significant variations across different laboratories.[5,13] In this study, inter-laboratory clinical interchangeability results at 12 and 24 hours showed that agreement was independent of time to measurement. The limits of agreement were similar when time to measurement was 12 hours or 24 hours. This finding mirrors the WHO laboratory recommendation that CD4 remains stable within 72 hours from time of venepuncture.[3,4] Clinicians using the laboratories in this study should therefore trust equally CD4 results done at 12 hours and 24 hours.

For CD4 percentage, both Lab B and Lab C were in agreement with the comparator laboratory, Lab A, at 12 and 24 hours with narrower limits of agreement than for CD4 count. Once again, stability of CD4 percentage and agreement with the comparator laboratory make it a potentially trustworthy and stable parameter to use in our setting for possible inclusion in guidelines to determine when to start ART, as suggested in some previous studies.[8,10]

The degrees of misclassification in this study were similar to findings from a study by Thakar and Kumar, which found a kappa factor range of 74% for a CD4 count below 350 cells/µl when two laboratories were being compared.[11] Repeating CD4 count measurement and not relying on single CD4 count results have been known to reduce disease misclassification.[6] One shortfall of this use of misclassification as done here is that it does not differentiate between low-magnitude inaccuracy, for example a count of 349 cells/µl being misclassified as >350 cells/µl, which may be reasonably expected from any test, and high-magnitude inaccuracy. A study that includes many CD4 values falling close to the defined cut-off (as measured by the reference test) will show higher rates of misclassification by the new test than a study in which the majority of values lie away from the threshold.[4]

The clinically significant ranges used in this study were ±250 cells/µl, ±19.5% and CV <10%, because these were the ranges used in similar studies which had pre-defined ranges.[7,8,10,11] The results of repeatability and agreement therefore relied on this pre-defined range. However, the choice of clinically significant ranges is debatable, and a narrower range of ±100 cells/µl could have changed the interpretation of these results greatly. However, the magnitude of CD4 count or CD4 percentage variability that can affect clinical decision making remains poorly defined.[13] The author felt that based on the new ART initiation threshold, 350 cells/µl, a range of ±250 cells is reasonable.

In conclusion, CD4 count and CD4 percentage appeared to be repeatable for all the three laboratories. Lab B and Lab C were clinically interchangeable with the comparator laboratory, Lab A, for both CD4 count and CD4 percentage but not between themselves. Time to measurement does not affect the inter-laboratory agreement within 12 and 24 hours. The clinical implications of inter-laboratory variation on disease misclassification were comparable to those from previous studies.

Table 4. Impact of CD4 variations at ART initiation threshold on treatment decision

Laboratories	Agreement* (%)	Expected agreement (%)	Kappa	Misclassified (%)
Lab A/Lab B	81.1	48.4	0.6	18.9
Lab A/Lab C	88.7	49.9	0.8	11.3
Lab B/Lab C	77.4	50.2	0.6	22.6

*Strength of agreement according to Byrt's criteria for assessing Kappa strength: excellent agreement = 0.93 to 1; very good agreement = 0.81 to 0.92; good agreement = 0.61 to 0.80; fair agreement = 0.41 to 0.60; slight agreement = 0.21 to 0.40; poor agreement = 0.01 to 0.20; no agreement <0.00.[12]

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Competing interests by authors. None.

Research ethics committee approval. Ethics approval was given by the University of Pretoria Ethics Committee 74/2010 on 21 April 2010, and institutional ethical approval was also obtained.

Author contributions. Dr Ganizani Mlawanda conceived the study, and formulated the study design, data collection, statistical analysis and manuscript design. Prof. Paul Rheeder and Dr Jacqui Miot were active supervisors throughout from conception to final manuscript. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Lung fibrosis in deceased HIV-infected patients with *Pneumocystis* pneumonia

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Background. Pneumocystis pneumonia (PcP) is one of the most common opportunistic infections found in patients with HIV. The prognosis if ventilation is required is poor, with mortality of 36 - 80%. Although more recent studies have shown improved survival, our experience has been that close to 100% of such patients die, and we therefore decided to investigate further.

Methods. All patients with confirmed or suspected PcP who died owing to respiratory failure were eligible for the study. Where consent was obtained, trucut lung biopsies were performed post mortem, stored in formalin and sent for histopathological assessment.

Results. Twelve adequate lung biopsies were obtained from 1 July 2008 to 28 February 2011 – 3 from men and 9 from women. The mean age was 34.7 years (range 24 - 46), and the mean admission CD4 count was 20.8 (range 1 - 68) cells/µl and median 18.5 cells/µl. All specimens demonstrated typical PcP histopathology; in addition, 9 showed significant interstitial fibrosis. Three had co-infection with cytomegalovirus (CMV), two of which had fibrosis present. There was no evidence of TB or other fungal infections.

Conclusion. The high mortality seen in this cohort of PcP patients was due to intractable respiratory failure from interstitial lung fibrosis. Whereas the differential includes ventilator induced lung injury, drug resistance or co-infections, we suggest that this is part of the disease progression in certain individuals. Further studies are required to identify interventions that could modify this process and improve outcomes in patients with PcP who require mechanical ventilation.

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Since the introduction of antiretroviral therapy (ART) for individuals who are HIV-infected with AIDS, there has been a dramatic decline in the number of these patients presenting with Pneumocystis jerovicii pneumonia (PcP) in the developed world. In South Africa, the antiretroviral (ARV) rollout was delayed for political reasons until 2004; consequently, significant numbers of patients are still presenting with PcP as a cause of respiratory failure. These patients are either unaware of their diagnosis or have not started ARVs for reasons that include poor access to medical facilities and drugs, denial and lack of education. If patients with PcP require mechanical ventilation, the prognosis is poor, with mortality ranging from 36 - 80%.[1,2] In fact, prior to the availability of ARVs, such patients were not mechanically ventilated in South Africa as no definitive therapy was available. Once these agents became available to all HIV-positive patients with CD4 counts <200 cells/µl, it became feasible for them to be considered for ventilation. At the Charlotte Maxeke Johannesburg Academic Hospital, it soon became apparent that few of these patients survived, despite early initiation of both ART and effective chemotherapy for PcP. Management included use of ARDSNET low tidal volume strategies,[3] conservative fluid protocols, adjunctive corticosteroids and minimal sedation. Despite these, mortality remained extremely high while other units were reporting 50 - 79% cure rates.[1,4] It was consequently decided to prospectively investigate the patients who had died in the unit, with the aim of determining the causes of failure of therapy. Possibilities that had been considered for this failure were concurrent infections including cytomegalovirus,[5,6] *Cryptococcus neoformans*, mycobacterial or bacterial infections such as *Streptococcus pneumonia*, drug resistance, as well as pulmonary Kaposi's sarcoma.[7]

Methods

This was a prospective study to investigate histological findings of patients who died from confirmed or suspected

PcP. All patients in these two categories, with respiratory failure, were considered for the study. PcP was suspected in patients with clinically advanced HIV presenting with hypoxic respiratory failure with typical chest radiograph changes, including bilateral diffuse alveolar infiltrates, granular opacities or, occasionally, unilateral or focal infiltrates.[7] *Pneumocystis* was confirmed *ante mortem* on sputum from 4 of the patients using the Giemsa stain; and 9 had organisms seen on histological samples. The remaining 3 had markedly elevated beta-D-glucan (BDG) levels >500 pg/ml.[8] Pre-mortem biopsies or bronchial washings were not possible owing to the severity of the hypoxia. With family consent, multiple trucut biopsies were taken from different regions of the lungs of each patient after death. The specimens were stored in formalin and subsequently stained with Grocott, Gordon and Sweets, Alcian blue, Ziehl-Neelsen and haematoxylin and eosin. Ethics approval was given by the University of the Witwatersrand Ethics Committee.

Results

Sixteen lung biopsies were obtained from 1 July 2008 to 28 February 2011. Table 1 lists patient demographics and laboratory characteristics. Four were inadequate samples and therefore not included. The final 12 were from 3 male and 9 female patients. Mean age of patients was 34.7 years (range 24 - 46 years). Mean admission CD4 count 20.8 (range 1 - 68) cells/µl, and the median CD4 was 18.5 cells/µl.

ICU details

All 12 patients were admitted to the intensive care unit (ICU), where 10 were mechanically ventilated; none developed pneumothoraces. All received appropriate high-dose trimethoprim-sulfamethoxazole (TMP-SMX) with high-dose corticosteroids as primary management. None were on ART at the time of presentation.

Histopathology

All 12 of the final specimens demonstrated the typical histopathological pattern of PcP, including alveoli filled with frothy material, type 2 cell hyperplasia and pneumocystis organisms. In addition, 9 of the 12 showed evidence of interstitial fibrosis with expansion of the interstitium by fibroblasts and collagen of varying degrees of severity. There was significant destruction and distortion of the lung architecture, resulting in a marked decrease in available alveolar-endothelial surface area for diffusion (Fig. 1). Three had evidence of CMV co-infection with intracellular inclusion bodies, and 2 of these also showed evidence of fibrosis. One of the latter 2 had a super-added bacterial infection that was not evident in any of the other specimens. There was no evidence of TB or other fungal infection in any of the specimens.

Discussion

Pneumocystis pneumonia is still one of the most common opportunistic infections found in patients infected with HIV.[9] *Pneumocystis* is primarily an alveolar pathogen that does not invade the pneumocyte to which it tightly adheres. The histopathological changes that are seen are explained by the exuberant host inflammatory response to the organism, which promotes pulmonary injury in only some patients during infection. Severe *pneumocystis* pneumonia can result in a significant neutrophilic response that leads to diffuse alveolar damage, impaired gas exchange and respiratory failure.[10] *P. jirovecii* has specific proteases that have the ability to damage the lung interstitium, and endogenous host proteases including matrix metalloproteinases (MMP) are also secreted in response to an influx of pro-inflammatory mediators (Interleukin-6 (IL-6), Interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and tumour necrosis factor alpha (TNF-α)) from alveolar epithelial cells.[11,12] This can explain the extensive capillary leak and frothy hyaline material that fills the alveolus in typical PcP. It is possible that the extensive effacement of normal alveolar architecture with fibrosis demonstrated in these biopsies is part of a reparative process that may occur only in those individuals genetically predisposed to the development of fibrosis, so that not all patients with PcP behave similarly.

In our sample, 75% of the patients who died of refractory respiratory failure revealed varying degrees of interstitial fibrosis resulting in obliteration of the alveolar capillary interface and loss of surface area for diffusion with the remainder, demonstrating unresponsive PcP. The pattern of the former is similar to that of the fibrotic stage of acute respiratory

Table 1. Patient demographics and laboratory characteristics

Patient	Sex	Age (years)	CD4 cells/µl	PcP on sputum	BDG pg/ml	PcP on histo	CMV on histo	Fibrosis
SM	♂	27	11	N/A	N/A	Yes	No	Present
NM	♀	46	19	Yes	N/A	Yes	Yes	None
MS	♂	40	31	Yes	402	Yes	No	Present
DM	♀	33	1	Yes	>500	Yes	Yes	Present
NS*	♀	44	29	Yes	>500	No	No	Present
XD*	♀	24	n.a.	N/A	>500	Yes	No	Present
NM	♀	28	68	N/A	>500	Yes	No	None
TR	♀	24	22	N/A	>500	Yes	No	Present
NM	♀	25	7	N/A	>500	No	No	Present
AM	♀	46	18	N/A	n.a.	Yes	No	None
TM	♀	41	n.a.	N/A	>500	No	No	Present
JN	♂	38	2	N/A	51	Yes	Yes	Present

*Not ventilated.
N/A=not available.

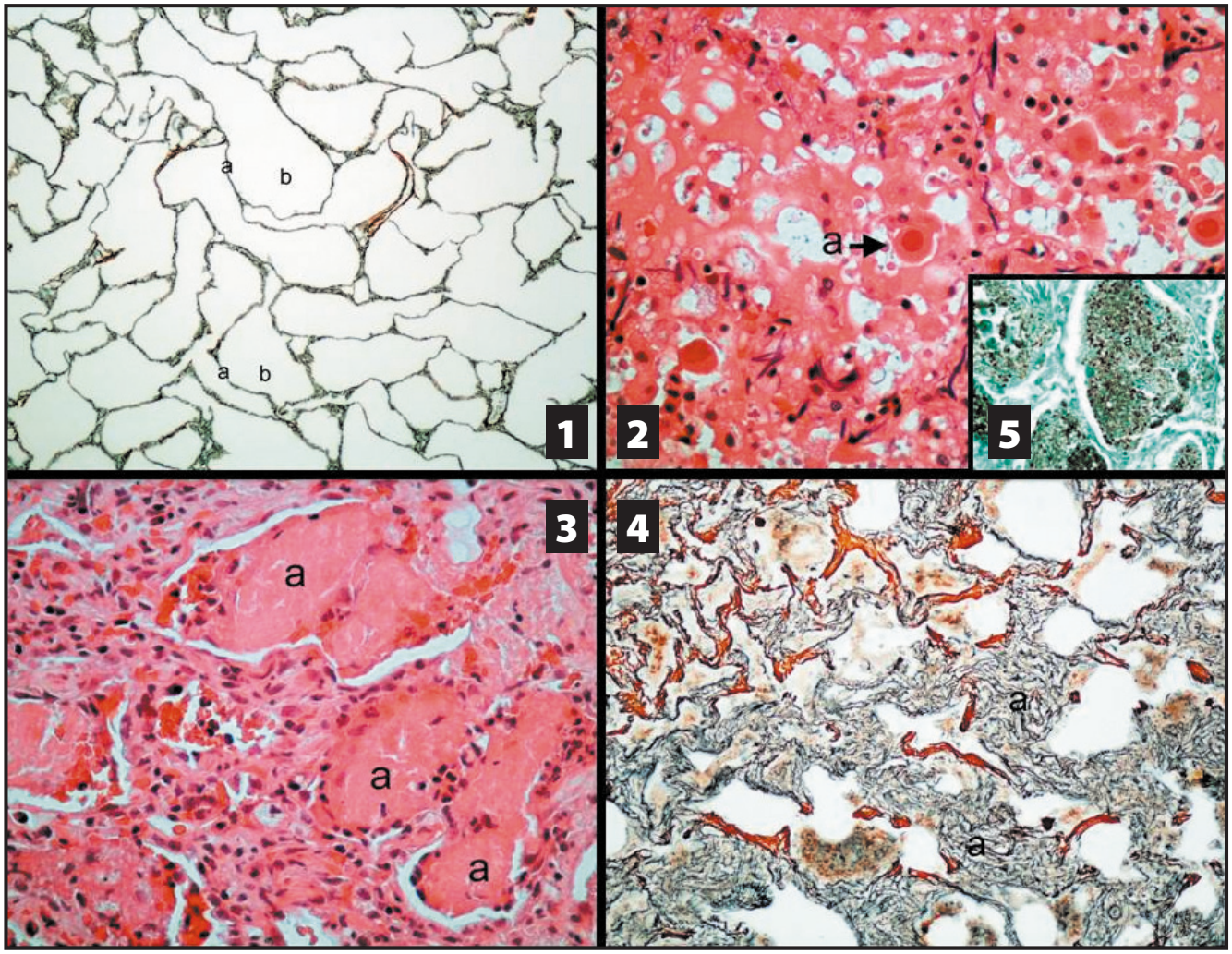

Fig. 1. Low magnification of alveoli showing normal interstitium of (a) alveolar walls and (b) alveolar spaces (Gordon and Sweet stain).
Fig. 2. High magnification showing an enlarged pneumocyte with (a) an intranuclear CMV inclusion (haemotoxylin and eosin stain).
Fig. 3. Low magnification showing (a) frothy exudate filling the alveolar spaces (haemotoxylin and eosin stain).
Fig. 4. Low magnification showing (a) marked expansion of the interstitium by fibrous tissue (Gordon and Sweet stain).
Fig. 5. Higher magnification showing pneumocystis organisms within frothy intra-alveolar exudates (Grocott stain).

distress syndrome (ARDS); and, whereas it might be argued that this could be consistent with ARDS following an infection by a more virulent organism such as *Streptococcus pneumoniae*, this organism was not cultured *in vivo*, and patients all received standard empiric therapy for community-acquired pneumonia. Importantly, none presented with the secondary organ dysfunction or systemic inflammatory response syndrome (SIRS), more typical of severe infections with this organism. In addition, all the patients received corticosteroids as a component of therapy for PcP that may be effective in the therapy of refractory ARDS owing to other causes.[13]

Another factor that could be responsible for the fibrotic injury is ventilator-induced lung injury (VILI). However, 2 of the 13 patients were not ventilated, and their biopsies showed similar interstitial fibrotic changes to those who were, and the other 11 were ventilated with tidal volumes ≤6ml/kg, and were recruited and maintained on appropriate PEEP, making this explanation unlikely.

In South Africa, where medical resources are limited, the majority of patients with PcP and respiratory failure (most of whom have PaO_2/FiO_2 ratios <200) are treated with oxygen via a re-breathing mask and appropriate pharmacological therapy in the general wards. Only the most severely hypoxic patients or those who fail therapy are considered for ventilation. This observation highlights a weakness in our sample, with a selection bias for those with a worse prognosis. Patients who were not considered candidates for ICU admission might have developed respiratory failure and died in the general medical wards, or more usually might have made a full recovery despite initial low P/F ratios. The latter, who in more resource-rich environments would have been admitted to ICU, could account for the high survival rates in other studies. Those admitted to ICU in South Africa are preselected, with most having already received and failed appropriate pharmacotherapy. It has previously been reported in the pre-ART era that patients who required ventilation despite adequate and appropriate therapy, have a poor prognosis.[4]

The reasons for the failure of therapy and the failure to benefit from mechanical ventilation have not previously been well described. Why some patients and not others develop fibrosis has also not been adequately elucidated. It could be argued that these patients might have had 2 disease processes: PcP superimposed on a more chronic condition or that this was an acute exacerbation of a more chronic underlying inflammatory process similar to

those that occur in the idiopathic interstitial pneumonias.[14] However, this does not explain why these patients had elevated β-D glucan levels in the absence of fungal infection elsewhere, and X-ray features not compatible with the interstitial pneumonias; and in the latter case, why the histological features were typical of PcP. CMV has been postulated to be more than a 'fellow traveller' in patients with PcP, and treatment with gancyclovir has been reported to improve outcome.[5,6] It is conceivable that infection by both organisms could be synergistic regarding the fibro-proliferative effects. However, in only 3 of our cohort was there evidence of CMV co-infection, 2 of whom did have fibrosis.

An important consideration for treatment failure is the possibility of resistance to sulfa drugs owing to mutations of *Pneumocystis* dihydropteroate synthase (DHPS) gene due to increased drug pressure from the widespread use of TMP-SMX prophylaxis. DHPS, the enzyme responsible for folate synthesis and the target of TMP-SMX, has undergone gene mutations that have been identified in 56% of *P. jirovecii* strains in South Africa.[15] However, as human strains of PcP cannot be cultured *in vitro*, it is difficult to prove that these mutations confer drug resistance. A number of studies have evaluated the effect of these mutations on clinical outcomes with conflicting results. Helweg-Larsen *et al*. demonstrated that patients infected by organisms with a DHPS mutation had a threefold increased risk of death.[16] Navin and colleagues, however, found no association with mortality at 6 weeks nor with treatment failure.[17] In fact, they found that 85% of patients with DHPS mutations treated with TMP-SMX responded to treatment.

A limitation of our study is the small sample size. In view of our resource-limited setting, these patients are not often viewed as good ICU candidates. Therefore, even though the PCP burden in South African hospitals is high, the available ICU PcP population is restricted. We feel that these are important data and will add to the understanding of the clinical course of these patients, even taking into account the small sample size.

Interstitial fibrosis has previously been demonstrated in patients who have survived an episode of PcP, as well as on previous necropsy studies.[4,18] There have also been histological reports of cryptogenic organising pneumonia, granulomatous inflammation and diffuse alveolar damage.[19] Our cohort, however, was unusual in that the majority of patients with PcP, most of whom were ventilated, had evidence of extensive pulmonary fibrosis – which was associated with an extremely poor prognosis. This phenomenon has been described previously; however, it has not been highlighted as the probable underlying cause for treatment failure and death. We suggest that, if we want to improve the dismal outcome of these patients, we need to consider the state of the underlying lung, and realise that treatment of the organism alone is insufficient. Primarily, we need to expand the rollout of ARVs and, failing this, try to both recognise and treat the condition sooner, prior to the development of fibrosis. Ideally, we should also develop a management protocol that addresses the lung fibrosis once it has occurred.

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ORIGINAL ARTICLE

Localised treatment and 6-month outcomes in patients with cytomegalovirus retinitis at a tertiary ophthalmology service in Ga-Rankuwa

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Objective. There are few data from before the antiretroviral therapy (ART) era for cytomegalovirus retinitis (CMV-R) from settings where cost limits use of systemic treatment. This study examines CMV-R treatment and survival outcomes in a public hospital ophthalmology service in Ga-Rankuwa, South Africa.

Methods. From October 2009 to October 2010, voluntarily consenting participants over the age of 15 years with incident clinically diagnosed CMV-R seen at the Dr George Mukhari Hospital ophthalmology clinic were prospectively enrolled in an observational study. Treatment was per clinic protocols and patients were followed up with structured data collection for up to 6 months.

