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- Timing of antenatal care and ART initiation in nurse-driven services
- Genital tract HIV shedding
- CSF findings in patients presenting with meningitis
- Antiretroviral therapy-induced optic neuropathy
- New consent norms may undermine health research with children

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MESSAGE
From the Editor

Most editions of *SAJHIVMED* are posted to you with a range of different inserts – materials sent in hard copy in addition to the Journal itself – related to different aspects of HIV prevention and treatment. Several times a year, this includes HIV Treatment Bulletin (HTB) South, an invaluable guide to recent developments in HIV medicine and antiretroviral therapy (ART). Other materials have included guides on specific aspects of HIV medicine, such as those assisting in the management of HIV-TB co-infection. With this edition of the Journal, we are sending a particularly important insert: the National Department of Health's new *Contraception & Fertility Planning Guidelines*. Experience over the last decade has demonstrated that unintended pregnancies are commonplace in HIV-infected women, and that preventing such pregnancies is a critical but neglected 'upstream' intervention to promote the health of HIV-infected women. In turn, these new guidelines place special emphasis on appropriate contraceptive choices for HIV-infected women, and feature integration of family planning services as a key intervention within HIV care and treatment programmes. Delivering appropriate counselling and contraception (when indicated) is a basic responsibility of every healthcare provider working in adult HIV services, and we hope that this month's insert will contribute towards this end.

This edition of the Journal features several important contributions that demonstrate the interplay of HIV medicine with an array of health systems and social concerns. For patients presenting with meningitis symptoms, lumbar puncture (LP) is a routine investigation in most parts of the country.[1] But laboratory access and the ability to examine cerebrospinal fluid is limited in many primary healthcare settings across Africa, and evidence-based approaches to help identify patients who require LP (often through referral) are needed.[1] To help address this, Loughborough *et al.*[1] used routine hospital audit data from KwaZulu-Natal to investigate factors associated with positive LP findings. In addition to providing local insights into the aetiology and predictors of positive LP results, this original article demonstrates the value of thoughtful analysis of routinely collected clinical data. In a similar vein, Mnyani *et al.*[2] use routine antenatal and ART clinic data from Johannesburg to examine how delays in ART initiation in HIV-infected pregnant women changed after the integration of nurse-initiated management of ART (NIMART) services into antenatal care. The results are surprising: of the five health facilities surveyed, four experienced increased delays in antenatal ART initiation after the start of NIMART, suggesting that integrating ART into other primary care services is not always straightforward. In addition, in reviewing the implications of new national legislation governing health research, Strode *et al.*[3] explain how HIV prevention and treatment research involving children will be hindered, limiting the ability of research efforts to improve the health of young people across the country.

In addition to these examples of HIV-related health programme and policy research, the Journal continues to present high-quality clinical research around HIV prevention and treatment. Apalata *et al.*[4] present the results of research on the association between vaginal infections and cervicovaginal shedding of HIV. While they demonstrate no association between vulvovaginal candidiasis and HIV shedding, their data confirm that plasma viraemia is a strong – but by no means complete – predictor of HIV shedding in the female genital tract.

Also in this issue, three case reports explore the potential for iatrogenic injury in HIV-related healthcare services. First, Zingela *et al.*[5] present an important case of Stevens-Johnson syndrome in a patient receiving both antipsychotic medications and a nevirapine-based antiretroviral regimen, reminding us of the pharmacological complexities of managing mental disorders in HIV-infected patients. Meanwhile, while we usually think of healthcare providers as being at risk of HIV transmission due to needlestick injuries, Ngene *et al.*[6] present a case of a needlestick injury causing HIV exposure between patients. They attributed this particular case to overcrowding within health facilities, another demonstration of how patient safety may be compromised in overburdened health systems. Finally, Moodley *et al.*[7] present two cases of a hereditary cause of optic neuropathy, here associated with nucleoside reverse transcriptase inhibitor use. While the mutations reported here are relatively rare, this report demonstrates the complex differential diagnoses of progressive visual impairment in HIV-infected patients.

Happy reading.

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MESSAGE
From the Executive

When the Minister of Health announced that the National Department of Health was introducing fixed-dose combinations (FDCs) into the antiretroviral programme in December 2012, as HIV clinicians we welcomed the decision. The FDC simplified prescription, dispensing and adherence messages.

As of 1 October 2013, the National Department of Health announced that FDCs should be offered to all HIV-infected adults on first-line therapy in the absence of any contra-indications. The previous priority groups no longer apply as the stock levels have now reached the required amounts.

As the President of the Southern African HIV Clinicians Society, I encourage all healthcare workers who are responsible for antiretroviral prescriptions to look actively for any remaining patients who are on single drugs and to change them to the FDCs, if clinically appropriate, as soon as possible.

- Patients who are receiving TDF, 3TC and EFV as single drugs can be changed to the FDC at their next dispensing or medical visit. Their monitoring visits and blood tests will remain on the same schedule as if they were still on the single drugs.
- For patients who were receiving d4T, 3TC and EFV prior to the switch, clinicians should please ensure that they have a recent undetectable viral load (VL) (~1 000 copies/ml) and normal creatinine clearance within the previous 3 - 6 months.
- For patients who were receiving d4T, 3TC and EFV who have a detectable VL, clinicians should do additional adherence counselling and repeat the VL in 2 - 3 months. If the repeat VL is undetectable (~1 000 copies/ml), the patient can be changed to the FDC. If the repeat VL is detectable, the patient should be changed to second-line therapy.

Please remember to reinforce these patient counselling messages:

- The dosage is one pill once daily, not three pills once daily.
- Although the FDC is 'one pill once a day', it does contain three different ARV medications.
- The FDC is easy to take, highly effective and in no way inferior to taking three individual drugs.

For any clinical questions or advice on other scenarios, please email us at sahivsoc@sahivsoc.org. Also check out our guidelines on FDCs on the Society's website, http://www.sahivsoc.org.

Let us work together to make sure that all patients who are eligible for an FDC receive it as soon as possible. Let us stand behind the Minister and the Department of Health and get this done.

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Failing the vulnerable: Three new consent norms that will undermine health research with children

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The South African National Health Act (No. 61 of 2003) provides a legal framework for the regulation of the health system across the country. Within the Act, section 71 introduces a number of legal norms relating to research or experimentation with human subjects, including research on HIV prevention and treatment. These norms have been criticised for the negative impact they will have on research involving children. This article describes three of the new consent requirements in section 71 of the Act. It shows, using a range of case studies, how important HIV-related research will be halted or undermined if the current provisions are implemented. The article argues that the new consent requirements are out of step with other statutory provisions and ethical guidelines, and as a result they will exclude a large population group – children in diverse settings – from much-needed evidence-based healthcare interventions. The article concludes with a clarion call for support of advocacy on this issue with the Minister of Health and the Health Portfolio Committee.

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Section 71 of the South African (SA) National Health Act (NHA),[1] which deals with research on or experimentation with human subjects, was put into operation on 1 March 2012.[2] This section fundamentally changes the way in which research with children may be undertaken across the country by introducing highly restrictive and inflexible standards into the current SA ethical-legal framework.[3,4] As a result, it has come under heavy criticism for limiting important research with children and containing impractical and unrealisable provisions.[3-9]

The full impact of section 71 has yet to be felt, as very few research ethics committees (RECs) require researchers to comply with its standards. However, this grace period may be coming to an end; on 29 May 2013, draft *Regulations Relating to Research on Human Subjects*[10] were published for public comment, indicating that the full implementation of section 71 is imminent.

This article focuses on three aspects of section 71, which we believe will have far-reaching consequences for research on children. It shows, using a range of case studies, how important research will be halted or undermined if the current provisions are implemented. The article concludes with a call to support advocacy in law reform.

The importance of health research with children

There is a global trend towards greater inclusivity in research practices and to facilitate research with children, while recognising that they need to be protected.[12] This approach flows from a recognition of the following:

- The number and severity of diseases that affect children is growing: for example, 17% of all 15 - 49-year-olds are HIV-positive.[13] Furthermore, mortality among children is unacceptably high, with one out of every ten deaths in the entire population being a child under the age of 14.[14]
- Some disorders occur only in children or are more common in children; for example, type 1 diabetes[15] and juvenile rheumatoid arthritis.[16,17]

A note on terminology: This article uses the term 'children' to refer to persons under the age of 18.[11] However, the NHA uses the term 'minors' in section 71; therefore, when we refer directly to this section we use 'minors' rather than children. We also limit our discussion to 'health research' on a 'living person', as the regulations in section 71 only apply to these types of studies.