Results. Eight individuals, all HIV infected and 50% female, were identified and enrolled. At enrolment, median age was 38 years (interquartile range (IQR) 32 - 39 years), median CD4 count 20 cells/µl (IQR 13 - 46.5 cells/µl), and 50% were currently receiving ART (mean duration of ART use 18 days, standard deviation (SD) 2.99 days). No participant received systemic ganciclovir, but 6 reported symptom combinations suggesting systemic CMV: shortness of breath (*n*=3), diarrhoea (*n*=3) and/or central nervous system complaints (*n*=3). Ten eyes had visual impairment less than counting fingers at enrolment. Treatment combinations were: ART plus intravitreal ganciclovir (*n*=5), intravitreal ganciclovir alone (*n*=2), and ART alone (*n*=1). Six-month outcomes were: death (*n*=1), survival (*n*=6), loss to follow-up (*n*=3), untraceable (*n*=1), systemic symptom resolution (4/4), visual acuity deterioration (0/5), and persisting uveitis (2/3).

Conclusion. In the ART era, incident CMV-R appears to be uncommon in this setting. CMV-R may occur within the first 3 weeks after ART initiation. Even in CMV-R patients with suggestive systemic symptoms, 6-month survival is good despite no systemic CMV therapy.

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South Africa has both a high burden of HIV disease[1] and a large, expanding antiretroviral therapy (ART) programme.[2] It is noteworthy that many South African HIV patients present for treatment when the CD4 count is less than 50 cells/µl.[3] Particularly in these individuals with advanced immune compromise, opportunistic infections such as cytomegalovirus (CMV) may present. The most commonly recognised manifestation of CMV is CMV retinitis (CMV-R), but pneumonitis, colitis, oesophagitis, adrenalitis, and neurological involvement are also described.[4]

In Africa, data describing the disease burden of CMV-R in the ART era are limited. In the pre-ART era, a 20-month longitudinal study in AIDS patients in Togo confirmed a 21.4% incidence.[5] Also in the pre-ART roll-out era in South Africa, 90 AIDS patients were treated for CMV-R at the University of Natal, and the incidence was noted to increase with time.[6] A cross-sectional study screening all HIV patients with CD4 counts of <50 cells/µl in Khayelitsha, South Africa, found 2% of these patients to have CMV-R.[5]

CMV-R treatment strategies include localised and systemic therapies optimally used in combination and in initiation and maintenance phases, and the use of ART in the context of HIV diagnosis. CMV has been called the 'neglected disease of the AIDS pandemic', in part because of limited access to treatment, especially in the developing world, where localised intra-ocular ganciclovir implants and systemic oral valganciclovir are unavailable or too costly.[5] Intravitreal ganciclovir injections, although more cost-effective, require highly skilled administration and may be inconvenient and/or unacceptable to patients.[7]

ART combined with CMV treatment strategies is associated with an improvement in median survival after CMV-R diagnosis from 6 months to over 1 year.[8,9] There are few ART-era data for CMV-R from African countries where cost limits the use of systemic CMV therapy.

This study describes the incidence of CMV-R in a public hospital outpatient ophthalmology clinic in Ga-Rankuwa, South Africa, over a 1-year period and examines treatment

and survival outcomes of CMV-R cases over a 6-month follow-up period, so as to inform future CMV-R care.

Methods

Setting

A prospective longitudinal observational study was conducted at the ophthalmology outpatient clinic of the Dr George Mukhari Hospital, a state tertiary academic service situated in Ga-Rankuwa, Gauteng province, South Africa. The hospital has a catchment population of 1 800 000 people from the surrounding Tshwane and Metsweding areas. In 2007, the antenatal HIV seroprevalence in Tshwane was 27% and in Metsweding it was 33%.[10] At the time of the study, the hospital also offered an on-site HIV clinic with free ART access for HIV-infected adults with CD4 counts less than 200 cells/µl or World Health Organization (WHO) stage 4 disease. ARV roll-out began in August 2004. In this setting, ophthalmology referrals are made by healthcare workers in the hospital and HIV clinic or in secondary and primary level facilities. Referral is based on presentation with any visual symptoms. In 2011, the HIV clinic saw 7 853 patients of whom 193 initiated ART during that year.

Eligibility

Patients were eligible for entry into the study if they were aged 15 years or older; able to provide voluntary written informed consent (for those 18 years or older) or assent with parent/guardian consent (for those between 15 and 17 years old); and presented at Dr George Mukhari Hospital ophthalmology clinic with new clinically diagnosed CMV-R during the 1-year enrolment period of the study.

Case definition

The CMV-R case definition was visualisation of at least one of the following on dilated pupil indirect ophthalmoscopy: indolent retinitis characterised by mild granular retinal opacification which may be associated with a few punctuate haemorrhages but absent vasculitis, or fulminating retinitis characterised by mild vitritis, vasculitis with perivascular sheathing and retinal opacification, dense, white, well-demarcated, geographical area of confluent opacification often associated with retinal haemorrhages, and slow relentless brushfire-like extension along retinal vascular arcades that may involve the optic nerve.[11]

Data collection

From 6 October 2009 to 6 October 2010, participants identified by clinic staff as having a possible CMV-R diagnosis from routine retinal screening (indirect ophthalmoscopy with fully dilated pupils) were referred to study representatives at the same clinic for assessment of eligibility, voluntary informed consent procedures and enrolment into the study.

Structured data collection by ophthalmologists was done at enrolment on the day of referral, and at months 3 and 6. This involved two components: (*i*) a clinical interview to record demographics, HIV status, CD4 count results, use of ART and CMV treatments, ocular symptoms, and a brief systemic symptom screen probing shortness of breath, diarrhoea, fever, headache, change in personality and decreased concentration; and (*ii*) visual acuity measurements using a Snellen chart or gross visual tests for vision worse than 6/120, and an ophthalmological examination including indirect ophthalmoscopy with fully dilated pupils to categorise CMV-R zonal location and to assess the presence of CMV-R complications, namely vitreous haemorrhage, cataract, endophthalmitis and uveitis.

Treatment was neither influenced nor provided by the study, but was managed by the clinic according to local standard of care. Patients were referred to the HIV clinic for ART per South African guidelines. Management of CMV-R in this setting was predetermined by availability of ganciclovir only. Current accepted practice in the developing world is intravitreal ganciclovir injection twice a week for the first 2 weeks, and then weekly until the CD4 count recovers to over 150 cells/µl or retinitis becomes quiescent. However, intravitreal ganciclovir is not recommended for patients who will not have recoverable vision, who have

Table 1. Summary of participant characteristics, treatment and symptoms at baseline and 6 months

	Baseline characteristics						
Participant	Gender	Age (years)	Presenting ocular symptoms	Presenting systemic symptoms	CMV-R treatment	ART use	Six-month follow-up of symptomatic outcomes
1	F	27	Decreased vision	Shortness of breath, diarrhoea, fever, headache, personality changes, decreased concentration	No	Yes	Ocular symptoms unresolved but systemic symptoms resolved
2	F	31	Ocular dryness	Diarrhoea	Intravitreal ganciclovir	Yes	Ocular and systemic symptoms resolved
3	M	44	Ocular redness and photophobia	Shortness of breath	Intravitreal ganciclovir	Yes	Defaulted follow-up but confirmed alive
4	F	39	Decreased vision	Nil	Intravitreal ganciclovir	Yes	Death
5	F	31	Ocular dryness	Diarrhoea	Intravitreal ganciclovir	Yes	Ocular and systemic symptoms resolved
6	M	39	Blind spots, visual distortions	Headaches, generalised pain	Intravitreal ganciclovir	Yes	Ocular and systemic symptoms resolved
7	M	44	Blind spots, 'floaters'	Nil	Intravitreal ganciclovir	No	Defaulted follow-up and survival not ascertained
8	M	37	Blind sports, photophobia	Shortness of breath, decreased concentration	Intravitreal ganciclovir	No	Defaulted follow-up but confirmed alive

less than 3 clock hours of disease in retinal zone 3 and no fundal view, and who cannot come for regular injections.[6]

Telephonic tracing was undertaken for participants who did not attend follow-up appointments.

Statistical analysis

Descriptive statistics are reported. There were insufficient cases reported to analyse risk factors for outcomes.

Ethical considerations

Ethical approval was received from the Medunsa Research and Ethics Committee before initiating the study.

Results

Baseline characteristics

Over the 1-year enrolment period, 8 individuals were eligible and all agreed to participate in the study (Table 1). All were HIV infected and 50% were female. The median age at first appearance of CMV symptoms was 38 years (intraquartile rage (IQR) 32 - 39 years). At enrolment, the median CD4 count was 20 cells/µl (IQR 13 - 46.5 cells/µl) and 50% were currently receiving ART (mean duration of ART use prior to CMV presentation 18 days, standard deviation (SD) 3 days).

Presenting ocular symptoms were either one or a combination of: blind spots (*n*=3), decreased vision (*n*=2), dry eyes (*n*=2), photophobia (*n*=2), eye redness (*n*=1), visual distortions (*n*=1) and 'floaters' (*n*=1).

Six participants (75%) reported systemic symptoms: shortness of breath (*n*=3), diarrhoea (*n*=3) and/or central nervous system complaints of headaches, personality changes and/or decreased concentration (*n*=3).

A summary of visual acuity is presented in Table 2. Ten eyes were classified as having visual impairment less than counting fingers at enrolment.

Indirect ophthalmoscopy at enrolment revealed bilateral retinal involvement in 7 participants (88%). The most common retinal zone affected was zone 3 (10 eyes), then zone 2 (8 eyes) and zone 1 (6 eyes). Uveitis was found in 7 cases, and vitreous haemorrhage was present in 1 case (Figs 1 and 2).

Treatment

None of the participants received systemic therapy, although 6 complained of systemic symptoms. The combination of ART and intravitreal ganciclovir injections was given to 5 participants. Intravitreal ganciclovir alone was given to 2 male participants who both refused ART and defaulted from study follow-up. One patient, who had no light perception bilaterally, received only ART because visual benefit with intravitreal ganciclovir was not deemed likely.

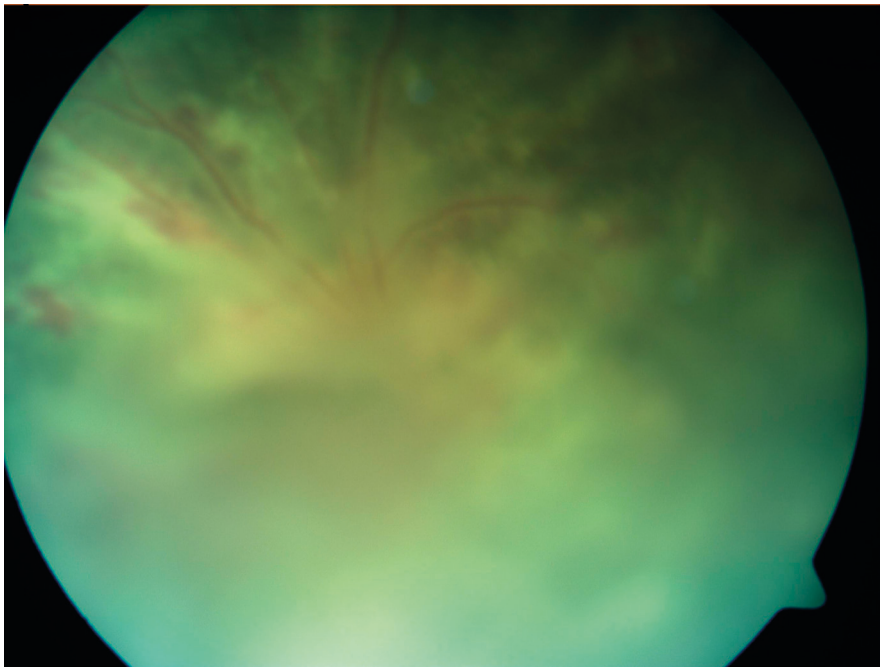

Fig. 1. Fundus photograph of the right eye of a 40-year-old woman with CMV-R. Vitreous haemorrhage, retinal detachment and retinitis are shown by the retinal opacification with associated retinal haemorrhages indicative of active CMV-R. The picture appears out of focus because the vitreous haemorrhage is anterior to the retina.

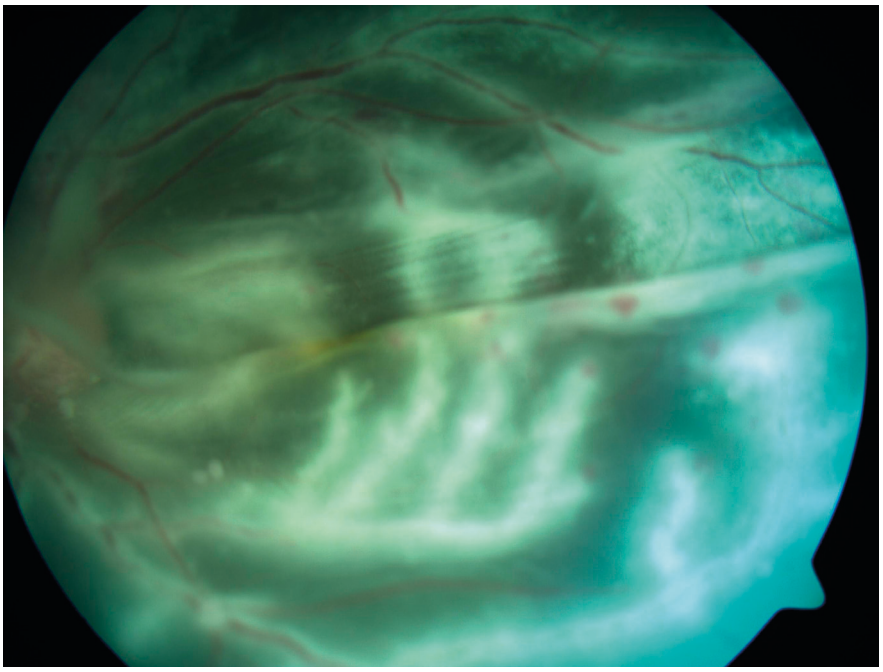

Fig. 2. Fundus photograph of the left eye of the same patient as in Fig. 1. Retinal detachment and retinitis are demonstrated by the retinal opacification and frosted branch angiitis, indicating active CMV-R disease.

Six-month outcomes

By month 6, 1 participant had died, 6 were alive and 1 was untraceable. Three participants had defaulted from follow-up.

Resolution of symptoms and complications at 6 months is described for the 4 participants who remained in follow-up. Ocular symptoms resolved for all 3 participants who received

Table 2. Visual acuity at enrolment and month 6

	Right eye (*N*=8)		Left eye (*N*=8)	
Visual acuity category	**Enrolment**	**Month 6**	**Enrolment**	**Month 6**
6/6 - 6/12 vision	0	0	4	2
6/18 - 6/60 vision	2	2	0	0
Counting fingers to hand motions vision	3	1	2	2
Light perception to no light perception	3	2	2	1
Defaulted/died	Not applicable	3	Not applicable	3

both intravitreal ganciclovir and ART, but did not resolve in the participant who received ART only. All 4 participants reported resolution of their systemic symptoms.

Visual acuity outcomes are presented in Table 2. Among the 5 participants who were followed up to month 6, there was no recorded deterioration in visual acuity from enrolment; 6 eyes demonstrated no change and 4 eyes showed slight improvement.

Uveitis resolved in 2 of 3 cases, and in the third case where it persisted, a cataract developed. There were no cases of endophthalmitis.

Discussion

This study found a low incidence of CMV-R at a resource-constrained public hospital outpatient ophthalmology clinic in Ga-Rankuwa, South Africa: only 8 cases were identified in a 1-year period. The low incidence may be explained by the availability of ART in this population.

In this setting, CMV-R remains an AIDS manifestation, and the late presentation to care is highlighted not only by the low median CD4 count of 20 cells/µl but also by the advanced stage of visual loss at enrolment.

Several studies from the pre-ART era describe improved or stable visual outcomes with intravitreal ganciclovir injections for CMV-R lesions.[12,13] Importantly, even though none of the 75% of participants who reported systemic symptoms received systemic ganciclovir, the combination of ART and intravitreal ganciclovir did ameliorate visual symptoms in our study. However, possibly owing to severe visual loss, no patient had complete recovery of vision.

Currently the authors are unable to predict whether the 6-month outcomes reported here would have been better if state-of-the-art treatments such as ganciclovir implants and systemic valganciclovir had been available in our setting. A pressing question in the field of cytomegalovirus medicine is the relative efficacy of localised versus systemic treatment when CMV-R has been diagnosed, and until this is answered, management remains individualised. When comparing localised with systemic treatments, the latter carry an increased risk of systemic side-effects but also a reduced risk of systemic CMV dissemination.

There are several limitations to our study. First, the small sample size prevents statistical comparison of outcomes by treatment combination. Second, incidence may be underestimated because of missed diagnoses and lack of presentation to hospital. Third, when screening for systemic symptoms of CMV infection, no attempt was made to discriminate from co-morbidities. It has been noted, however, that systemic CMV symptoms have been misdiagnosed as *Pneumocystis jirovecii* pneumonia and tuberculosis, among others.[14] Last, though we attempted in our mortality assessment to control for treatment defaulting by telephonic tracing, loss to follow-up may signify an underestimation of mortality, a phenomenon well described in studies of ART programmes.[15]

In conclusion, CMV-R is an uncommon disease in the ART era in Ga-Rankuwa, South Africa. Intravitreal ganciclovir, complemented with ART, was an effective option to treat CMV-R.

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ORIGINAL ARTICLE

Association of -308 TNF-alpha promoter polymorphism with viral load and CD4 T-helper cell apoptosis in HIV-1 infected black South Africans

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Objective. To determine whether the -308 TNF-α promoter polymorphism is associated with markers of HIV progression in the South African population.

Methods. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the -308 TNF-α polymorphism in 75 patients and 76 healthy controls. Serum TNF-α concentrations were measured using ELISA in each cohort. $CD4^+$ T cell apoptosis and HIV-1 RNA viral load were determined using Annexin-V-FITC assay and Nuclisens Easy Q HIV-1 assay respectively. $CD4^+$ T cell counts were measured flow cytometrically.

Results. The frequency of -308 G allele was similar in the HIV-1 and control cohorts. The -308GG genotype was associated with lower TNF-α concentrations and markers of increased HIV progression indicated by higher T_H lymphocyte apoptosis, lower T_H lymphocyte count and higher plasma viral load, irrespective of treatment.

Conclusion. The presence of the TNF-α -308 G allele in HIV-1 patients may be associated with increased risk of HIV-1 progression. Further research is required to investigate the nature of this association.

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Patients infected with human immunodeficiency virus (HIV) show a decline in $CD4^+$ T-helper (T_H) lymphocyte levels and an increase in viral load that ultimately results in compromised immune function and increased susceptibility to various opportunistic infections.[1] In early stages of infection, HIV-1 has the ability to manipulate the immune response to ensure its own replication and survival.[2] Consequently, there has been much controversy as to whether eliciting a robust immune response towards the virus early in infection will be beneficial or detrimental for the patient.[2]

The differential rate of HIV progression and chronic inflammatory disorders[3-5] may be induced by viral, environmental and host genetic factors. Dean *et al.* observed a 32 base pair deletion in the chemokine receptor 5 (CCR5) that showed better protection against HIV and slower progression to AIDS.[6] Another study investigated a chemokine receptor 2 (CCR2) polymorphism with a G→A transition at position 190, that also resulted in slower progression to AIDS.[7] Crawley *et al.* found that a polymorphism associated with IL-10 at the -592 position resulted in decreased production of IL-10, inhibition of macrophage growth and decreased proliferation of HIV-1 in infected individuals.[8,9] The molecular mechanisms of most polymorphisms have not been fully elucidated. There is a need to explore more the role of host genetics in understanding HIV disease.

In vitro and *in vivo* studies have shown that HIV-1 infection can induce the secretion of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α).[10-12] TNF-α is the central mediator of the inflammatory response, and high concentrations of TNF-α may influence HIV-1 replication via clonal expansion of infected T lymphocytes.[13] In addition, TNF-α is also a potent inducer of apoptosis, which is a function dependent on the death receptor configuration of immune cells.[1,14] HIV-1 induces immune suppression by rapid apoptosis of bystander T_H lymphocytes.

TNF-α production is tightly controlled but genotypic differences may influence transcriptional regulation.[15,16] Reports have shown that promoter polymorphisms affect TNF-α gene expression.[17-19] A common polymorphism occurs at the -308 locus in the promoter region that results in

a guanine (G) to adenine (A) transition.[20] The -308 A allele has been associated with higher transcriptional activation and, therefore, increased TNF-α expression in different populations.[4,17,19] This association has also been linked to pathogenesis of various inflammatory disorders and, consequently, poorer disease prognosis.[4,17] The presence of various allelotypes, especially in promoter regions of cytokines, may severely affect immune responses to infection, given that they exert a large degree of transcriptional control over cytokine production. These effects, however, have not been comprehensively investigated in the context of infection. The precise mechanisms of genotypic influences on transcriptional regulation are currently unknown. However, it is thought that the G to A transition at the -308 locus is associated with conformational changes that increase binding affinity of transcription factors such as nuclear factor-kappa B (NF-κB).[15-17]

Considering the influence of the -308 TNF-α promoter polymorphism on TNF-α concentration, CD4 T_H lymphocyte apoptosis and HIV-1 replication, genotype may severely influence clinical outcomes in HIV-1 infected patients. The influence of the -308 TNF-α promoter polymorphism on HIV-1 infected black South Africans has not been studied. This is important as South Africa has the highest burden of HIV-1 infected individuals, and polymorphic variation may not only affect disease progression, but also response to treatment.

The aim of this study was to investigate genotypic frequencies of the -308 TNF-α promoter polymorphism in a cohort of HIV-1 infected black South African patients and determine whether genotype at this locus influenced serum TNF-α concentrations. In addition, the influence of this promoter polymorphism on CD4 T_H lymphocyte apoptosis and HIV-1 burden was investigated.

Materials and methods

Patient recruitment

This cross-sectional study was approved by the University of KwaZulu-Natal, Biomedical Research Ethics Administration (H129/04). Patients (*N*=75) were recruited by purposeful sampling from an antiretroviral (ARV) rollout clinic at King Edward VII Hospital, Durban, after obtaining informed consent. All patients had confirmed HIV-1 infection. Twenty-five patients were on NRTI-based HAART (NRTI: nucleoside reverse transcriptase inhibitor; HAART: highly active anti-retroviral therapy); 50 patients were HAART-naive. Healthy controls (*N* =76) were sourced from the South African National Blood Service. There was no follow-up of patients to assess changes in measures or outcomes over time.

Peripheral lymphocyte preparation

Buffy coats containing peripheral blood lymphocytes (PL) were extracted as previously described by our laboratory.[21] Cell density was adjusted to 1×10^6 cells/ml with the trypan blue exclusion test.

DNA extraction

Genomic DNA was extracted from PLs for each patient. Cells were transferred to 500 µl lysis buffer containing 0.5% SDS, 150 mM NaCl, 10 mM EDTA, and 10 mM Tris-HCl (pH 8.0). To this, RNase A (100 µg/ml, DNase-free) was added, and the solution was incubated at 37°C for 1 hour. Following the RNase A step, proteinase K (200 µg/ml) was added to the solution and thereafter incubated for 3 hours at 50°C. Protein contaminants were then precipitated by addition of 0.1 volume 5 mM potassium acetate and centrifuging (5 000 ×g, 15 minutes). Supernatants containing genomic DNA were transferred to fresh tubes and extracted with 100% isopropanol on ice and then washed with 70% ethanol. DNA samples were then dissolved in 10 mM Tris and 0.1 mM EDTA (pH 7.4) at 4°C overnight. To verify DNA extraction, equal amounts of DNA (300 ng) were electrophoresed (150 V, 50 min.) on a 1.8% agarose gel containing 0.5 mg/ml ethidium bromide. DNA bands were visualised by UV light and digitally photographed using a gel documentation system (Chemi-Doc XRS, Bio-Rad) and Quantity One Image Analysis software (Bio-Rad). The concentration of each sample was determined spectrophotometrically.

Genotyping for the -308 TNF-α promoter polymorphism

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the -308 TNF-α promoter polymorphism. A 107bp PCR product was amplified using 20 pmol of forward and reverse primer in a 25 µl reaction containing 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1X Green GoTa0071 Flexi buffer (Promega), 1 U GoTaq DNA polymerase (Promega) and 100 ng genomic DNA template. The forward and reverse primers were those according to Wilson *et al.*[20] (5'AGGCAATAGGTTTTGAGGGCCAT 3'; 5' TCCTCCCTGCTCCGATTCCG 3').

DNA was amplified for 35 cycles with denaturation at 94°C for 3 minutes, annealing at 60°C for 1 minute, extension at 72°C for 1 minute and a final extension at 72°C for 5 minutes. The PCR product was then digested with the restriction enzyme *Nco*I for 12 hours at 37°C. Digestion of the PCR product confirmed 2 alleles viz. -308 G allele which resulted in 2 fragments (87 bp and 20 bp), and –308 A allele which resulted in a single 107 bp fragment (Fig. 1).[20]

TNF-α enzyme-linked immunosorbent assay (ELISA)

Plasma was collected by centrifuging whole blood. Plasma TNF-α concentration was measured using the human TNF-α Max

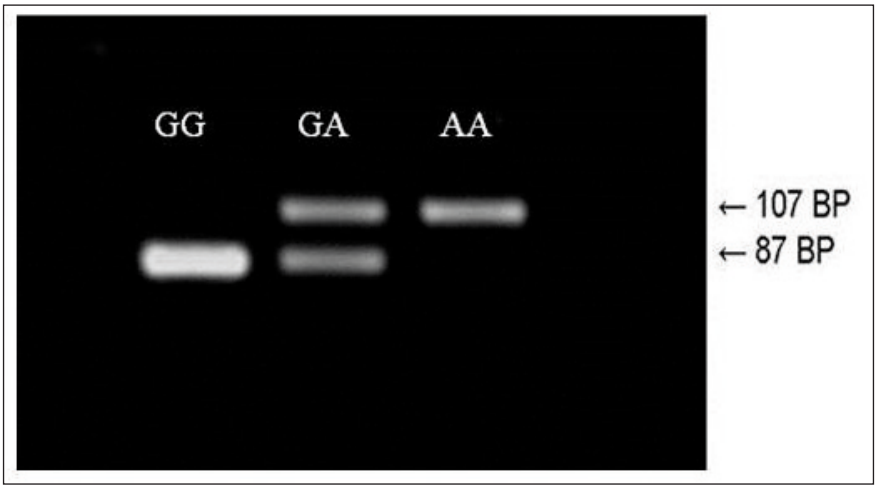

Fig. 1. Restriction fragment length polymorphism (RFLP) showing alleles of the -308 TNF-α promoter polymorphism. The -308 G allele gave rise to a 87 bp and 20 bp fragment, and the -308 A allele to a 107 bp fragment.

Standard ELISA kit (Biolegend). A high-affinity microtitre plate was coated with TNF-α capture antibody (100 µl/well, 18 hours at 4°C). Plates were washed and treated with 200 µl assay diluent. Thereafter, 100 µl standards and samples were added. Biotinylated anti-human TNF-α detection antibody and avidin-horseradish peroxidase were then added, followed by the TMB substrate and the stop solution. Absorbance was measured at 450 nm (570 nm reference) (Bio-Tek µQuant ELISA plate reader). Plasma concentrations of TNF-α were calculated by extrapolation from the standard curve.

CD4 T_H cell apoptosis, CD4 T_H cell counts and viral loads

CD4 T_H lymphocyte apoptosis, CD4 T_H cell counts and viral loads were determined as described previously.[21]

Statistical analysis

Genotype and allelic frequencies of the TNF-α -308 polymorphism for the control and HIV-1 cohort were compared by direct counting. Hardy-Weinberg statistics were used to determine whether our study cohort was representative of the larger population. Statistical analyses and correlations were done using Graphpad Prism Software (version 5).

Results

-308 TNF-α promoter polymorphism

Genotypic distribution did not deviate from those predicted by the Hardy-Weinberg equilibrium (HIV-1: p=0.331, chi-square statistic=0.946; controls: p=0.194, chi-square statistic=1.688). There were no significant differences in genotypic distribution between the HIV-1 and control cohorts respectively (GG 60% and 65.8%; GA 37.3% and 27.6%; and AA 2.7% and 6.6%). However, when allelic distribution was investigated, we found that the -308 G allele was more frequent in the control population (79.6% v. 78.7%) but this difference did not reach statistical significance (chi square test p=0.888, odds ratio=1.06, 95% CI (confidence interval) 0.607 - 1.84; see Table 1).

Table 1. Genotypic and allelic frequencies of the -308 TNF-α promoter region polymorphism in both HIV-positive and control populations

	HIV (N=75)	p value	Controls (N=76)	p value
Genotype frequency				
G/G	60%	0.331*	65.8%	0.194*
G/A	37.3%		27.6%	
A/A	2.7%		6.6%	
Allelic frequency				
A	21.3%		20.4%	0.888
G	78.7%		79.6%	

*Hardy-Weinberg equilibrium (HIV-1: chi-square statistic=0.946; controls: chi-square statistic=1.688).

Table 2. Mean TNF-α concentration and markers of HIV-1 progression in the HIV-positive and control cohorts. Markers of HIV-1 progression within the -308 GG and -308 GA genotypes of the HIV-positive cohort

	HIV-positive patients	Controls	p value
TNF-α concentration (pg/ml)	10.87±0.73 (14.40)	3.57±1.36 (0.00)	p<0.0001
% apoptosis of $CD4^+$ T cells	25.98±1.82 (24.30)	8.52±0.90 (6.94)	p<0.0001
HIV-positive patients	GG	GA	
TNF-α concentration (pg/ml)	15.01±1.40 (14.04)	15.52±1.05 (15.39)	p=0.403
Plasma viral load (log copies/ml)	3.69±0.337 (4.66)	3.92±0.321 (4.36)	p=0.970
$CD4^+$ T cell count (cells/µl)	256.10±25.04 (243.00)	288.60±20.97 (275.00)	p=0.242
% apoptosis of $CD4^+$ T cells	28.04±2.57 (24.52)	22.57±2.45 (23.30)	p=0.223

All values reported as mean±SEM (median).

Plasma TNF-α concentration

Mean plasma TNF-α concentration was determined in patients and controls by ELISA. The HIV-1 infected subjects showed significantly higher TNF-α concentration than controls (10.87 pg/ml and 3.57 pg/ml, p<0.0001, 95% CI: HIV-1 infected patients 9.39 - 12.36 pg/ml, controls 0.74 - 6.41 pg/ml; see Table 2).

We then investigated whether genotypic variation at the -308 locus influenced plasma TNF-α concentration in the HIV-1 infected cohort. Mean TNF-α concentrations were determined after grouping patients according to genotype. Higher plasma TNF-α concentrations were recorded in the -308GA genotype than in the -308GG genotype (15.52 pg/ml v. 15.01 pg/ml). This difference did not reach statistical significance (Mann-Whitney test, p=0.404, 95% CI: GA 13.35 - 17.70 pg/ml, GG 12.19 - 17.83 pg/ml; see Table 2). The mean TNF-α concentration in patients with the -308AA genotype was 19.35 pg/ml.

Genotype and clinical parameters

Since genotypic differences in TNF-α concentration were noted, we investigated whether genotype influenced viral load and CD4 T_H cell counts. Lower mean plasma viral load and lower mean CD4 T_H cell counts were observed in the -308GG genotype than in the -308GA genotype (3.69 log copies/ml v. 3.92 log copies/ml and 256.10 cells/µl v. 288.60 cells/µl respectively), with no significant difference (Mann-Whitney, p=0.970, 95% CI: GG 3.00 - 4.38 log copies/ml, GA 3.25 - 4.58 log copies/ml and p=0.242, 95% CI: GG 204.80 - 307.40 cells/µl, GA 245.30 - 331.90 cells/µl; Table 2). Mean plasma viral load and CD4 T_H cell counts in patients with the -308AA genotype were 3.59 log copies/ml and 197.00 cells/µl respectively.

Genotype and HAART

Following the observation of genotypic differences in the clinical markers of infection, we investigated whether genotype influenced patient response to treatment. Patients were grouped into HAART-naive and HAART-treated cohorts, and these groups further

stratified according to genotype. Mean plasma viral load and CD4 T_H cell counts were analysed according to genotype and treatment.

In the HAART-naive cohort, higher plasma viral loads and lower CD4 T_H cell counts were observed in the -308GG genotype than in the -308GA genotype (4.92 log copies/ml v. 4.54 log copies/ml and 244.30 cells/µl v. 283.80 cells/µl) but there were no significant differences (Mann-Whitney test, p=0.101, 95% CI: GG 4.68 - 5.16 log copies/ml, GA 4.17 - 4.90 log copies/ml and p=0.250, 95% CI: GG 179.70 - 308.80 cells/µl, GA 233.80 - 333.80 cells/µl; see Table 4).

Higher CD4 T_H cell counts and statistically significant lower plasma viral loads were recorded in the HAART-treated cohort than in the HAART-naive cohort (288.64 cells/µl v. 264.80 cells/µl and 1.19 log copies/ml v. 4.72 log copies/ml) (Mann-Whitney test, p=0.451, 95% CI: HAART-naive 226.80 - 302.80 cells/µl, HAART-treated 216.70 - 360.60 cells/µl and p<0.0001, 95% CI: HAART-naive 4.51 - 4.93 log copies/ml, HAART-treated 0.940 - 1.44 log copies/ml; Table 3). This result was expected as HAART is associated with lower plasma viral loads and higher CD4 T_H cell counts. Interestingly, we noticed genotypic differences in the HAART-treated cohort in the -308GG genotype. The -308GG genotype showed higher plasma viral loads and lower CD4 T_H cell counts than in the -308GA genotype (1.22 log copies/ml v. 1.13 log copies/ml and 278 cells/µl v. 314.0 cells/µl); however, the differences did not reach statistical significance (Mann-Whitney, p=0.251, 95% CI: GG 0.855 - 1.58 log copies/ml, GA 1.02 - 1.23 log copies/ml and p=0.374, 95% CI: GG 177.70 - 379.30 cells/µl, GA 185.40 - 442.60 cells/µl; see Table 4).

Genotype and apoptosis

Since genotypic differences were observed in TNF-α concentration, we investigated whether genotype influenced CD4 T_H cell apoptosis. Significantly higher mean apoptosis levels were observed in HIV-1 infected patients than in controls (25.98% v. 8.52%; Mann-Whitney test, p<0.0001, 95% CI: control 6.71 - 10.32%, HIV-1 infected 22.35 - 29.61%; see Table 2). In the HIV-1 cohort, higher apoptosis levels were observed in the -308GG genotype (28.04%); however, there was no statistical difference between genotypes (Mann-Whitney, p=0.223, 95% CI: GG 22.87 - 33.21%, GA 17.56 - 27.58%; see Table 2).

We investigated mean apoptosis levels in patients on treatment, and observed higher apoptosis levels in the HAART-naive cohort than in the HAART-treated HIV-1 infected cohorts; however, the differences did not reach statistical significance (27.13% v. 23.68%, Mann-Whitney test, p=0.482, 95% CI: HAART-naive 22.14 - 32.13%, HAART treated 18.99 - 28.38%; see Table 3). The -308GG genotype showed higher apoptosis levels in both the HAART-naive and HAART-treated HIV-1 infected cohorts than in the -308GA genotype (32.12% v. 29.58% and 23.77% v. 21.57%); however, differences in both cohorts were not statistically significant (Mann-Whitney test, p=0.404, 95% CI: GG 25.17 -39.07%, GA 22.79 - 36.37% and p =0.786, 95% CI: GG 18.19 - 29.35%, GA 4.82 - 38.32%; see Table 4). The mean apoptosis level in the patients with the -308AA genotype was 27.77%.

Table 3. Markers of HIV progression in HAART-naive and HAART-treated groups in HIV-positive patients

	HAART-naive	HAART-treated	*p* value
Plasma viral load (log copies/ml)	4.72±0.105 (4.85)	1.19±0.115 (1.06)	p<0.0001
CD4⁺ T cell count (cells/µl)	264.80±18.80 (256.00)	288.64±33.31 (293.00)	p=0.451
% apoptosis of CD4⁺ T cells	27.13±2.49 (24.77)	23.68±2.27 (22.86)	p=0.482

All values reported as mean±SEM (median).

Discussion

TNF-α is an immune regulatory cytokine that is released in response to viral antigens to combat infection.[10-12] However, chronically high concentrations of TNF-α may facilitate progression of HIV-1 and apoptosis of bystander T cells.[22]

Table 4. Markers of HIV progression in the -308 GG and -308 GA genotypes in the HAART-naive and HAART-treated groups

	GG	GA	*p* value
HAART-naive			
Plasma viral load (log copies/ml)	4.92±0.115 (4.91)	4.54±0.173 (4.57)	p=0.101
CD4⁺ T cell count (cells/µl)	244.30±30.72 (188.00)	283.80±23.97 (273.00)	p=0.250
% apoptosis of CD4⁺ T cells	32.12±3.40 (26.49)	29.58±3.34 (24.54)	p=0.404
HAART-treated			
Plasma viral load (log copies/ml)	1.22±0.16 (1.06)	1.13±0.03 (1.15)	p=0.251
CD4⁺ T cell count (cells/µl)	278.50±44.57 (273.50)	314.00±40.42 (314.00)	p=0.374
% apoptosis of CD4⁺ T cells	23.77±2.67 (22.36)	21.57±5.26 (22.48)	p=0.786

All values reported as mean±SEM (median).

TNF-α indirectly induces viral replication by activating NF-κB[23] which binds to the long terminal repeat (LTR) of HIV.[23,24] This may lead to production of viral proteins such as Tat and Nef which further induce TNF-α production via the inflammatory response.[23,24] The -308 TNF-α promoter polymorphism has been associated with altered TNF-α concentration.[15,16] Genotypic variation may induce conformational changes in the promoter region that increase binding affinity of transcription factors, such as NF-κB.[15,16,23]

Ours is the first report on the -308 TNF-α promoter polymorphism in HIV-1 infected black South Africans. It is probable that elevated levels of TNF-α may alter clinical outcomes in the patient.[4,17] A previous study showed lower CD4 T_H cell apoptosis and plasma viral load in a cohort of HIV-1 infected patients on HAART.[21] The current study aimed to investigate whether the -308 TNF-α promoter polymorphism influenced TNF-α concentration, CD4 T_H cell count, CD4 T_H cell apoptosis and plasma viral load in HIV-1 infected black South Africans.

It is well established that TNF-α concentration is elevated early in infection.[10,11] However, during HIV-1 infection, consistently high levels of TNF-α may be attributed to constant antigenic stimulation from viral proteins such as Tat and Nef.[23,24]

Our study shows that the -308 G allele was similar in both the HIV-1 infected and control cohorts. This finding is consistent with other studies that reported similar allelic frequencies in different demographic groups.[4,25-27] The -308 G allele in the HIV-1 infected cohort was associated with significantly high levels of TNF-α, which may be due to increased binding affinity of transcription factors.

In addition to high TNF-α concentration, this study showed a cross-sectional association between allelic frequency and markers of HIV disease progression, which was indicated by high bystander T_H cell apoptosis and viral replication. High TNF-α concentration is involved in HIV-1 replication via clonal expansion of infected T_H cells.[13,23] It is also involved in rapid apoptosis of bystander T_H cells, which may account for the high viral titres and high levels of apoptosis observed in this study. During HIV-1 infection, TNF-α may act as a molecular rheostat that switches between clonal expansion and bystander T_H cell apoptosis, depending on membrane receptor profile.[1,14] Genotypic differences in the TNF-α promoter that influence a cell's inherent ability to produce the cytokine may exacerbate these functions during HIV-1 infection. In response to rapid apoptosis, the immune system may compensate by increasing bone marrow turnover of mononuclear cells. These may, however, not reach complete maturation and lead to impaired T_H cell recovery, ultimately contributing to HIV-1 progression.[1,22,28]

This study differs from previous studies which have associated the -308 A allele with high TNF-α concentration and disease.[4,17,19] The -308AA genotype has been widely associated with poorer clinical outcomes and disease progression in *Leishmaniasis*, cerebral malaria and insulin-dependent diabetes mellitus.[29-31] Interestingly, some reports showed no association between this genotype and disease severity.[25-27,32,33] In studies that showed the association between the -308AA genotype and disease severity, frequencies of the -308 A allele were low, which may have conferred low statistical power and, as such, these conclusions warrant confirmation in other populations.[4,34] Furthermore, the bulk of these studies were performed in populations of white ancestry. No studies to date have investigated the influence of the -308 TNF-α promoter polymorphism in infectious diseases in a black African population.

Conclusion

In contrast with other studies, our study reports for the first time that the -308 G allele may contribute to mechanisms that lead to poorer response to HAART therapy in Black South Africans infected with HIV-1. Similarly, we found the -308AA genotype to be least frequent (*N*=2), which may preclude disease association studies until adequate sample sizes are collected. Comparable clinical outcomes were observed in heterozygote individuals, providing further evidence that the presence of the -308 G allele may be associated with markers of HIV-1 progression in this study.

Single nucleotide polymorphisms that affect regulation of cytokines may affect host response to HIV-1 infection. This effect may influence disease progression and clinical outcomes. To provide holistic management of patients infected with HIV-1 and develop individual treatment strategies, it is imperative to study genotypic differences between individuals. Such approaches may curb the advent of adverse drug reactions, minimise therapeutic failures and also address not only the medical, but also the economic burdens of this disease.

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ORIGINAL ARTICLE

Levels of procalcitonin, C-reactive protein and neopterin in patients with advanced HIV-1 infection

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Objectives. To compare the value of procalcitonin, C-reactive protein (CRP) and neopterin as indicators of immune deficiency, co-infection, efficacy of treatment, and disease progression, in patients with advanced HIV-1 infection.

Design. Cross-sectional, investigating baseline blood measurements and clinical observations in 82 HIV-positive patients divided into an antiretroviral treatment (ART) group and an ART-naïve group.

Setting. Secondary general hospital in Pretoria.

Results. Procalcitonin and CRP levels showed no significant differences between the ART and ART-naïve groups, and no correlations with CD4 counts or viral loads. CRP levels were significantly higher with TB co-infection ($p<0.05$). Neopterin levels were raised above normal in 92% of the ART-naïve group and in 75% of the ART group. The levels were significantly higher ($p<0.05$) in the ART- naïve group. Negative correlations were found between neopterin and CD4 counts for the total patient group ($r=-0.482$; $p<0.001$). Neopterin was significantly ($p<0.05$) higher in the HIV/TB co-infection group than in those without TB. Higher neopterin levels at baseline were associated with a decline in CD4 counts over the ensuing 6-month period, and patients with higher baseline neopterin levels developed more complications over the 6-month period.

Conclusions. Compared with procalcitonin and CRP, neopterin appears to be associated with the degree of immunodeficiency and of co-infection with TB. Neopterin levels may be investigated further as a measure of disease progression or treatment response.

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Procalcitonin, C-reactive protein (CRP) and neopterin are three of the markers most commonly used, with varying degrees of success, as diagnostic or prognostic indicators to monitor disease progression and to estimate the efficacy of therapeutic interventions in infectious diseases and non-infectious inflammatory conditions. All three are, to a lesser or greater extent, used among HIV-positive patients.

Procalcitonin is the pro-hormone of calcitonin. In normal conditions, transcription of the procalcitonin gene occurs in the C-cells of the thyroid under conditions of hypercalcaemia and neoplastic disease.[1] However, in the presence of bacterial infection or endotoxins, virtually all cells produce calcitonin precursors.[1] Recent indications are that, in infectious or inflammatory conditions, procalcitonin may in fact be considered an acute phase reactant, with the liver being the major source of procalcitonin.[2] Procalcitonin levels increase in certain pro-inflammatory conditions, especially bacterial infections, but are thought not to show significant increases with viral and non-infectious inflammatory conditions.[3] The levels are often used to differentiate between patients with sepsis and those with systemic inflammatory response syndrome (SIRS).[4] Procalcitonin levels have been recommended for distinguishing between bacterial and non-bacterial infections, and therefore as a guideline in the prescription of antibiotics.[5,6] One disadvantage in the use of procalcitonin is that the levels in healthy individuals are below the reliable detection limit (10 pg/ml) of most clinical assays.

C-reactive protein is an acute-phase protein, and its levels are upregulated in viral, bacterial and fungal infections, as well as in non-infectious inflammatory conditions. The cytokine profile found with raised CRP levels is predominantly pro-inflammatory, and CRP levels are often used as a non-specific indicator of inflammatory activity, irrespective of the cause.[7] The levels of CRP in bacterial and viral infections differ, and high levels (e.g. >100 mg/l) can be found with bacterial infections, while lower levels (usually <10 mg/l) are more commonly associated with viral infections.[8] As an acute-phase reactant, macrophage- and perhaps adipocyte-derived IL-6 is a major stimulant for the production of CRP, and liver failure is the major cause for a decline in CRP synthesis.[9,10]

Neopterin (6-D-erythro-hydroxy propyl pteridine) is a catabolic product of the purine nucleotide guanosine triphosphate. Neopterin is produced in macrophages from guanosine 5'-triphosphate (GTP) which is cleaved by GTP-cyclohydrolase 1 to 7,8-dihydroneopterin triphosphate, followed by conversion of 7,8-dihydroneopterin triphosphate

to neopterin and 7,8-dihydroneopterin under the influence of phosphatases.[11] GTP-cyclohydrolase 1 is stimulated, predominantly, by T-helper cell type-1 derived interferon-g, but co-stimulation by tumour necrosis factor alpha may contribute.[11] Neopterin is used as indicator of both macrophage function and cell-mediated immunity. When cell-mediated immunity dominates, circulating neopterin levels are usually high and, when humoral immunity dominates, neopterin levels are low.[11] Increased neopterin levels are found with viral infections, intracellular bacterial infections, intracellular parasites, a number of auto-immune diseases, malignancies, rheumatoid arthritis, systemic lupus erythromatosus, acute cellular graft rejection or graft-v.-host disease, and in almost every condition where cellular immunity dominates.[12,13] In HIV-1 infection, serum neopterin has been described as an immune activation marker and predictor of disease progression.[14]

In HIV/AIDS, plasma HIV-1 RNA concentration reveals the degree of viral replication, and CD4 counts reflect the degree of immune deficiency and, it is speculated, end-organ damage. The outcome is, however, largely influenced by the co-existence of other complications, especially co-infection with TB. Although viral load and CD4 counts are considered the diagnostic gold standards for HIV, soluble markers may add valuable information about immune activation status and prognosis. In addition, cost-effective reliable serum markers would be of benefit in resource-limited settings where restrictions are placed on the frequency of laboratory investigations such as viral loads. The aim of this investigation was to compare the associations of procalcitonin, C-reactive protein and neopterin and measures of HIV disease status and co-infection with TB.

Methods

HIV-positive outpatients were randomly recruited from the Immunology Clinic at the Kalafong Hospital, Pretoria. The study took place during 2010 - 2011, and patients were followed-up 6 months after baseline, wherever possible.

Informed consent was obtained from 82 adult patients who were attending the clinic on a Friday, who freely gave informed consent to take part, and who were not ruled out by the exclusion criteria. Exclusion criteria included patients <18 years of age, patients with CD4 counts >400 cells/µl, patients on antiretroviral treatment (ART) for <2 months, treatment defaulters from the ART group and, for the ART-naïve group, patients previously on any ART. Ethical approval was obtained from the Faculty of Health Sciences Research and Ethics Committee, University of Pretoria.

The patients were firstly divided into a group on active ART (*N*=57) and a group not on ART (ART-naïve; *N*=25). The ART group was further subdivided into groups depending on their time on treatment prior to baseline investigation (2 months - 1 year; 1 - 2 years, and >2 years). At the 6-month follow-up, patients were subdivided into 2 groups according to baseline neopterin levels, and the groups were compared in terms of the CD4 counts and development of complications diagnosed by the attending physician and confirmed by the specialist involved in the study.

Blood specimens collected at baseline were centrifuged on site; plasma aliquots were stored at -70°C until analysis. Procalcitonin (RayBiotech Inc., USA) and neopterin (Immuno-Biological Laboratories Inc., USA) were measured by commercial enzyme-linked immune-absorbent assay (ELISA) kits. CRP and other routine blood investigations (CD4 count, WBC count, haemoglobin etc.) at baseline were determined according to standard procedures of the National Health Laboratory Service (NHLS), and results were extracted from the laboratory reports and patient files.

Student's *t*-test and nonparametric Mann-Whitney U-test were used to determine group differences. Kruskal-Wallis one-Way ANOVA indicated variance across multiple groups. Correlations were determined by regression analysis and Spearman rank correlation co-efficient. Statistical analysis was performed using NCSS/PASS (Hintze J 2001) software, and all testing was done at a significance level <0.05 unless otherwise specified.

Table 1. Patient demographic information at baseline

	ART	ART-naïve
N	57	25
Females	35 (61.4%)	15 (60%)
Age (years)	36.6±8.2	36.8±10.8
Race	57 black	25 black
BMI	22.6±5.0	21.2±3.5
Married	10 (17.5%)	7 (28%)
Employed	22 (38.6%)	12 (48%)
Alcohol (number of patients)	3 (5.3%)	3 (12%)
Smoking (number of patients)	9 (15.8%)	5 (20%)
Average months on treatment	13.6±16.2 (2 - 63)	-
TB positive at baseline	10 (17.5%)	8 (32%)

Table 2. Comparison of baseline blood measurements for the two groups

	ART	ART-naïve	*p*-value
Procalcitonin (pg/ml)	13.2±3.3	12.9±1.5	0.767
Neopterin (nmol/l)	39.5±38.9	64.4±39.4	0.001*
CRP (mg/l)	25.3±38.5	34.9±82.9	0.567
CD4 count (cells/µl)	288.2±196.4	157.5±181.9	0.027*
Viral load (log_{10} copies/ml)	2.4±0.9	3.6±1.7	0.005*
Red cell count (x10^{12}/l)	3.6±0.5	3.9±0.7	0.048*
Haemoglobin (g/dl)	14.2±15.2	11.1±2.0	0.345
White cell count (x10^9/l)	4.9±1.5	5.7±2.8	0.107
Neutrophils (x10^9/l)	2.7±1.2	3.8±2.7	0.026*
Lymphocytes (x10^9/l)	1.6±0.8	1.4±0.8	0.211
CD4 % of lymphocytes	17.4±7.6	9.2±7.3	0.0006*

Note: Viral load measured within 2 months of baseline (*p<0.05; mean±SD).

Table 3. Comparisons for patients who were followed up after 6 months

	Complications after 6 months	No complications after 6 months
N	29 (61.7%)	18 (38.3%)
ART	12 (41.4%)	15 (83.3%)
Baseline CD4 count (cells/µl)	237.0	327.7
6 month CD4 count (cells/µl)	232.5	325.1
Baseline viral load (log_{10} copies/ml)	2.34±0.9	2.3±1.0
Baseline CRP (mg/l)	43.2±87.4	9.5±0.7
Baseline neopterin (nmol/l)	53.9±33.9	10.8±7.6
Baseline PCT (pg/ml)	13.7±4.5	12.6±0.43

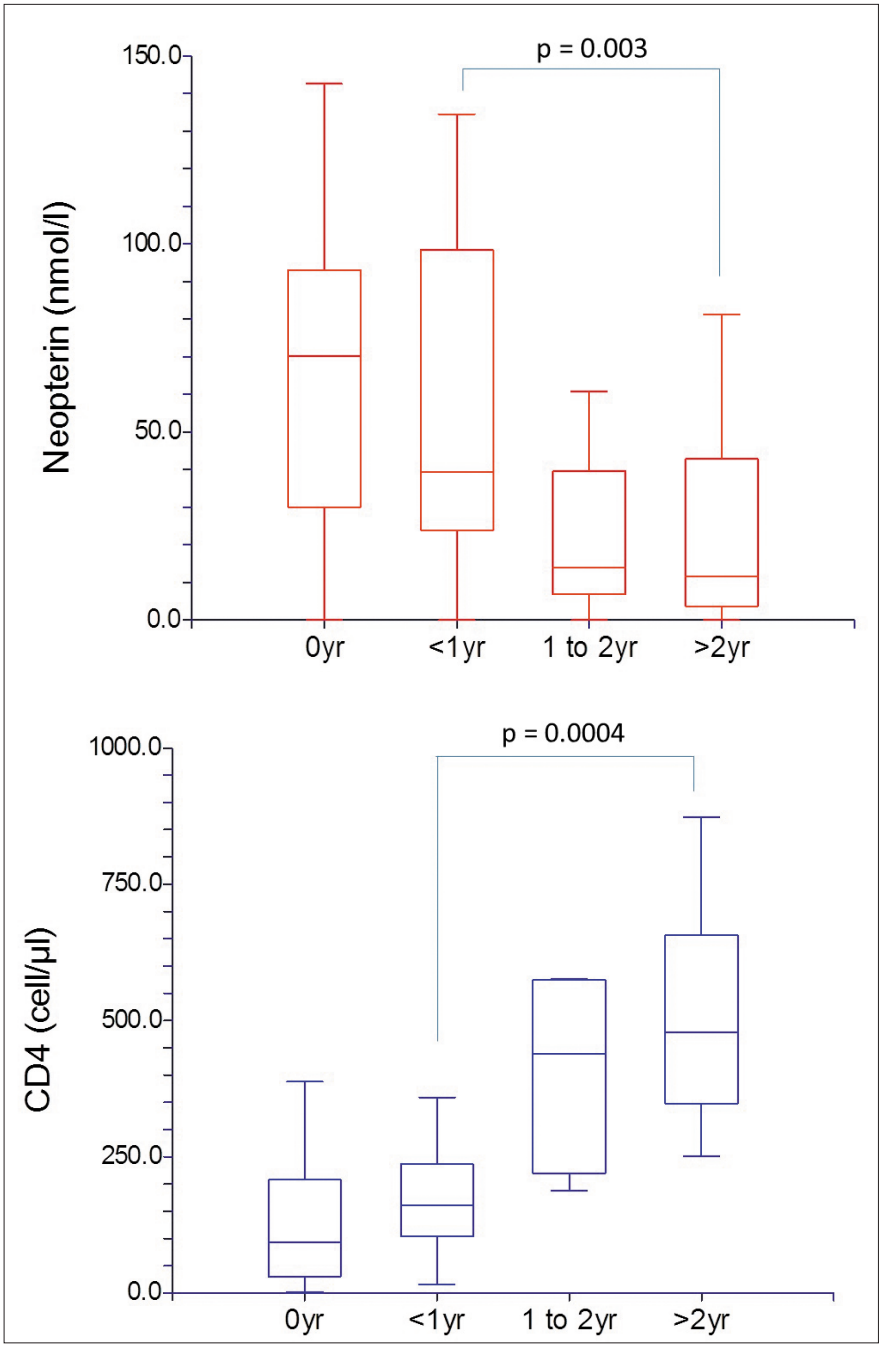

Fig. 1. Box plots illustrating neopterin and CD4 levels for patients after 0 years (n=25), <1 year (n=30), 1 - 2 years (n=10) and >2 years (n=10) on ART.

Results

The demographic profiles for the patient groups are presented in Table 1. The 2 groups were comparable in age, body mass index (BMI), gender distribution, race and employment status. Results of the baseline blood measurements and the comparison between the ART and ART-naïve groups are presented in Table 2. Neopterin levels were significantly higher (p=0.0096) in the ART-naïve group than in the ART group. Negative correlations were found between neopterin and CD4 counts for the total group of patients (r=-0.482; p<0.0001; N=82), as well as for the ART group (r=-0.451; p=0.0045; n=57). Neopterin also correlated negatively with haemoglobin levels for the total patient group (p=-0.597; p<0.0001; N=82).

Six months after the baseline measurements, 47 of the original 82 patients were still available and could be followed up with regard to CD4 counts and the development of complications. A comparison between patients with complications and those without complications, at baseline and at follow-up, is shown in Table 3. Additional complications at follow-up consisted of TB (n=6, 2 of whom had extrapulmonary disease); pneumonia (n=5); severe lymphadenopathy (n=4); cardiac/renal disease (n=4) and haematological complications such as anaemia, thrombocytosis or neutrophilia (n=10).

The relationship between neopterin and CD4 counts over the 6-month period following the baseline assessments was examined. Patients who developed additional complications, stopped taking anti-retroviral drugs or ART-naïve patients who started ART during this period were excluded. Seven patients stopped ART over this period; the reasons included non-compliance and drug side-effects. This cessation resulted in a drastic decline in sample sizes, i.e. 11 patients (8 on ART) had a decrease, and 9 (all on ART) had an increase in CD4 over the period. Mean baseline neopterin was significantly higher in the patients whose CD4 counts were decreased at follow-up (35.09 v. 10.82 nmol/l; p=0.035). In the group whose CD4 counts decreased over the 6-month period, baseline neopterin levels correlated negatively with both baseline CD4 count (r=-0.68; p=0.03) and follow-up CD4 count (r=-0.58; p=0.07).

As shown in Fig. 1, the patients were subdivided into groups according to the period of time they had been on treatment prior to the baseline investigations. Analysis

of variance showed that neopterin levels were significantly ($p<0.01$) lower and CD4 counts significantly higher ($p<0.001$) in the patients who had been on treatment >1 year.

Discussion

This study examined the associations of 3 laboratory markers of disease in HIV-positive patients. The key findings are that neopterin is more strongly associated with the degree of immunodeficiency and of co-infection with TB than CRP or procalcitonin. Higher neopterin levels at baseline were associated with a decline in CD4 counts and the development of more complications over the ensuing 6-month period.

Limitations of this study include the fact that not all patients could be traced for the 6-months follow-up, that the groups became progressively smaller as patients who had a change in treatment over this period were excluded from the statistical comparisons, and that disease progression could only be estimated from CD4 counts and not viral loads.

The results of this study suggest that CRP levels are not specifically associated with immune deficiency, the effects of ART, or disease progression. These results are in agreement with those of a study in India in which CRP measurement in HIV-positive patients was found neither to be of value as diagnostic aid nor as prognostic marker in HIV/AIDS.[15] However, in view of the fact that CRP levels in HIV-positive individuals are generally significantly lower in viral than in bacterial infection, significantly raised levels of CRP could be an indication to investigate for a possible co-infection, keeping in mind that other conditions marked by a pronounced pro-inflammatory response can also lead to increases in the levels of CRP. This finding is in line with the results of a South African study by Wilson *et al.* who showed that normal CRP levels, in combination with clinical evaluation, could be useful to rule out TB in populations with a high prevalence of HIV.[16]

Procalcitonin (PCT) is known for its increase in bacterial infections and is used by some to differentiate between viral and bacterial infections.[17] One explanation as to why procalcitonin levels remain low in purely viral infections is based on the fact that the production of PCT is primarily stimulated by tumour necrosis factor. It is suggested that increases in procalcitonin do not occur with viral infections because alpha interferon, synthesised as a result of viral infections, inhibits synthesis of tumour necrosis factor.[1] Should this be true, the question remains whether procalcitonin would be of much use for the detection of bacterial co-infection in HIV-positive patients. In developing countries such as South Africa, co-infections with TB and other bacterial infections in HIV-positive individuals are common – even major sources of morbidity and mortality – especially at CD4 counts <200 cells/µl. The level of circulating PCT in normal healthy individuals is generally below the limit of detection (10 pg/ml) of most clinical assays.[18] According to sensitive research assays, the normal level for plasma/serum PCT is 33±3 pg/ml.[1] The analytical sensitivity for the assay of this study was typically below 30 pg/ml and, from linear extrapolation, individual PCT levels were all >10 pg/ml. However, the mean PCT levels for the total group of patients were normal, with no significant difference between the ART and ART-naïve groups, and no significant correlations between PCT and CD4 counts or viral loads. Although the value of PCT as a reliable marker of active TB has on occasion been questioned,[19] the overriding assumption is that PCT is indeed a valuable marker of *Mycobacterium tuberculosis* in non-immunocompromised patients.[20] The procalcitonin findings of this study are in line with studies that showed suppression of the procalcitonin response in HIV-positive individuals.[20,21] Although some diagnostic and prognostic value for the measurement of PCT in HIV/TB-co-infection has been described in a South African study, only 58% of their HIV-positive patients with TB had PCT levels marginally above 100 pg/ml.[22] This is, in view of better performing markers, not adequate for clinical use in individual patients. Although procalcitonin induction in HIV-positive individuals is known to occur in sepsis, and reports exist of significant increases in procalcitonin in pneumococcal and a number of other non-viral infections,[23] it would appear that secondary infections in HIV-positive patients do not in general trigger overt increases in procalcitonin synthesis,[21,23] provided that the infections are localised or organ-related without systemic inflammation.

Neopterin levels were increased above normal (10 nmol/l) in 92% of the ART-naïve group and in 75% of the ART group. The levels were significantly higher ($p<0.01$) in the ART-naïve group and were inversely associated with CD4 counts. These results confirm the value of neopterin levels as a reflection of the degree of immunodeficiency. Fig. 1 shows the increase in CD4 counts that occurred over the same periods on ART as the decrease in neopterin. This implication (that neopterin may be an indicator of the efficacy of ART) warrants further investigation.

Among the 18 patients (>26% of the study population; 50% on ART) in whom active TB-co-infection was confirmed at the baseline investigations, neopterin levels were significantly higher ($p<0.001$), and CD4 counts significantly lower ($p=0.028$), than among the patients without TB co-infection. These results are in agreement with previous indications that neopterin levels are significantly higher in HIV-positive patients with TB-co-infections and that, although neopterin levels may decrease with anti-TB therapy, high levels of neopterin persist with progression of the immune deficiency and a poor prognosis.[24]

As neopterin levels reflect the degree of immune deficiency in HIV-positive patients, and perhaps the response to ART, the question was asked whether neopterin has indeed, as claimed elsewhere, prognostic value concerning disease progression.[25] Baseline neopterin was significantly higher ($p<0.01$) in the group of patients in whom other complications were present 6 months after baseline investigations, than patients who progressed well (53.9±39.9 v. 10.8±7.6 nmol/l; $p<0.01$). When all patients who stopped ART over the 6-month period were excluded, the mean neopterin levels were significantly higher in the group with complications than in the group without complications (59.29 v. 30.9 nmol/l; $p=0.018$). When those patients who did not change antiretroviral status were split into groups, the mean neopterin levels were significantly higher in the group that developed complications than those who did not, both for the ART (45.9 v. 24.13 nmol/l; $p=0.04$) and the ART-naïve (75.02 v. 30.99 nmol/l; $p=0.001$) groups. Although these results do not necessarily imply a direct relationship, they warrant further investigation. The possibility that neopterin levels could perhaps be predictive of disease progression was further examined by looking at the changes in CD4 counts. The baseline neopterin values were compared between patients whose CD4 counts decreased and those that increased over the 6-month period following baseline assessments. To minimise the number of confounding factors, any patient who had additional complications or a change in ART during the 6 months was excluded. This resulted in a drastic decline in sample sizes, i.e. 11 patients (8 on ART) had a

decrease, and 9 (all on ART) had an increase in CD4 counts over the period. Mean baseline neopterin levels were significantly higher in patients whose CD4 counts decreased, and significantly lower in patients whose CD4 counts increased. In the group whose CD4 count decreased over the 6-month period, baseline neopterin levels correlated with both baseline CD4 counts (r=-0.68; p=0.03) and follow-up CD4 counts (r=-0.58; p=0.07). Although the group divisions, owing to the exclusion criteria, were small, the association of neopterin levels with CD4 counts is nonetheless seen. These results warrant further investigation into the value of neopterin as a possible predictor of disease progression.

In view of the stimulatory role of IFN-γ in neopterin synthesis,[11] the link between chronic elevation of IFN-γ and HIV-1 progression, as well as the active role of neopterin in the disease,[25] the value of neopterin is not surprising. Neopterin has previously been described as one of the better immunological markers in patients with HIV-1 infections.[14,25] It has even been said that neopterin levels increase before other markers of HIV infections have risen.[25] In the present study, 40% of ART, and 75% of ART-naïve, patients had CD4 counts <200 cells/μl, and all had CD4 counts <400 cells/μl. Therefore, with regard to patients in the advanced stages of the disease, the results of this study support the notion of neopterin as an inexpensive indicator of CD4 status and as an indicator of bacterial co-infection. The results warrant further investigation into neopterin as an indicator of disease progression and of the success of ART.

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LETTERS

Is stavudine worth saving?

To the Editor: The ultimate goal of HIV therapy in resource-constrained settings must be to keep as many people alive with the best possible quality of life using the resources available. The question debated between Andrieux-Meyer *et al.*[1] and Venter *et al.*[2,3] might therefore be: 'With the resources available, can we keep more people alive with the best possible quality of life using stavudine 20 mg bd or tenofovir 300 mg od as standard first line therapy?' Quality of life is extremely important but unlikely to be the overriding factor if budgetary constraints restrict access to ART and therefore increase mortality. Both groups seem to agree that tenofovir is superior to stavudine for most patients and that the crux of the argument is about cost. Venter *et al.* describe tenofovir as 'the gold standard' and state that 'these arguments [about the benefits of tenofovir] are likely to be irrelevant when the cost of medication is considered'. Andrieux-Meyer *et al.* acknowledge that 'the rationale for this [proposed] trial is to lower treatment costs'.

The question of whether stavudine or tenofovir will ultimately save the most lives within the budget is complex. Many of the variables change over time and differ between countries. The 'resources available' may even be influenced by the choice of ART regimens if, for example, a government tries to save money by using inferior but cheaper drugs. In some settings, the rate-limiting factors for saving lives may not actually be financial resources to buy medications but a lack of human resources or logistic challenges. Despite these complexities, it is important that each side provides as clear a picture as possible. In addition to cost-effectiveness estimates, we need absolute cost estimates for competing regimens including the estimated minimum cost of tenofovir once costs are driven primarily by raw materials. We need estimates of the number of patients requiring ART over time, and we need to know how much funding is available. In particular, we need to know how the recent cuts to funding from donors such as the Global Fund will affect the provision of ART in different countries. Without such figures, any discussion about drugs in phase II trials that have a high attrition rate and long time delay before affordability is frankly irrelevant.

Venter *et al.* have conceded that 2 years may be too short to show differences between the groups, and it will be vital to motivate for lengthening the trial if non-inferiority is shown at 2 years. Upon completion of the trial, it would seem sensible to use the cheaper option if non-inferiority is shown. However, even if stavudine is not non-inferior (i.e. is inferior), it still might be preferred in some settings if the alternative is running out of money and restricting access to ART. We commonly use inferior treatments owing to cost constraints; just one example is the use of amphoteracin B monotherapy to treat cryptococcal meningitis rather than the superior but more expensive combination of liposomal amphoteracin B and flucytosine. Activists will argue that we should continue to lobby for increases in funding and reductions in drug cost. Of course we should, but we must also be mindful to look at the problem through the eyes of future patients. They will not thank us if our lobbying efforts fail to prevent ART rationing owing to shortfalls in funding.

It is clear that both sides of the debate have the best interests of patients in mind. To make an informed decision on the merits of trialling stavudine against tenofovir in the Southern African context, we need a clearer description of the costs of each strategy and the likely available resources. In short, we need to know whether choosing tenofovir over stavudine in first-line therapy is likely to lead to restricted access to care in some settings.

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CPD QUESTIONNAIRE
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Two CPD points are awarded for the correct completion and submission of the questions below.

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This programme is available free of charge to members of the HIV Clinicians Society and SAMA only.

TRUE (A) or FALSE (B) – click on the correct answer:

Regarding levels of soluble inflammatory markers in HIV infection

1. The kidneys are the major source of increased levels of procalcitonin during acute infection.

2. Levels of C-reactive protein may be used to distinguish bacterial from viral infections.

3. Neopterin is produced by macrophages and is a marker of cell-mediated immunity.

4. Levels of neopterin may be lower in individuals with untreated HIV infection, compared with individuals on antiretroviral therapy.

Regarding reliability of CD4 cell count enumeration

5. CD4 cell counts vary naturally according to the time of day (diurnal variation) and by the site of phlebotomy.

6. The time delay between phlebotomy and laboratory testing can influence CD4 cell count results, and the World Health Organization recommends conducting tests within 72 hours of specimen collection.

7. Data from Swaziland show that there is variability in CD4 count results within and between laboratories; this variability may lead to incorrect decisions to initiate antiretroviral therapy in up to 20% of patients.

Regarding tumour necrosis factor-alpha

8. High levels of TNF-alpha may help to reduce HIV viral replication.

9. TNF-alpha levels may be increased in individuals with particular genetic variations, such as -308 TNF-alpha polymorphisms.

10. There is clear evidence that -308 TNF-alpha polymorphisms occur more commonly in HIV-infected individuals than uninfected individuals.

Regarding cytomegalovirus (CMV) retinitis

11. Intravitrial gancyclovir injections are the gold-standard for managing CMV retinitis.

12. With management through gancyclovir injections and antiretroviral therapy, the vast majority of patients with visual loss due to CMV-retinitis will regain normal visual function.

13. CMV disease in the retina occurs early in the course of HIV disease, and the incidence of CMV-retinitis is not affected by use of antiretroviral therapy.

Regarding pneumocystis pneumonia

14. Patients with pneumocystis pneumonia who require mechanical ventilation have a high risk of mortality.

15. Lung fibrosis may help to explain the poor prognosis of patients with pneumocystis pneumonia who require ventilation.

16. *Pneumocystic jirovecii* is universally sensitive to trimethoprim-sulfamethoxazole, and antibiotic resistance is unknown.

Regarding anaemia in the context of HIV infection

17. Bone marrow infiltration from AIDS-related conditions is common in advanced, untreated HIV; this may be evidenced by a pancytopaenia, or an anaemia with minimal reticulocyte production.

Regarding pre-exposure prophylaxis to prevent HIV infection

18. Use of tenofovir or Truvada for PrEP requires monitoring of renal function and annual bone mineral density scanning in all individuals (e.g. DEXA).

19. Real-world adherence to PrEP regimens is a critical determinant of their effectiveness in preventing HIV infection, and ongoing adherence counseling is required.

20. Careful consideration needs to be given to drug-drug interactions in PrEP, as tenofovir may interact with cimetidine, metformin and some aminoglycosides.

REVIEW

A review of the use of blood and blood products in HIV-infected patients

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Dr Vis Poovalingam, who had a critical role in motivating for this review, is acknowledged for her valuable contribution.

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Despite numerous publications on the appropriate use of blood and blood products, few specifically consider the role of transfusion in the management of HIV. This review is a synthesis of conditions encountered in the management of HIV-infected patients where the transfusion of blood or blood products may be indicated. A consistent message emerging from the review is that the principles of transfusion medicine do not differ between HIV-negative and -positive patients. The aim of the review is to provide clinicians with a practical and succinct overview of the haematological abnormalities and clinical circumstances most commonly encountered in the HIV setting, while focusing on the rational and appropriate use of blood and blood products for HIV patients. Important ethical considerations in dealing with both the collection and transfusion blood and blood products in the HIV era have also been addressed.

S Afr J HIV Med 2012;13(2):87-103.

'Blood transfusion is like marriage: it should not be entered upon lightly, unadvisedly or wantonly, or more often than is absolutely necessary.'[1]

Blood services have been at the forefront of raising awareness of the HIV pandemic. The emergence and recognition of HIV as a transfusion transmissible infection (TTI) in the early 1980s had a profound impact on blood services worldwide. There has been concerted international effort, expending considerable resources, to prevent transmission of TTIs through blood products and to provide safe blood products for transfusion. One means of reducing the risk of TTIs and properly managing haematological symptoms, especially in immunocompromised individuals, is appropriate clinical use of blood. This is contingent upon an appreciation of the risks of unnecessary transfusion. Clinicians should consider non-transfusion options such as haematinics in the management of anaemia. The decision to transfuse should be based on an individual patient's co-morbid and clinical status, rather than on laboratory indices only. Specific and careful consideration of each patient is therefore required when HIV-related anaemia or associated conditions raise the possibility of transfusion.

There have been numerous requests from members of the Southern African HIV Clinicians Society for guidance and direction on blood transfusion in HIV-infected patients. While the role of blood transfusion in the management of haematological conditions such as anaemia and thrombocytopaenia does not differ substantially between HIV-negative and HIV-positive patients, it is the authors' experience that there is a general need to promote rational transfusion practice. The objective of this review is not to provide a novel approach to the management of haematological conditions; rather, it is intended to provide a practical and succinct review on the rational use of blood transfusion in the management of haematological abnormalities, focusing on conditions either unique to, or more frequently encountered in, HIV-infected patients. This review should be read in conjunction with published national and international clinical guidelines.

(For a review of the ethical and legal considerations of transfusion and HIV, with a specific focus on South Africa, please refer to Appendix 1.)

Cytopaenias in HIV

Clinically significant cytopaenias (anaemia, thrombocytopaenia, neutropaenia) are common in persons with HIV.[2] Many factors may contribute to the development of cytopaenias in HIV, including the virus itself that can infect progenitor cells directly, cytokine effects, reticulin fibrosis, altered immune function with auto-antibody production, micro-nutrient deficiency (folate, vitamin B_{12}, iron), co-infection with other agents both opportunistic (e.g. TB, mycobacterium avium complex (MAC), cytomegalovirus (CMV), Ebstein-Barr virus) and conventional (e.g. bacteria, parvovirus), bone marrow infiltration by malignancy, and anaemia of chronic disease. Numerous drugs used in the management of HIV – including anti-retroviral therapy (ART) (e.g. AZT) and prophylactic therapies (e.g. co-trimoxazole) both may cause and exacerbate cytopaenias in HIV. In addition, bone marrow suppression in HIV occurs due to the effect of cytokines as well as reduced erythropoietin production and function.[3-5]

The decision to transfuse a patient is a clinical decision that needs to be individualised, informed by the patient's clinical status, the laboratory findings where available, and available resources. This decision may incorporate the patient's socio-economic circumstances: a patient with borderline

decompensated anaemia, living in a remote area with poor access to follow-up care, might warrant transfusion; whilst the same patient might otherwise be managed conservatively in an urban setting with access to care.[6] **In general, indications for transfusion in HIV-positive patients are the same as for HIV-negative patients.** HIV-positive patients may, however, have compromised bone marrow function and require additional haematological support until such time that ART results in improved bone marrow function.

Bone marrow involvement

Bone marrow infiltration can result in anaemia through destruction of the haematopoietic environment. Infiltration is reflected by pancytopaenia and a reticulocyte production index (RPI) <1% and sometimes by a leuco-erythroblastic reaction where immature red and white blood cells are visible on the peripheral blood smear. Ideally, evidence of a leuco-erythroblastic reaction requires consideration of a bone marrow biopsy and specialist input. Common infiltrative processes in the setting of HIV include granulomatous infection (e.g. mycobacterial and fungal), lymphoproliferative disorders and fibrosis. Management will be determined by the underlying cause, with transfusion limited to patients who are symptomatic.

Dysplastic changes in the bone marrow are common, and may occur at any stage of HIV infection. Where available, bone marrow biopsy may be informative in investigating various diagnoses e.g. neoplasia (lymphoproliferative or myeloproliferative disorder) and opportunistic infections such as TB. Special stains and cultures for fungi, viruses and mycobacteria may help to resolve the cause of unexplained fever or source of infection.[7] Although the same indications for bone marrow biopsy apply independently of HIV status, certain indications (e.g. pancytopaenia, pyrexia of unknown origin (PUO), and lymphadenopathy) feature more prominently in patients with HIV. Similarly, certain diagnoses are more prevalent in the HIV population (e.g. immune thrombocytopaenia, disseminated mycobacterial infection, disseminated fungal infection).

A bone marrow biopsy is an invasive procedure; it may be painful and is not without risk. The latter includes infection, bleeding and haematoma. It should **not** be a first step in investigation and, regarding haematological disorders, should ideally be accompanied, where available, by additional testing i.e. bone marrow aspiration, cytogenetics and immunophenotyping (e.g. flow cytometry) to provide maximal diagnostic yield. Availability of this testing is often confined to tertiary academic and private referral laboratories. Bone marrow investigations may be helpful in the following situations:

- bone marrow failure (pancytopaenia and reticulocyte production index (RPI) <1%)
- cytopaenias unresponsive to treatment
- investigation of opportunistic infection
- pyrexia of unknown origin (PUO) after initial investigations fail to identify the cause
- exclusion/staging of malignancy
- diagnosis of specific pathology, such as pure red cell aplasia
- atypical and/or abnormal blood cells noted in the peripheral blood smear.

Specimen adequacy is important for identifying a focal process (e.g. granuloma, metastatic carcinoma or lymphoid infiltrate). Both the focal nature of the pathology and increased marrow fibrosis (a component of granuloma formation) increases the risk of a non-diagnostic specimen.

Anaemia

Anaemia is **not** a diagnosis, and management should focus on investigation and treatment of the underlying cause, independent of HIV status. Anaemia refers to a reduced red cell mass as reflected by a decreased haematocrit or haemoglobin level. It is a clinical sign that reflects an underlying disease process that requires appropriate investigation and management that is specific to the underlying process. Anaemia is common to a diverse array of pathologies with similarly broad therapeutic options. Consequently, generic treatment (e.g. blood transfusion or haematinics) without knowledge of the specific cause is considered bad practice. As an example, iron-deficiency anaemia can be due to dietary deficiency (a simple problem of insufficient intake). However, it can also be due to chronic blood loss (e.g. menorrhagia, helminth infestation, visceral malignancy). Simply treating with iron supplements without a root-cause analysis ignores the differential diagnosis and may delay time-sensitive treatment of the actual cause (e.g. colonic carcinoma).

Table 1. Anaemia and HIV-infection

Decreased production	Increased loss and/or destruction
Deficiencies	Haemolysis
Erythropoietin	Autoimmune haemolytic anaemia
Iron	Thrombotic thrombocytopaenic purpura (TTP)
Folate	Disseminated intravascular coagulation (DIC)
Vitamin B_{12}	Infections: Malaria
Drugs	Pre-existing conditions
Zidovudine	Glucose-6-phosphate dehydrogenase deficiency
Co-trimoxazole	Sickle cell disease
Anti-mycobacterial therapy	Thalassaemia
Amphotericin B	Gastrointestinal bleeding
Ganciclovir	Infections (CMV, Candida, parasites)
Dapsone	Kaposi's sarcoma
Chemotherapy	GIT lymphoma
Infections	Hypersplenism
HIV	Infection
Cytomegalovirus (CMV)	Haemophagocytosis
Epstein-Barr Virus (EBV)	Lymphoma
Parvovirus B19	Idiopathic
Mycobacterium tuberculosis (MTB)	
Mycobacterium avium complex (MAC)	
Histoplasma capsulatum	
Neoplasia	
Hodgkin's disease	
Non-Hodgkin's lymphoma	
Kaposi's sarcoma	
Miscellaneous	
Anaemia of chronic disease	
Pure red cell aplasia (PRCA)	
Hypoplastic/aplastic anaemia	
Haemophagocytic syndrome	
Secondary myelodysplastic syndrome	

Anaemia may occur at any stage of HIV infection, and 63 - 95% of infected persons will develop anaemia during the course of their illness; furthermore, the incidence of anaemia increases with disease progression.[2] The presence of anaemia is an independent, yet reversible, predictor of mortality.[6,8,9] Table 1 lists some of the causes of anaemia in HIV; it is by no means complete. The main aetiologies for HIV-related anaemia are dyserythropoiesis (anaemia of chronic disease), infections and drugs.[2-5] In addition, anaemia may be compounded by co-morbid haematinic deficiency (iron, folate and vitamin B_{12}), suggesting a need for early replacement in the management of anaemia in HIV. Zidovudine (AZT), especially when used as a single agent, is the drug historically most frequently implicated in HIV-related anaemia; a dose-dependent macrocytic anaemia is characteristic.[10] With lower doses, and in combination therapy, the haematological adverse events occur less frequently. Anaemia at baseline should not preclude the use of Zidovudine in patients initiating ART in resource-limited settings.[11]

The following revised haemoglobin (Hb) and haematocrit (Hct) levels are based on recommendations published by Lawrie *et al.*[12] in 2009, in which the normal ranges for Hb were found to be: 13.4 - 17.5 g/dl in males and 11.6 - 16.4 g/dl in females. Lower values may be accepted as normal in selected settings (e.g. evaluation during pregnancy or at sea level). There is significant variability in the definition of anaemia in relation to Hb level. Although anaemia may be strictly defined as a Hb level <13.4 g/dl for males and <11.6 g/dl in females, investigating all patients with Hb levels that approximate these values are both impractical and of questionable value in resource limited settings.

A baseline full blood count (FBC) with a differential count should be performed on all newly diagnosed HIV-positive patients. Clinicians are advised to monitor the patient's Hb level regularly in accordance with local guidelines. All patients with anaemia should be investigated for the underlying cause(s) and treated appropriately. While reduced red blood cell (RBC) production frequently underlies HIV-related anaemia, it is important to exclude other causes e.g. haemolysis or blood loss.

The reticulocyte count and corrected reticulocyte production index (RPI) will guide one with regard to the underlying aetiology (e.g. decreased production or increased destruction). This discrimination is important as management differs accordingly. The reticulocyte count is a useful index of bone marrow function, and should increase in response to any blood loss (e.g. haemorrhage or haemolysis). Failure to increase appropriately may reflect a primary production problem that can affect multiple cell lines (e.g. aplastic anaemia) or be specific to one cell line (e.g. Parvovirus B19-related pure red cell aplasia (PRCA)). The reticulocyte count is normally 0.6 - 1.83%,[13] but different textbooks and laboratories use different reference ranges. With anaemia, the corrected reticulocyte count (cc) is adjusted for the lower Hct. A reticulocyte production index of 1 - 2% indicates an appropriate marrow production; 3% or more indicates haemolysis; and levels <1% suggest reduced production.

Mean corpuscular volume (MCV) changes with age. From age 1 to 8 years, the lower limit of the MCV can be roughly calculated using the formula: age in years + 70 fl. In adults, MCV is typically 80 - 100 fl. An MCV less than this range suggests microcytosis, while greater levels suggest macrocytosis.

Fig. 1 provides a practical investigative approach to the causes of anaemia in the HIV positive patient.

Iron deficiency is the most common cause of microcytic anaemia. Iron deficiency is not a definitive diagnosis; rather, it reflects an underlying pathology that must be identified and treated appropriately and specifically. The mechanisms for iron deficiency include reduced intake (e.g. nutritional deficiency and inflammatory bowel disease) as well as increased loss (either acute haemorrhage or chronic, e.g. menorrhagia, helminthic infestation, gastrointestinal bleeding). Iron deficiency requires iron supplementation administered orally or parenterally. The latter may incur risk of, among others, anaphylaxis, and should only be reserved for severe cases of iron deficiency where oral supplementation is not tolerated (e.g. inflammatory bowel disorders, malabsorption). Patients should respond to iron replacement therapy within 2 months if the underlying cause has been addressed. Failure to respond within this period should prompt review of initial diagnosis with attention to patient adherence. Adherence is a recognised problem of iron supplementation, given gastrointestinal side effects. Excessive iron therapy can be deleterious and should not be given as a routine supplement in the absence of a clinical indication.[14]

In proven iron deficiency, one should – especially in children – consider administration of an anti-helminthic agent, in conjunction with iron therapy, in view of the high prevalence of co-morbid helminthic infestation in low-resource settings. Helminthic infestation (e.g. hookworm) is known both to cause and exacerbate underlying iron deficiency.

Transfusion should not be a first line of intervention in iron deficiency; oral iron is generally effective in managing stable patients. Transfusion is reserved for patients with decompensated anaemia (e.g. patients with signs of hypoxia, angina, cerebrovascular compromise, heart failure). Transfusion with red cell concentrate ('packed cells') can ameliorate symptoms in these patients. Each unit of packed red cells contains (replaces) approximately 250 mg of iron.

Macrocytic anaemia is often attributed to deficiencies in Vitamin B_{12} (cobalamin) and/or folate. In stable patients with proven vitamin B_{12} deficiency (pernicious anaemia), the patient should be treated with injectable vitamin B_{12} for at least 3 days before starting folate therapy. This is essential to avoid permanent neurological complications. In all other cases, start supplementation with folate and vitamin B_{12}. The response to vitamin B_{12} occurs within 48 to 72 hours. Serum potassium levels can fall during initial therapy for severe Vitamin B_{12} or folate deficiencies owing to increased utilisation of potassium by new haematopoietic cells; potassium levels should be monitored during therapy, with potassium supplementation where indicated.[15,16]

Blood transfusion should be avoided as patients with macrocytic anaemia generally tolerate extremely low haemoglobin levels very well. Transfusion can lead to circulatory overload especially in critically ill patients who require stringent maintenance of fluid volume status. In severely symptomatic anaemic patients, a maximum of 1 - 2 units of red cell concentrate may be administering slowly (over 4 hours per unit), followed by intravenous furosemide (20 - 40 mg), or be given in an isovolemic manner (i.e. removing 250 ml of the patient's whole blood, which has a low haematocrit, and replacing with 250 ml of packed RBCs, which has a high haematocrit).

Anaemia of chronic disease (ACD) is common in HIV; the mechanism is complex and is caused, in part, by the release of cytokines, resulting in iron blockade and dyserythropoiesis. In ACD, haemoglobin

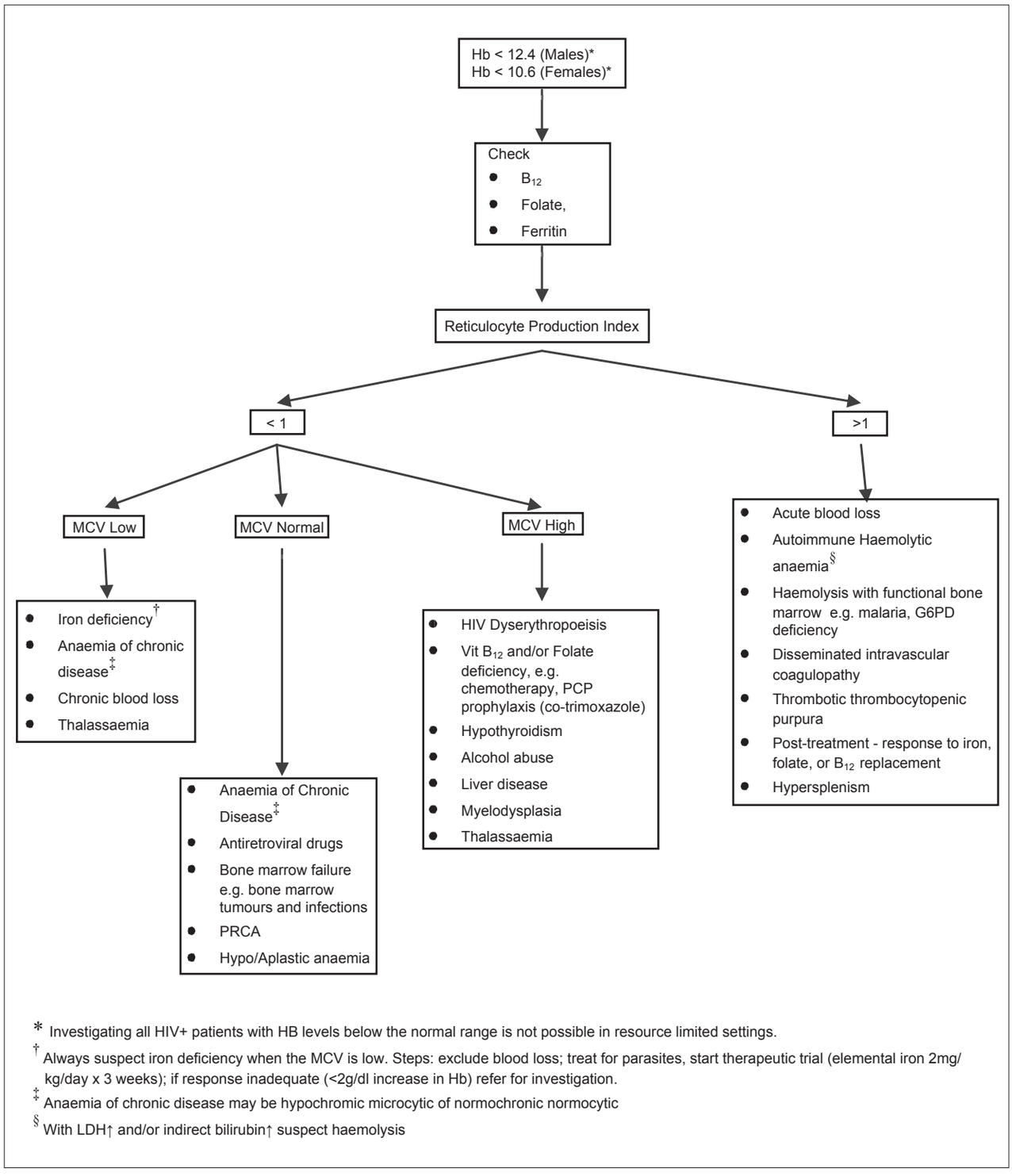

Fig.1. Diagnostic algorithm for anaemia in HIV infection.

levels seldom drop below 7 g/dl. Additional causes should be considered if haemoglobin levels fall below 7 g/dl. ACD does not respond to haematinics and requires treatment of the underlying condition (e.g. ART in the setting of HIV). Where treatment is not possible and the patient remains symptomatic, judicious use of erythropoietin can be considered.[17,18]

Autoimmune haemolytic anaemia is more common in HIV-infected patients than in HIV-negative patients. The direct anti-globulin test (DAT) (Coombs test) is positive in up to 20 - 40% of HIV-positive patients;[19] few patients, however, demonstrate signs of haemolysis.[20] The presence of a high reticulocyte count, unconjugated hyperbilirubinaemia, elevated LDH, spherocytes on the peripheral smear, and falling haemoglobin, suggests haemolysis and should prompt further investigation. Primary management of AIHA is similar to that of the HIV-negative patient (i.e. treatment of the underlying cause (e.g. ART), corticosteroids, IVIG, and immunosuppressive therapy).[21] These therapies generally require specialist

input and/or support. AIHA can complicate compatibility testing and the rapid access to blood for transfusion. Red cell auto-antibodies can mask clinically significant allo-antibodies that have developed owing to prior antigen exposure (e.g. pregnancy and/or previous blood transfusion). In non-urgent cases, a full serological investigation should be done to resolve the specificity of the auto-antibody as well as to exclude any co-existing allo-antibodies. Once an allo-antibody has been excluded, it is generally acceptable to administer red cell transfusions to patients whose DAT is positive and where the indirect anti-globulin test (IAT) phase of the cross-match is also positive; a haemolytic transfusion reaction in these patients is unlikely.[21] These patients do, however, warrant slow transfusion and careful monitoring.

Importantly, the reticulocyte count and peripheral smear must be requested before a transfusion, since the value of the count as well as the appearance of the peripheral blood morphology and the MCV may change after a transfusion and not truly reflect the patient's haematological status.

Table 2. Thrombocytopaenia and HIV infection

Decreased production	Increased loss/destruction/sequestration
Drugs	Immune thrombocytopaenia (secondary – HIV associated)
AZT	Thrombotic thrombocytopenic purpura (TTP)
Co-trimoxazole	Disseminated intravascular coagulation (DIC)
Fluconazole	Haemolytic uraemic syndrome (HUS)
Ganciclovir	Hypersplenism
Acyclovir	Infection
Rifabutin	Haemophagocytosis
Clarithromycin	Drugs
Didanosine	Interferon
Chemotherapy	Saquinavir
Deficiencies	Secondary anti-phospholipid syndrome
Vitamin B_{12}	
Folate	
Infections	
HIV	
CMV	
MTB	
MAC	
Histoplasma capsulatum	
Neoplasia	
Hodgkin's disease	
Non-Hodgkin's lymphoma	
Miscellaneous	
Hypoplastic/aplastic anaemia	
Haemophagocytic syndrome	
Secondary myelodysplastic syndrome	

Thrombocytopaenia

Thrombocytopaenia occurs commonly (often as one of the presenting symptoms), increases with disease progression, and is associated with shortened survival in HIV-positive patients.[22-25] The most common cause of primary HIV-associated thrombocytopaenia is immune-mediated destruction.[25] This is frequently attributed to high levels of auto-antibodies directed against platelet-associated antigens. HIV can also infect megakaryocytes directly, given megakaryocyte expression of CD4 and CXCR4 receptors which are known docking points for HIV.[25] Consequently, platelet production and lifespan are reduced in the HIV-positive patient. Additional causes of reduced platelet survival and decreased production are listed in Table 2.

As with anaemia, thrombocytopaenia is associated with a poor prognosis, while the treatment of the cause of the thrombocytopaenia confers improved survival.[26-28] Most patients with a platelet count >30 $x10^9$/l do not require treatment but do warrant investigation to elucidate the underlying cause.

Patients do not generally bleed spontaneously at platelet counts >10 - 20$x10^9$/l. Prophylactic platelet transfusion is seldom indicated. Some exceptions include: prematurity or neonatal thrombocytopaenia; intracranial pathology or risk of intra-cranial haemorrhage; functional platelet disorders independent of platelet count. Therefore, platelet counts <100$x10^9$/l may warrant investigation as to the underlying cause with appropriate management, but do not necessarily require platelet transfusions in the absence of bleeding. A unit of platelet concentrate containing >3$x10^{11}$ platelets usually results in an increment in the platelet count, in an adult, of ~ 30$x10^9$/l. In the absence of factors that shorten the lifespan of transfused platelets (such as fever, splenomegaly and/or anti-platelet antibodies), the lifespan of transfused platelets is ~3 - 4 days.

Immune mediated thrombocytopaenias

Immune thrombocytopaenia (ITP) can occur at any stage of HIV but most commonly in early disease. Auto-antibodies directed against platelet antigens are readily found in most people with HIV, but they are not necessarily clinically significant. Laboratory confirmation of ITP is complex; consequently, ITP is a diagnosis of exclusion. First line therapy is oral prednisone (1 mg/kg each day). Prednisone at this dosage does not appear to significantly affect viral replication; it may, however, promote Kaposi sarcoma growth.[29] If there is no response to steroids after one week, prednisone dosage can be increased to 2 mg/kg per day. Patients should not receive high-dose steroids for more than 2 weeks without referring the patient to a specialist unit. Patients on steroids require proton pump inhibitor (PPI) or H2-receptor antagonist prophylaxis for the prevention of peptic ulcer disease related to steroid therapy. If the platelet count drops below 30$x10^9$/l, IVIg therapy, with or without steroids, should be considered (exception: patients with haemophilia or patients on anticoagulation therapy, where the lowest accepted platelet count is usually 50$x10^9$/l). Response to therapy should be monitored, and ART needs to be initiated or continued. A high index of suspicion of concomitant TB should be maintained. Tranexamic acid and progesterone should be considered in female ITP patients presenting with a platelet count <50$x10^9$/l and genito-urinary bleeding – remembering, though, that tranexamic acid in patients with haematuria may result in blood clots and urinary colic. It is prudent to give patients prophylactic pneumococcal vaccination in anticipation of potential need for splenectomy. It is recommended that refractory patients be referred to a tertiary unit for further management.

Thrombotic thrombocytopaenic purpura (TTP) is a medical emergency with a high mortality rate. Despite being frequently encountered in HIV, the diagnosis is often missed. With timely and appropriate management, the prognosis is significantly improved. TTP should be considered in all patients presenting with signs of micro-angiopathic haemolytic anaemia, thrombocytopaenia, fever, renal and liver dysfunction, and fluctuating neurological signs. Where possible, patients should be referred urgently to a tertiary facility for further aggressive management. Therapeutic plasma exchange (TPE) is ideal as it allows large volume plasma transfusions. If TPE is not available, fresh frozen plasma (FFP) or cryo-poor plasma at ~30 ml/kg per day should be infused in divided doses. TTP associated with HIV has been shown to respond well to FFP infusion alone and is appropriate in resource-limited settings without TPE.[5,30] Prednisone therapy at 1 mg/kg per day is also recommended. Tranexamic acid should be avoided. Platelet transfusions are **not routinely** given to patients with TTP as they may potentiate thrombotic events and complicate monitoring therapeutic response.

Neutropaenia

The incidence of neutropaenia similarly increases with HIV disease progression.[31,32] The aetiology of neutropaenia is often multi-factorial and attributable to conditions considered under the pathogenesis discussed above.

Transfusion medicine best practice

- Transfusion is only one element of the patient's management.
- The decision to prescribe blood products should be based on individual patient needs, informed by best practice as well as the transfusing institution or national guidelines (e.g. Clinical Guidelines for the use of Blood Products in South Africa).[33] Blood should only be transfused when clinically indicated and where the benefits outweigh the recognised risks.
- Appropriate management of chronic anaemia through investigation and treatment of the underlying cause may help to reduce the need for blood transfusion.
- Blood loss should be minimised to reduce the need for transfusion.
- A patient with acute blood loss should receive effective resuscitation with intravenous replacement fluids, oxygen and immediate measures to stop further blood loss, while the need for transfusion is being assessed.
- An appropriately trained healthcare worker must monitor the transfused patient and respond immediately and appropriately if any adverse event occurs.
- To avoid wastage and unnecessary risk, the clinician should prescribe the **minimum** effective volume of blood and blood products necessary to stabilise the patient. Routine transfusion to predefined haemoglobin (Hb) levels should be avoided.

Blood should be transfused or discarded within 6 hours of breaking the seal on the blood bag to prevent risks associated with bacterial contamination.

Special considerations

Rate of transfusion. The rate of transfusion depends on the indication for the transfusion, patient's co-morbidities and prior response to transfusion, if known. For example, those with acute haemorrhagic shock require rapid rates of transfusion as part of urgent resuscitation management. In contrast, patients with longstanding chronic anaemia should not be transfused rapidly (rate should not exceed 2 ml per minute), given adaptation to their longstanding anaemia where rapid transfusion can precipitate cardio-respiratory failure. Particular caution needs to be taken for patients at age extremes (e.g. neonates, children, and age ≥60) and those with co-morbid disease (e.g. renal and cardio-pulmonary disease). These patient groups are at particular risk of volume overload, also referred to as transfusion associated circulatory overload (TACO). Precautions to minimise the risk of volume overload include spacing of transfusions where possible, small volume infusion, and/or low dose furosemide following transfusion (where not contraindicated).

Filters. Red blood cells, whole blood, FFP and cryoprecipitate must be administered through a standard blood administration set. These sets have 170 - 240 µm mesh filters to prevent the transfusion of clots or coagulation debris. The filter should be covered ('primed') with blood to ensure that the full filtering area is used. Platelets should be transfused with a platelet giving set (a standard filter may be used in an emergency). Standard filters incur significantly greater platelet loss owing to adhesion to the comparatively larger surface area, larger chamber and longer tubing. Administration sets should be changed:

- following reported transfusion reactions; this prevents further introduction of potentially harmful blood entering the patient. This is particularly important in the setting of suspected septic transfusion reactions where bacteria from the implicated unit can similarly contaminate the original administration set.
- between red cells and other blood products
- between red cell units of different ABO groups (e.g. group O and group A red cells administered consecutively)
- prior to infusing other fluids (e.g. Dextran, Ringers lactate)
- every 12 - 24 hours (or according to the package insert/instruction) in patients requiring on-going transfusion.

Temperature of the blood. Blood warmers are **not** indicated for routine blood transfusion; cold blood transfused at a slow rate is unlikely to adversely affect the patient. Selected indications for blood warming include:

- massive transfusion >50 ml/kg/hr
- infants transfused at >15 ml/kg/hr
- neonates receiving exchange transfusion or large volume transfusion
- patients with high titre cold haemagglutinins reactive *in vitro* at temperatures >30°C.

In these select settings, large volume or rapid infusion of cold blood can precipitate cardiac arrhythmias and impair haemostasis. Blood warmers should be designated only for transfusion use, and require a temperature-monitoring device that has been properly maintained in line with the manufacturer's instructions.

Improper or excessive warming can induce haemolysis of the red cells, with consequent renal failure and even death. **Blood must never be placed in a microwave or oven.** Rapid warming in a water bath is ineffective as only the outer cells are warmed and, at temperatures >37°C, may cause damage to these cells. Moreover, this practice risks bacterial contamination of the transfusion ports.

Patient identification. Near-miss or actual misdirected transfusions remains the greatest risk of a haemolytic reaction to any patient receiving a blood transfusion. It is the responsibility of each person involved in the transfusion of the patient to avoid identification errors. This begins with the prescribing doctor and extends to all staff involved in administering the transfusion. Patient identification should be repeated at every step of the transfusion process to ensure

that errors do not occur. Where possible, the patient should be positively identified in the presence of 2 staff members. The patient needs to state his or her name, which should be an identical match with that on the chart and with the name on the blood packs for transfusion. Where the patient is unable to state his or her name, confirm the identity by using at least 2 or, if possible, 3 identification points, e.g. full name, hospital number, date of birth and/or identification number. Scrupulous attention to detail can help to prevent serious harm. Clerical error (i.e. through patient or blood product misidentification) is the foremost reason for severe haemolytic transfusion reactions.

Paediatric patients. Anaemia in paediatric patients is very common in Africa.[34] In Ghana and Malawi, for example, more than 50% of children <5 years have a haemoglobin level <11 g/dl, and 20 - 47% of those admitted to hospital, reportedly receive blood transfusions.[35-37] Furthermore, 6 - 13% of patients admitted with severe anaemia (Hb<5 g/dl) die – many before transfusion is possible. Anaemia in southern Africa disproportionately affects the young, given pervasive malnutrition, and helminthic and infectious diseases (specifically HIV and malaria, both endemic to southern Africa). This has a major adverse effect on childhood morbidity and mortality, particularly where resource constraints and the relatively high cost of blood transfusion limit availability of safe blood.

Both anaemia and thrombocytopaenia are common among South African HIV-positive children. Of those referred with cytopaenias, ~35% are anaemic (Hb <11 g/dl), ~12.5% have thrombocytopaenia, ~35% have combined anaemia and thrombocytopaenia, and ~10% are pancytopenic.[38] The need for blood transfusion places a significant burden on transfusion inventories and hospital budgets.

Critically ill paediatric patients and neonates have special transfusion requirements. Neonates specifically, require small-volume transfusions that are relatively fresh (collected within the previous 14 days) and, when feasible, leucodepleted and/or CMV-negative. Neonates are particularly susceptible to volume overload, and measures should be adopted to counteract the risk e.g. small-volume and slow rates of transfusion. Given the risks of transfusion, both the decision to transfuse and the required volume should be carefully considered and individualised.[39]

In stable HIV-infected anaemic children, it is reasonable to consider a Hb <6 g/dl as the transfusion trigger.[40,41] A threshold Hb of 7 - 8 g/dl is recommended in haemodynamically stable, critically ill patients (e.g. trauma, intensive supportive care, or surgery).[42-44] Higher thresholds are required in premature infants and children who are actively bleeding.[45] The recommended volume of transfusion is 10 ml/kg for red cell concentrate and, in severe malnutrition or heart failure, 5 ml/kg. For each 1 g/dl rise in Hb desired, 3 - 5 ml/kg are needed. A paediatric unit of red cell concentrate contains 60 - 80 ml of red cells, compared with an adult unit of approximately 265 ml red blood cells. The calculated transfusion volume needed should be rounded off to the nearest volume of bag available, to not waste this scarce resource.

Appropriate clinical use of plasma products.[33] Plasma products produced by component processing laboratories at the blood service centres through physical separation techniques are referred to as plasma components. Products derived from large pools of plasma by chemico-physical processing techniques (fractionation) are referred to as plasma derivatives.

Fresh frozen plasma (FFP) refers to plasma that is separated from anticoagulated whole blood and frozen within 18 hours of donation. FFP contains all coagulation factors at normal physiological levels. The indications for transfusion of FFP are outlined in Table 3 and are identical to those for HIV-negative patients. FFP should be transfused judiciously. In addition to infectious risk, it is associated with transfusion-related acute lung injury (TRALI), which has significant morbidity and mortality. **Inappropriate** uses of FFP include volume expansion and nutritional supplementation.

Cryoprecipitate is the cold insoluble fraction of FFP and contains Factor VIII and von Willebrand Factor (100 IU per unit), fibrinogen (150 - 250 mg per unit), fibronectin, and Factor XIII. It is indicated primarily for the treatment of hypofibrinogenaemia (acquired or congenital) as found mainly in DIC states. Owing to the relative low levels of Factor VIII and von Willebrand Factor, it is not indicated in von Willebrand 's Disease or in Haemophillia A.

Freeze dried plasma (FDP) is produced from pooled fresh human plasma that has been subjected to a pathogen inactivation procedure that inactivates lipid-enveloped viruses. FDP has the same indications and dosage as FFP.

Intravenous immunoglobulin (IVIg) is prepared by a fractionation process which inactivates lipid-enveloped viruses. The two principal indications for IVIg are antibody replacement therapy and immunomodulation. In the setting of HIV, IVIg is primarily used in the management of ITP and PRCA.

Intramuscular hyperimmune immunoglobulin is produced by a method of fractionation similar to that of intravenous preparations. Most preparations have a high titre of antibodies for passive immune prophylaxis against selected infections (e.g. chicken pox, hepatitis B, rabies etc).

Oncology patients. Independent of HIV status, the oncology population is a major user of blood and blood products. Certain malignancies are AIDS-defining conditions (e.g. high-grade B-cell non-Hodgkin's lymphoma, Kaposi's sarcoma and carcinoma of the cervix). However, HIV-positive patients are susceptible to the full range of malignancies encountered in HIV-negative patients, such as breast cancer, colorectal cancer etc. With the advent of HAART, as HIV-positive patients live

Table 3. Clinical indications for FFP

Indications
Multiple coagulation factor deficiencies e.g. DIC Massive blood transfusion Liver disease
Active or ongoing bleeding with abnormal coagulation tests
Replacement of inherited single factor deficiencies, where single factor concentrate is not available
Thrombotic thrombocytopaenic purpura
Reversal of warfarin with active bleeding where prothrombin complex concentrate (PCC) is not available
Vitamin K deficiency associated with active bleeding
Scoline apnoea

longer, they will be at risk of the same spectrum of malignancies as seen in negative individuals.

General transfusion principles apply to the HIV-positive oncology patient. The decision to transfuse should be individualised to the patient's needs; this can be challenging in patients with uncertain outcomes. For example, transfusion in patients with poor expected short-term outcomes may be inappropriate if it is unlikely to change the outcome. However, transfusion has a role in palliative care where it can be used to maintain or improve quality of life in terminally ill patients. Adjunctive therapy with haematinics, erythropoietin and other alternatives to transfusion should be considered in patients who require active management of their anaemia.

Transfusion thresholds can differ to accommodate planned therapies. For example, radiotherapy requires a higher tissue oxygenation to be effective; therefore these patients require a higher haemoglobin level, and a target of 10 g/dl should be used. Similarly, maintenance of higher haemoglobin levels has been shown to improve radiotherapy outcomes in head and neck cancer.[46]

Surgical patients. Perioperative indications for blood transfusion are the same for both HIV-positive and -negative patients. However, HIV-positive patients are more likely to be anaemic and consequently more likely to require transfusion pre-operatively. All reasonable measures must be taken to treat and correct anaemia and its causes before surgery. Estimating transfusion needs and communicating with the hospital blood bank is especially important. Algorithms to help predict need for transfusion may be useful[47] but, in practice, if the patient presents at surgery with a haemoglobin level <7 - 8 g/dl, and the predicted blood loss is >500 ml, then perioperative transfusion should be anticipated and planned according to the patient's condition.[48]

A study of Jehovah's Witness patients undergoing surgery who refused transfusion noted that mortality in elective surgery depended on estimated blood loss rather than on preoperative haemoglobin levels. Furthermore, the study found that elective surgery could be performed safely in patients with a preoperative haemoglobin level as low as 6 g/dl if estimated blood loss was maintained below 500 ml.[49] Other studies in Jehovah's Witness patients have demonstrated a significant increase in mortality and morbidity in patients with a postoperative haemoglobin level <7 g/dl and 8 g/dl respectively.[50]

It has been shown that the risks of surgery and anaesthesia in HIV-positive patients is similar to that of surgery in immunocompromised or malnourished patients, with increased risk with disease progression.[51] As for any patient, the preoperative physiological status (including an assessment of nutritional reserve) is considered the best predictor of surgical morbidity and mortality.[52] HIV-associated thrombocytopaenia can potentially increase the risk of bleeding, but regional anaesthesia is not contraindicated.[52,53]

Haemophiliac patients. In the late 1970s and early 1980s, patients with haemophilia (PWH) were infected with HIV through the use of contaminated blood products, specifically clotting factor concentrates, FFP and cryoprecipitate. This tragedy has since lead to a complete revision of transfusion practice, policy and procedures. With robust donor selection, sensitive laboratory testing and good manufacturing practice for the production of fractionated factor concentrates, the current risk of transfusion-transmitted HIV is very low. However, development of thrombocytopaenia, lymphadenopathy or splenomegaly in PWH still warrants testing for HIV infection.

PWH who are found to be HIV-positive can develop thrombocytopaenia consequent to their HIV disease. Therefore, the development of purpura or increased mucosal bleeding in an HIV-positive PWH may be due to thrombocytopaenia rather than factor deficiency. Investigation and identification of the cause is important: factor administration alone may not be sufficient, and therapy may need to be directed toward the HIV thrombocytopaenia (as a primary cause). Management is similar to that of patients without haemophilia, and includes steroids, platelet transfusions and, in the HIV-positive patients, the initiation of HAART.

Obstetric patients. Regarding blood transfusion, the management of HIV-positive obstetric patients should not differ from that of HIV-negative obstetric patients. Transfusion should be used sparingly in obstetrics. Pregnancy may, however, be associated with sudden massive blood loss – obstetric haemorrhage remains the third most common cause of maternal mortality in South Africa.[54] Hospital maternity sections frequently rank among the highest in terms of demand for blood and blood products, along with trauma and ICU.[55]

There is a high prevalence of underlying anaemia in pregnancy owing to various causes. This anaemia must be anticipated, recognised and treated to lessen the risk associated with blood loss at delivery and to lessen the requirements for transfusion. Specific physiological changes in the haematological system occur in pregnancy, including haemodilution (there is an increase in plasma volume by 45 - 50% that reaches a maximum at about 34 weeks' gestation and exceeds the 18 - 30% increase in the red cell mass).[56] In the anaemic patient on oral haematinics, a static Hb may represent a response to the anaemia masked by haemodilution. Plasma volume increases more in multiple pregnancies.

The fetus must also be considered in terms of the potential effects of the anaemia. Folic acid deficiency (a cause of pregnancy-related anaemia) has been associated with neural tube defects. Periconceptual folic acid supplementation is recommended.[57]

Pregnancy is also associated with specific disorders that affect the haematological system, increasing the need for transfusion. These include HELLP syndrome (haemolysis, elevated liver enzymes and low platelets) associated with pre-eclampsia/eclampsia and pregnancy-associated thrombocytopaenia.

Although these guidelines contain recommendations for complex investigations and discuss current blood product use, it is also recognised that a large proportion of pregnant patients in South Africa and elsewhere in Africa are cared for at primary care centres where only basic haematological investigation may be available. In parts of southern Africa, the available blood supply does not meet the clinical demand, and clinicians face a chronic shortage of blood. In all circumstances, much can be achieved by the prompt use of standard obstetric protocols to minimise blood loss. Where possible, the more complex patient who requires specialised antenatal or intra-partum care should be transferred to better-resourced settings.

The prevalence and cause of anaemia in pregnancy varies throughout Africa. In Malawi and Zimbabwe (and in the Limpopo and northern KwaZulu-Natal provinces of South Africa), for example, malaria and/or gastrointestinal parasite infection may be a cause of anaemia in addition to underlying nutritional deficiencies. Should a patient in such areas become infected with HIV, these underlying causes of anaemia will persist and require treatment in accordance with the principles mentioned before.[58] Anaemia should be identified, appropriately investigated, and treated early in pregnancy.

Other haematological complications such as bleeding tendencies and cardiac failure warrant urgent admission or referral to a secondary/tertiary centre. Routine obstetric assessment includes the gestational age, which indicates the time available to treat a low Hb. Risk factors for obstetric haemorrhage may indicate referral to a secondary/tertiary centre for delivery, where transfusion, if required, will be available.

According to the South African National Prevention of Mother to Child Transmission of HIV (PMTCT) Clinical Guidelines (2010),[59] **all** HIV-positive pregnant women should receive iron and folate supplementation. In South Africa, folate should be available at the treatment dose of 5 mg and not the prophylactic dose. In countries where folate fortification of food takes place, folate deficiency is rare. The recommended daily dosage for iron is 60/65 mg elemental iron in the 2nd trimester and 120 mg elemental iron, in divided doses, in the 3rd trimester. If there is intolerance, replacement can be deferred since, in the 1st trimester, oral iron may cause increased nausea and vomiting. If calcium is given antenatally, **it must not be given at the same time** as the iron, as it blocks absorption.[60,61] **There is an exception:** Patients with a known haemolytic anaemia – e.g. thalassaemia or sickle cell anaemia (particularly from malaria-endemic areas) – should **not** be given iron routinely.

Anaemia must be recognised and managed promptly before the time of likely delivery. The time intervals for assessment of response are therefore shorter than in the non-pregnant patient. A review after only 3 months is not appropriate.

1. For treatment, dosage: 200 - 250 mg elemental iron daily in divided doses, i.e. one tablet of iron sulphate or iron fumarate 3 times daily. If vitamin C is available, it should be given with iron, also in divided doses.[61] Iron taken with food increases tolerance.
2. There is an increased absorption of iron in pregnancy. If the anaemia is a result of iron deficiency, an Hb increase of up to 0.7 g/dl per week may result. The lower the starting Hb, the more rapid the anticipated response.
   - 2.1 With an inadequate (<0.5 mg/dl per week) or absent response, a full blood count (FBC) and reticulocyte production index (RPI) should be performed. The RPI is frequently >2 in the early response to iron deficiency anaemia in pregnancy.
   - 2.2 The FBC may direct further investigation of the anaemia as mentioned above.
   - 2.3 In iron deficiency anaemia responding to iron **but with a delayed Hb response**, the red cell distribution width (RDW) may also increase, usually to >20%. The increase in RDW is not valuable if a recent transfusion or drugs have been given that cause macrocytosis e.g. AZT[62] causing an artificial increase in RDW.
   - 2.4 If the MCV is high (>110 fl), Vitamin B_{12} deficiency, though rare, should be considered, especially if there is oral ulceration or neurological symptoms.

Intravenous iron is only indicated, remote from term, if iron deficiency anaemia, proven on FBC and iron studies, is associated with intolerance of oral iron or there is no improvement of the anaemia despite apparent compliance.[63] Intravenous iron should only be given where there is no other reason for immediate transfusion.

According to the National PMTCT Guidelines (2010), the newly diagnosed HIV-positive pregnant patient who has a CD4 count >350 cells/mm^3 or WHO Staging 1 or 2 should, after 14 weeks' gestation, commence Zidovudine (AZT), if consenting. If CD4 count <350 cells/mm^3, or WHO Stage 3 or 4, full ART should be offered.[59] AZT is associated with macrocytosis,[62] but rarely associated with severe anaemia as a result of a pure red cell aplasia.[64] If there is adequate time remaining in the pregnancy, patients who are treatment-naïve, do not qualify for full ART, and have an Hb <10g/dl, should receive a full course of haematinics and their response observed. Their response should be re-assessed after 2 weeks and, if there is a response, AZT be commenced. If the patient's Hb is <8g/dl, the South African PMTCT Guidelines suggest withholding AZT.[59] If, however, there is a rapid response of the anaemia to iron and folate, AZT can be given. If the Hb is <8g/dl and there is no response to haematinics, further investigation is warranted. In certain patients, there may be a case for considering full ART. These include cases where delivery is approaching and where the Hb remains low or is falling. Patients with a falling Hb on AZT (or AZT-containing regimen) should be investigated for other causes of anaemia, usually nutritional or infective, but autoimmune haemolytic anaemia should also be considered. Haematinics should be commenced if not yet initiated and the patient examined and investigated further for additional underlying pathology. If a pure red cell aplasia is confirmed, AZT should be stopped. In such cases, patients usually respond within a week. Blood transfusion may be required if the patient is symptomatic or if the Hb falls below 6g/dl with no response to treatment.

Many patients tolerate very low levels of Hb (5 - 6 g/dl). If there are no medical or obstetric complications, and it is early in pregnancy (<34 weeks), oral replacement with haematinics should be used and the patient reassessed after 1 - 2 weeks. Where patients are in cardiac failure, have worsening anaemia with no response to haematinics, or where delivery is imminent, transfusion should be considered. Transfuse one unit of red cell concentrate and reassess. There is no need to transfuse if the Hb >7 g/dl and there are no obstetric complications. A higher Hb (>8 g/dl) should be targeted in patients at increased risk of obstetric haemorrhage, e.g. previous PPH, multiple pregnancy and placenta previa (where a Hb >9g/dl should be targeted).

Routine obstetric intervention may prevent transfusion. Appropriate observation is critical. Timely caesarean section for antepartum haemorrhage, controlled delivery of the placenta in vaginal delivery, and the recognition and treatment of uterine atony may prevent the need for transfusion. If a transfusion is not to be given to a patient in whom the peripartum loss has been considerable (>500 ml in normal delivery, >1000 ml in caesarean section), the cause of bleeding must be controlled and the condition of the patient considered. Close observation is essential; any further blood loss must be accurately recorded and action taken if necessary. Blood loss is frequently underestimated.[63] In patients with physiological compromise (both HIV-negative or -positive), transfusion should be considered earlier than in otherwise healthy counterparts.

If blood products are required and not available at the point of care, the patient should be transferred to a unit with access to a blood bank as soon as possible. The patient should be oxygenated, kept warm, with adequate intravenous (IV) support and with adequate measures to control further haemorrhage.

There is no need to give blood to reach a particular Hb level. Transfusion practice depends on the availability and proximity of blood products in the case of an emergency; the American and British Anaesthetic Task Forces in Obstetrics recommend neither

routine type and screen, nor crossmatch of patients who undergo a routine normal delivery or routine caesarean section if these services are readily available.[63,65] Despite these recommendations, patients at risk for greater than average blood loss (e.g. caesarean section for placenta previa) should have blood crossmatched and should, wherever possible, give birth in a place where further blood products are readily available.[54]

Malaria. The risk of severe malaria appears to be greater in HIV-positive (non-immune) patients than in HIV-negative patients.[66] In addition, HIV-positive patients are at a significantly higher risk of developing severe anaemia. Pregnant women co-infected with HIV and malaria are at greater risk of complicated disease than women with either malaria or HIV infection alone. Peripartum complications include severe anaemia,[67] thereby increasing the need for blood transfusion.

Severely anaemic patients may benefit from transfusion early in the course of acute malaria but, once stable and in process of recovery, the benefit of transfusion[68] is limited. However, persistent worsening anaemia is a recognised complication in the weeks following clearance of parasitemia.[69] Clinicians should therefore monitor HIV-positive patients for at least 3 months following malaria treatment. Paediatric patients are at greatest risk. One study of HIV-1 and *Plasmodium falciparum* co-infected children aged 3 - 36 months demonstrated significantly worse anaemia (Hb <6.0 g/dl) and a nearly 10 times greater mortality within 3 months post treatment, compared with an HIV-negative cohort.[70]

HIV-malaria co-infected patients are particularly prone to invasive bacterial infection (IBI); this underscores the need for good transfusion practice and vigilance against bacterial contamination.[71] Improper handling of blood and blood products further increases the risk of Gram-positive bacteraemia. Broad-spectrum antibiotics (such as a third generation cephalosporin) should be routinely administered to HIV-malaria co-infected patients with severe malaria, to provide cover against both Gram-positive and Gram-negative bacteria; this should be instituted from the time of admission.[72] Aggressive initial management of malaria is essential. Blood transfusion has a role in management, but should not delay initiation of anti-malarial therapy.

Haemoglobinopathies. There is no evidence to suggest that patients with haemoglobinopathies who are HIV-positive should be managed differently to those who are HIV-negative.

Massive transfusions. The principles of management of patients requiring massive blood transfusion are the same for HIV-positive and HIV-negative patients.

Leukodepleted blood. The routine use of leukodepleted blood in HIV-positive patients is not recommended. Even though HIV/AIDS patients are immunosuppressed, there is no substantive data supporting improved outcomes in patients who routinely receive leukodepleted blood components. Currently, the indications for the transfusion of leukodepleted blood products are the same for HIV-positive and HIV-negative patients. While the use of leukodepleted blood products may reduce the risk of transmission of leucocyte-associated pathogens such as CMV and HTLV, the Viral Activation Transfusion Study (VATS) demonstrated no clinical benefit for HIV-positive persons, who received white-blood-cell-reduced transfusions.[73-75] The indications for use of leukodepleted products include:

- prevention of alloimmunisation:
  - patients on chronic transfusion regimens, such as aplastic anaemia or sickle cell anaemia
  - organ and stem cell transplant patients
  - haem-oncology patients
- patients at risk for CMV infection such as:
  - transplant patients receiving immunosuppressant drugs
  - infants <1 year old
- prevention of febrile non-haemolytic transfusion reactions
- other
- patients undergoing cardiac surgery.

Note: Where indicated and if available, prestorage leukodepleted products obtained from blood services are preferable to bedside leukodepletion; prestorage leukodepletion removes leucocytes prior to release of cytokines, which are responsible for adverse effects such as febrile non-haemolytic reactions.

Random-donor platelet concentrates are prepared from buffy coats and are not usually leukodepleted. Single donor (apheresis) platelet concentrates **are** routinely leukodepleted; the indications for this product are similar to those mentioned above for leukodepleted products.

Irradiated blood products. HIV-positive patients do **not** routinely require blood products to be irradiated. Blood products are irradiated to prevent transfusion-associated graft v. host disease (TA-GvHD). It has been postulated that, in patients with HIV infection, depletion of CD4 cells increases the number of donor cells needed to induce TA-GvHD. In HIV and AIDS, there has to date only been one reported case of TA-GvHD despite widespread use of blood transfusions in patients with profound HIV-associated immune suppression. The indications for irradiated blood products are the same for HIV-positive as for HIV-negative patients. Specific indications for irradiation include:

- blood donations from blood relatives
- HLA-matched platelet concentrates
- recipients of allogeneic bone marrow transplant
- Hodgkin's disease
- intrauterine transfusions
- patients (haematological/non-haematological disorders) receiving Fludarabine therapy.

Note: Please refer to the Clinical Guidelines for the use of Blood Products in South Africa or your national guidelines for additional information.

Blood conservation strategies

Blood conservation (restrictive transfusion practice) is clinically effective in most patient subsets. In particular, the TRICC study76 showed that restrictive transfusion practice applied to critically ill adult patients was at least as effective and potentially superior to that of liberal transfusion practice in terms of lower morbidity and mortality.[76] This was also shown in a major randomised control trial (PICU study) of stable, critically ill children.[43]

Low-cost and relatively simple preventative measures can be employed to minimise blood use. One example is that of judicious screening for anaemia with early intervention. All cases of clinically significant anaemia should be investigated, and the underlying cause addressed and appropriately managed. Early intervention is particularly important for surgical candidates where timely management of anaemia can help to minimise periopertaive transfusion.

Clinicians should employ alternatives to blood transfusion wherever appropriate e.g. haematinic therapy in chronic anaemia or the use of crystalloids/colloids to restore blood volume in resuscitation. Where indicated and where available, erythropoietin is another measure to be considered in patients refractory to standard therapy.[6,9,77] Erythropoietin has been shown to benefit HIV-positive patients. In the critical care setting, excessive phlebotomy

can exacerbate underlying anaemia; this can be avoided through considered testing, confined to that which directly influences patient management.

Good surgical and anaesthetic techniques, with particular attention to haemostasis and keeping patients warm, are essential transfusion conservation principles. Suitable alternatives or adjuncts to transfusion should be considered, e.g. anti-fibrinolytics and fibrin sealants. Bleeding, when it occurs, should be managed aggressively, avoiding a passive watch-and-wait approach. The use of medication that can impede haemostasis (e.g. anticoagulants, anti-platelet agents and non-steroidal anti-inflammatories) should be prescribed cautiously in the chronic patient at risk for bleeding, and stopped in the bleeding patient. The latter may require specific reversal of anticoagulation if bleeding does not stop with conservative management.

Pre-surgical autologous blood donation obtained from HIV-positive donors is not routinely available and should not be accepted. HIV-infected blood products pose significant risk, both to blood service staff as well as to other patients. Risk to other patients can occur through administrative or clerical error resulting in mis-transfusion (unintentional transfusion to the incorrect recipient).

Finally: acute normovolemic haemodilution and cell salvage are two intraoperative blood conservation techniques that can be used in HIV-positive surgical patients. These techniques should be considered particularly where significant blood loss is anticipated.

Lookback programmes

The Blood Service's Transfusion Transmissible Infection (TTI) Lookback Programme aims to trace all patients who are identified as recipients of blood from donors who test positive for a transfusion-transmissible infection on a subsequent blood donation, where the initial (index) donation might possibly have been donated in a window period. In such a 'donor-triggered' lookback investigation, the recipient/s of the previous TTI negative units is/are identified and their treating doctor notified. As far as possible, the patient must be recalled, counselled and tested for the relevant viral marker, and the result reported to the blood service. Despite diligent donor selection and laboratory screening, suspicion may arise that a patient might have been infected with HIV, HBV or HCV through blood transfusion. In such instances, the attending clinician should contact the blood service promptly to initiate a 'recipient-triggered' lookback investigation, a formal procedure designed to trace and confirm the status of the implicated blood donor(s).

Haemovigilance programme

Haemovigilance is the process through which information related to the transfusion of blood and blood products is monitored and centrally reported. It is a system to detect, gather and analyse information on untoward and unexpected effects of the transfusion of blood and blood products. Such programmes aim to improve blood systems and blood safety through the early detection and comprehensive reporting of untoward effects of blood transfusion e.g. transfusion reactions, transfusion-transmitted infections, etc. Ideally, a haemovigilance programme is integrated into blood transfusion practice to maximise the safety of not only the blood supply, but also all aspects of laboratory and clinical blood transfusion practice. In some countries such as Namibia and South Africa, data reported to the National Haemovigilance Programme are analysed and the results published in an Annual Haemovigilance Report. It is important that medical practitioners who transfuse blood and blood products report all adverse transfusion events to the blood service.

Laboratory testing of donated blood

Serological tests are performed on every blood donation to determine the donor's ABO group and Rh type and to detect irregular blood group antibodies. Every blood donation is tested for HIV, hepatitis B, hepatitis C and syphilis, using serological techniques. Nucleic acid testing (NAT) is used in combination with serological testing in a few well-resourced African countries. Clinicians should consider the following options when ordering blood for their patients:

- **Type and screen.** The clinician should select this option if their patient has a low probability of needing a transfusion or in, for example, certain elective surgical procedures where the extent of blood loss is unpredictable. The blood specimen submitted to the blood bank will be tested to determine the patient's ABO group and Rh type and will be tested to ensure that the patient does not have irregular blood group antibodies (a 'rare blood type') that could delay finding compatible blood. The specimen will be held for approximately 96 hours, depending on blood bank policy. Blood will only be crossmatched when requested by the attending doctor. The 'type and screen' expedites cross-matching and dispatch of blood from the blood bank should transfusion be required. If irregular antibodies are detected, the requesting doctor will be notified. The presence of irregular antibodies can delay procurement of compatible blood, and patient management needs to be changed accordingly. This could, for instance, necessitate delaying surgery.
- **A full crossmatch** refers to full compatibility testing between a patient's blood sample (intended recipient) and a given donor (unit of blood). This includes the type and screen as described above as well as confirmatory blood grouping on the intended donor unit. In addition, the patient's serum is 'crossmatched' with the red blood cells of the donor to ensure serological compatibility. Incompatibility between patient and donor is reflected by *in vitro* agglutination.
- In contrast, an **emergency crossmatch** refers to partial compatibility testing, given the urgent need to transfuse. Blood is issued after performing the ABO group, Rh type and antibody screen only. Further testing is completed after the unit has been issued.

Providing sufficient clinical detail, including the HIV status of the recipient, to the hospital blood bank staff, will expedite the crossmatching process and the timely availability of compatible blood. The direct antiglobulin test (DAT) is, for example, positive in up to 40% of HIV-positive patients. This will manifest as a positive crossmatch. Knowing the patient's HIV status will therefore assist the blood bank's medical and technical staff in interpreting the compatibility test results and, as indicated, expedite the release of compatible blood.

Adverse events associated with blood transfusion[33]

Evaluation of benefits and risks of transfusions should precede the decision to transfuse. All blood products carry risk of adverse effects. These include transfusion reactions, transfusion transmissible infections, alloimmunisation and immune modulation. The attending doctor must be familiar with best practice recommendations regarding transfusion practice, and is also responsible for obtaining and documenting informed consent.

Transfusion reactions are the most common hazard of blood transfusion, occurring with

Table 4. Products, services and glossary

RED CELL PRODUCTS: STORE AT 1°C - 6°C.
Definitions, products and services described below refer to those available in South Africa and may not be available in other countries and regions.

Product	Average vol.	Average unit price incl. VAT (2012)	Characteristics	Major indications
Whole blood leucodepleted (<5 days old)	485 ml	R2 499.00	WBC: <5x10^6/unit Leucocyte depleted at the time of processing.	Indicated for neonatal exchange transfusion.
Red cell concentrate in additive solution	300 ml	R1 369.00	Buffy coat removed WBC: <2.4x10^9/unit	To increase tissue oxygenation owing to reduced haemoglobin concentration.
Red cell concentrate (leucodepleted)	260 ml	R2 237.00	WBC: <5x10^6/unit Leucocyte depleted at the time of processing.	See indications for leucodepleted products.
Red cell concentrate in additive solution (<5 days old)	300 ml	R 1 484.00	Buffy coat removed WBC: <2.4x10^9/unit	
Red cell concentrate (leucodepleted) (<5 days old)	260 ml	R2 237.00	WBC: <5x10^6/unit Leucocyte depleted at the time of processing.	Suitable for neonatal exchange transfusion. See indications for leucodepleted products.
Red cell concentrate paediatric leucodepleted	75 ml	R1 265.00	WBC: <5x10^6/unit Leucocyte depleted at the time of processing.	For paediatric use.

PLATELET PRODUCTS: USE IMMEDIATELY AFTER ISSUE; DO NOT REFRIGERATE.

Product	Average vol.	Average unit price incl. VAT (2012)	Characteristics	Major indications
Platelet concentrate pooled non-leucodepleted	250 ml	R5 769.00	Platelets: ≥2.4x10^{11}/unit WBC: <5x108 /unit Prepared from buffy coat of 5 whole blood donations. Not leucodepleted.	Clinically significant thrombocytopaenia or platelet function abnormalities.
Platelet concentrate leucodepleted (apheresis)	200 ml	R7 936.00	Platelets: ≥2.4x10^{11}/unit WBC: <5x10^6/unit Prepared from a single donor by apheresis. If unavailable, leucodepleted pooled platelets will be supplied.	See indications for leucodepleted products.
Platelet concentrate paediatric leucodepleted		R1 741.18	Platelets: ≥ 5.5x10^{10}/unit WBC: <5x10^6 /unit Prepared from a single donor by apheresis.	For paediatric use.

PLASMA PRODUCTS – MUST BE TRANSFUSED IMMEDIATELY AFTER ISSUE.

Product	Average vol.	Average unit price	Characteristics	Major Indications
Cryoprecipitate	30 ml	R774.00	Fibrinogen content – >300 mg/unit	1. Hypofibrinogenaemia 2. Factor XIII deficiency
Fresh frozen plasma – paediatric	130 ml	R637.00	Contains physiological levels of most clotting factors. NB: Freeze-dried plasma is used as an alternative.	
FFP – cryo-poor	250 ml	R883.79	FFP from which the cryoprecipitate has been removed. Limited availability.	May be indicated for TTP.

Table 4. Products, services and glossary (continued 2)

SPECIAL REQUESTS: CONTACT THE BLOOD BANK – ADVANCE NOTICE IS REQUIRED.

Service/procedure	Average unit cost	Characteristics	Major indications
Irradiated products	R293.00	For the prevention of transfusion-associated graft-versus-host disease.	1. Intrauterine transfusion 2. Bone marrow transplant recipients 3. Directed donations from blood relatives
HLA-matched platelet concentrate	R1 073.00	HLA-matched single-donor apheresis platelet concentrate.	Prevention and management of platelet refractoriness.
Autologous programmes	R150.00	The collection, normal testing and processing of a patient's own blood for him- or herself.	For use in certain limited elective surgical cases in suitable patients
Directed programmes	R181.00	Programme where family members or friends donate for a specific patient. Chosen donors have to meet the same criteria as normal donors and must have a compatible blood group. Blood donated by first-line blood relatives requires irradiation to prevent transfusion associated graft v. host disease.	For use in certain limited cases. The blood must be tested and processed as usual, requiring 3 - 5 days before the unit is available for transfusion.
Washed products	R1 051.00	The product is suspended in isotonic saline and centrifuged; the saline from the first saline 'wash' is removed, and the red cells re-suspended in isotonic saline.	As washed cells are manipulated in an open system, with a possibility of bacterial contamination, they must be transfused within 24 hours of preparation.
Cryo-preserved cells	~R7 400.00	The storage of frozen rare donations for use locally and internationally.	As washed cells are manipulated in an open system, with a possibility of bacterial contamination, they must be transfused within 24 hours of preparation.
Leucocyte depleted (leucodepleted) products	Included in cost of product	Filtered under laboratory conditions. This ensures optimal removal of leucocytes to minimise cytokine release. Leucocyte depletion will result in a leucocyte count $<5x10^6$ per unit and usually $<1x10^6$ per unit.	1. Prevention of transfusion transmitted CMV. 2. Potential haemopoietic transplant recipients. 3. Intrauterine transfusions and children <1 year old. 4. Prevention of febrile non-haemolytic transfusion reactions.

TYPES OF CROSSMATCH

Test	Time-frame	Average cost	Comments
Type and screen	N/A	R272.00	The specimen will be grouped and tested to ensure that it does not contain antibodies that could delay finding compatible blood. The specimen will be held for 96 hours. Blood will only be crossmatched when requested by the attending doctor.
Standard crossmatch	Within 2 hours	R609.00	Crossmatched products will be held in reserve for 24 hours unless otherwise indicated by the attending doctor. Crossmatched products not collected, will incur the fee.

Table 4. Products, services and glossary (continued 3)

Emergency crossmatch	20 - 30 minutes	R114.00	Blood issued on emergency or without a compatibility test is transfused at the attending doctor's own responsibility. There are risks involved in emergency procedures – **use them only for emergencies.**
Uncrossmatched blood	5 - 10 minutes	R136.00	Blood issued on emergency or without a compatibility test is transfused at the attending doctor's own responsibility. There are risks involved in emergency procedures – **use them only for emergencies.**
GENERAL			
Blood on returnable basis (BRB)	Blood is transported in a temperature-controlled hamper. Provided the blood is returned within 10 hours of issue, remains sealed in the hamper, and the temperature of the hamper does not exceed 10°C, the fee for the blood will fall away. However, the service and laboratory test charge will be levied.		
Informed consent	As with any treatment, the patient has the right to decide whether or not to accept the treatment. As far as possible, the patient should understand the benefits, risks and alternatives to transfusion as explained by the prescribing doctor. It is recommended that transfusion transmissible infections and receiving of incorrect products be mentioned specifically. Informed consent is a process which must be acknowledged and documented.		
Blood administration set	For the infusion of whole blood and red cell concentrate.		
Platelet administration set	For the infusion of platelets.		
Blood pack without anticoagulant	For therapeutic venesections.		
Blood pack with anticoagulant	For blood salvage and subsequent autologous reinfusion.		
Voluntary donor	A person who donated blood or a blood components without compensation.		
Recipient	A person who receives blood or blood products.		
Look-back programme	A formal process for the identification of donors and recipients who test positive for viral markers after having donated or receiving, respectively, a unit which at the time of issue tested negative. In cases where the recipient tests positive, the donor/s of the original unit/s are traced and tested to confirm whether the infection was transmitted via the transfusion. In cases where the donor tests positive on a subsequent unit, the recipients of the previous unit are traced and tested to confirm whether or not the infection was transmitted through transfusion of blood or blood products		
Haemovigilance	A system to detect, gather and analyse information on untoward and unexpected effects of the transfusion of blood and blood products		

variable frequency depending on the type of reaction. Transfusion reactions fall broadly into the following categories:

- haemolytic – acute
- haemolytic – delayed (DHTR)
- febrile non-haemolytic
- allergic
- anaphylactic
- reactions due to bacterial contamination
- reactions due to 'citrate toxicity'
- reactions due to circulatory overload (TACO)
- transfusion-associated acute lung injury (TRALI)
- transfusion-associated graft versus host disease (TA-GvHD).

Signs and symptoms suggestive of a transfusion reaction include:

- chills/rigors
- fever/sweating
- tachycardia/bradycardia
- dyspnoea/bronchospasm
- hypertension/hypotension
- urticaria/pruritus
- chest/flank pain
- nausea/vomiting
- haemoglobinuria
- oliguria/anuria
- restlessness
- jaundice.

If a transfusion reaction is suspected, the transfusion **must be stopped immediately** pending further evaluation. The administration set must be changed and venous access should be maintained with normal saline unless it is a simple urticarial reaction. If the latter is the case, the transfusion can continue with symptoms or after use of an antihistamine (such as diphenhydramine 12.5 - 25mg for an adult patient).

The following additional steps should be taken:

- Both a member of the medical staff as well as the blood bank must be contacted immediately.
- The medical management of the transfusion reaction will depend on the type and severity of the reaction.
- The patient's temperature, pulse, respirations and blood pressure must be recorded.
- All clerical and identity checks must be repeated to ensure that the correct blood product was transfused to the intended patient. Clerical error is the foremost reason for major acute haemolytic transfusion reaction, i.e. blood given to the wrong patient.
- If a case of misdirected transfusion is noted, immediate steps must be taken to locate the units originally intended for the patient, as they may be in the process of being transfused to another incorrect patient.
- Send a fresh blood specimen for compatibility testing to the blood bank.
- All empty and non-transfused blood units should be returned to the blood bank.

Popular misconceptions

An HIV-infected patient doesn't warrant transfusion as the prognosis is poor anyway

Anecdotal reports of patients dying following transfusion led to the unjustifiable practice of withholding blood transfusion from HIV-positive patients. At the start of the HIV pandemic, patients presented with advanced disease and generally had little prospect of effective management. Today, with highly effective ART and prophylaxis, HIV is a chronic manageable disease with an excellent outcome. Transfusion best practice and a rational approach to the management of anaemia apply, independent of HIV status.

Moribund HIV+ patients require rapid correction of their anaemia

Again, best transfusion practice applies to the HIV-positive patient. Rapid correction of chronic anaemia increases the risk of transfusion-associated circulatory overload (TACO) and cardiac decompensation as would be encountered in any patient with severe chronic anaemia (see section on transfusion rates). HIV-positive patients with acute haemorrhage may require resuscitation and rapid transfusion, similarly to HIV-negative patients.

If a patient needs only one unit of blood, he/she does not need blood at all

The indication for transfusion is based on clinical symptoms and signs, and not on laboratory indices. Patients should be evaluated after each unit transfused. A single unit transfusion, in the right circumstances, may be sufficient to stabilise the patient. 'Topping up' the patient with additional blood after the indication for transfusion has been addressed, may confer additional unnecessary risk.

Disclaimer

Specific recommendations provided in this document are intended only as a guide to clinical therapy, based on expert consensus and best current evidence. Treatment decisions for patients should be made by their responsible clinicians, with due consideration for individual circumstances. The most current peer-reviewed literature, reference text books and local guidelines should always be consulted.

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Appendix 1: Legal and human rights considerations

The use of blood and blood products raises two broad categories of legal and human rights considerations: the rights of donors and the rights of recipients. Much of the debate regarding blood, blood products and HIV has historically focused on the rights of people to be protected from infection; very little focus has been placed on the rights of people living with HIV insofar as access to blood and blood products is concerned. This appendix expands on both categories of rights bearers – including the legal requirements of informed consent – as well as the issue of confidentiality as it relates to the communication of information on a patient's HIV status.

Informed consent

South African law has recognised the concept of informed consent for many years.[1,2] The right to informed consent has been fleshed out by way of case law,[3] regulatory council guidelines,[4] the Patients' Rights Charter, and legislation.[5] In particular, the National Health Act 61 of 2003 (NHA) has codified the law on informed consent and defines it as consent for the provision of a specific health service; the Act further stipulates that informed consent may only be provided by a person with legal capacity to do so, and that the person providing consent has been adequately informed.[6]

The law on informed consent is based on the notion that a patient – referred to in the NHA as a user – has the right to participate in any decision affecting his or her personal health and treatment.[7] If, however, the circumstances prevent the user from making the decision himself or herself, the NHA provides guidance on how to proceed.[8]

Confidentiality

The communication of any information pertaining to a patient's HIV status, whether positive or negative, is subject to the confidentiality provisions in the NHA.[9] Section 14 of that statute makes it clear that '[a]ll information concerning a user, including information relating to his or her health status … is confidential.' This guarantee is subject to the provisions of Section 15, which deals with access to health records. Simply put, Section 15(1) ensures that the guarantee of confidentiality should not stand in the way of running an efficient and effective blood service, including the appropriate handling of blood and blood products.

The rights of donors

In considering the rights of donors, two key questions arise: (*i*) Is there a right to donate blood?; and (*ii*) can blood services be used as a testing facility to determine an individual's HIV status?

Is there a right to donate blood?

There is no right to donate blood. On the contrary, blood services are constitutionally obliged to take all reasonable steps to ensure a safe supply of blood and blood products. By necessity, this implies refusal to accept as donors those at high risk of carrying a transfusion-transmissible pathogen (such as HIV). However, the manner in which donors are treated may not result in the violation of fundamental constitutional rights and values. The need to protect public health cannot be done in a manner that unreasonably and unjustifiably limits rights. If it did, potential donors would have legal recourse to vindicate their rights.

Can blood services be used as a testing site to test for HIV?

While potential donors should be discouraged from using blood donation services for the purpose of establishing their HIV status, there is nothing in the law that can be used to prevent this from happening. Both donor education and evidence-based self-exclusion questionnaires serve as appropriate means to discourage and defer those at risk of HIV infection from donating blood. Provision of alternative HIV counselling and testing services also help to prevent the blood collection centre from being used as a default testing site. This relies on the premise that alternative testing services, of high quality, rendered in a non-discriminatory way, are available outside of HIV-specific service points and are either free or affordable at point of delivery.

The rights of recipients

In considering the rights of recipients, two key issues arise: (*i*) the right to have access to safe blood and blood products; and (*ii*) the rights of terminally ill patients.

Right to have access to blood and blood products.

The right to have access to health services, which includes access to blood and blood products, is guaranteed in Section 27 of the Constitution. The central issue is whether access to blood and blood products can be denied solely on the basis of HIV status. This raises concerns of health rights as outlined in Section 27; it also breeches the constitutional guarantee of equality and protection against unfair discrimination. In this regard, two Constitutional Court decisions are relevant.[10,11,] Read together, the cases are clear: given that HIV infection is a chronic manageable condition for those with access to appropriate treatment and care, it would not be reasonable to limit access on the basis of HIV status alone.[12]

The right of access to safe and adequate blood and blood products obligates blood services to take all reasonable measures to ensure that such products are indeed available and safe for use; such measures do not require a blood service that is 100% safe, as the technology to ensure this does not exist. Where people have been exposed to unsafe blood and/or blood products despite blood services having acted reasonably, the latter cannot be held liable for any resultant harm. On the other hand, donors who have acted unreasonably – such as by misrepresenting their actual risk of infection in self-exclusion questionnaires – may indeed be sued for damages.[13] In addition to civil liability, such a donor may also be criminally liable.

Rights of terminally ill patients.

The right to have access to health care services does not impose an obligation on the state – and those who provide public services – to ensure that everyone receives every health service they need. Instead, it is an obligation to 'take reasonable legislative and other measures, within … available resources, to achieve the progressive realisation of [the right]'.[14] This was made clear in *Soobramoney v Minister of Health, KwaZulu-Natal*.[15] In its judgment on Mr Soobramoney's appeal, the Constitutional Court adjudicated the claim on the basis of the State's positive obligations under Section 26(2), holding that the guidelines according to which access was limited were reasonable and had been applied 'fairly and rationally'.[16] In the result, the State had complied with its Section 27(2) obligations.

The primary responsibilities of the physician are to assist the patient in maintaining an optimal quality of life by controlling symptoms and addressing psychosocial needs, as well as enabling the patient to die with dignity and in comfort.[17] It is considered ethically justifiable to discontinue life-sustaining treatment if the patient has the ability to make that decision, fully understands its consequences, and states that they no longer wish to continue treatment. A decision to withhold or withdraw life-prolonging treatment should be only be made by the senior clinician in charge of a patient's care, informed by the patient's views or those closest to him or her.[18]

There may be circumstances when withholding treatment, even if it is not requested by the patient, may be permissible. This may apply, for example, in cases akin to that of *Soobramoney*. Ordinarily, it would not be justifiable to discontinue life-sustaining treatment for cost reasons alone. That said, there may be cases in which the costs expended on one terminally ill patient could be better used on another patient with an improved outlook.[19] In such circumstances, a health care facility may have the right to limit access to life-sustaining interventions, provided that such a limitation is based on reasonable national admission criteria developed by expert professional bodies, such as the HPCSA.[20]

APPENDIX 1 CITATIONS

1. 1923 CPD 128.
2. Anneke Meerkotter, 'The Rights and Duties of Users of the Health Care System', in Adila Hassim et al, Health & Democracy: A Guide to Human Rights, Health Law and Policy in Post-Apartheid South Africa (Cape Town: Siber Ink, 2007:249.
3. See, for example, Castell v de Greef 1994 (4) SA 320 (C); C v Minister of Correctional Services 1996 (4) SA 292 (T).
4. Such as the Health Professions Council Guideline for Good Practice in Medicine, Dentistry and Medical Sciences
5. In particular, consider the Children's Act 38 of 2005 and the National Health Act 61 of 2003.
6. Above note 2 at page 252.
7. This is codified in section 8 of the NHA.
8. See generally sections 7 to 9 of the NHA (in Chapter 2 entitled Rights and Duties of Users and Health Care Personnel). See also sections 129 and 130 of the Children's Act.
9. As is the case with the provisions of the NHA dealing with informed consent, the NHA also codifies the common law – decisions of the courts – on confidentiality. This is underpinned by the constitutional guarantee of privacy.
10. 2001 (1) SA 1 CC.
11. 2004 (6) SA 505 (CC).
12. This position is supported by the ISBT Code of Ethics for Blood Donation and Transfusion which states that 'blood is a public resource and access should not be restricted' (see above note 10 at paragraph 10).
13. In Canadian Blood Services/Société Canadienne du Sang v Freeman [2010] ONSC 4885), a gay man who knowingly provided false information on a self-exclusion questionnaire was successfully sued by the blood services for negligent misrepresentation. His blood sample, which was tested and resulted in a false negative for syphilis, subsequently tainted an entire unit of blood. It is likely that the case, if decided under our Constitution, would have been decided differently. However, the principle of negligent (material) misrepresentation would stand.
14. Section 27(2) of the Constitution.
15. 1998 (1) SA 765 (CC).
16. At paragraph 25.
17. At principle 5.
18. At section 3.
19. See section 9.1.
20. At section 9.2. See also paragraph 3 of the preamble to the World Medical Association (WMA) Declaration on Terminal Illness, first adopted by the 35th World Assembly (October 1983) and revised by the WMA General Assembly in October 2006. That preamble recognises that many palliative and life-sustaining measures require technologies and/or financial resources that are simply not available globally. The declaration is available at http://www.wma.net/en/30publications/10policies/i2/.