- The dynamics in some diseases are different in children compared with adults. For example, 20% of untreated HIV-infected infants will die within 90 days of birth,[18] 40% within their first year of life, and 52% by the end of their second year.[19] This type of rapid mortality does not occur among newly infected adults.
- Certain medication has a different impact on children as opposed to adults, as they have differing biokinetics, metabolism, physiology and immunology, and metabolise medicines differently. This results in children needing different dosages, which can only be established through research.[20] Without research, limited information is available on the efficacy and safety of many of the medicines commonly used in children.[20]
- There is a developing trend against allowing the licensing of drugs, vaccines and other interventions for children before testing their safety and efficacy in this age group. There is also concern about the 'off label' use of medicines in children.[20]
- Using the results from clinical trials on children has resulted in significant health benefits for them.[21] For example, human papilloma virus (HPV) vaccine studies on children have enabled them to receive the vaccine, which can prevent cervical cancer and genital warts.[22]
- Laws such as the Children's Act emphasise that children have the right to participate in decision making.[11] Likewise, 'their participation in research is akin to respecting and promoting their entitlement to have their opinions heard. It assumes that they are persons of value, their experiences are of interest to themselves, and to others, and that they have a valuable contribution to make.'[23]

Against this backdrop, it is argued that an approach that excludes children from health research, including research related to HIV prevention and treatment, infringes on their constitutional rights to both 'basic health care' and access to 'healthcare services'.[24] For example, their exclusion results in ineffective and even harmful interventions being used owing to the lack of evidence on drug efficacy or dosage.[21] This also has unintended consequences, such as research being delayed or risking lack of funding due to extended enrolment periods that may be required in order to comply with a restrictive legal framework. This may result in research being undertaken in other countries, where the ethical-legal framework is more flexible.

New restrictive regulations for all forms of health research with human subjects

New standards on health research with children have been introduced, which will limit the circumstances in which they may participate in research. Three of the new consent regulations in section 71 are described and critiqued below.

Requiring written consent

Section 71 of the NHA provides that research participants must give written, informed consent to health research.[1] This will have serious implications for certain types of health research, such as telephonic interviews and postal or electronic studies, in which the voluntary completion of a questionnaire is commonly regarded as consent.[25] It also excludes the use of passive consent (informing parents of a study and assuming they have agreed to their child participating, unless otherwise instructed) – a practice frequently used with adolescent school-based studies.[9]

This approach is out of step with the more flexible approach in the National Health Research Ethics Council (NHREC) guidelines, which provide that consent may be given verbally or in writing. Consent may also, in certain circumstances, be waived, if prior approval of the REC is obtained.[26]

Prohibiting independent consent from minors

The NHA[1] provides in section 71 that consent must be obtained from parents or legal guardians, and minors if they have understanding. In other words, children under the age of 18 do not have the capacity to consent independently to any form of health research, but they may in certain circumstances provide dual consent alongside that of their parents or guardians.

Mandatory parental consent means that it will no longer be possible to undertake health research where it involves the following:

- *Certain socially marginalised groups*. For example, adolescent men who have sex with men are highly stigmatised in SA, and may face social harms if they are required to seek parental consent to participate in research focusing on their sexuality or sexual practices.
- *Behaviour that is legal, but which may incur parental disapproval or reprisal*. An example is termination of pregnancy in young girls, as it is likely that very few teenage girls would be willing to approach their parents for consent to a study on a decision they had made autonomously to terminate a pregnancy. Even though this is a lawful decision, studies have confirmed that teenagers will not use such services if they have to obtain parental consent for fear of disapproval.[27]
- *Illegal behaviours*. For example, studies into illegal practices such as child drug use or child prostitution would be complicated by concerns that: (*i*) children would not be prepared to seek parental consent, or (*ii*) parents are in fact not available to provide such consent.
- *Minimal or no-risk research with children over the age of 12, using a passive consent approach.*[9] For example, this could include completing surveys about drug, alcohol or sexual abuse, eating disorders, attitudes towards oral hygiene, exercise behaviour or even experiences of healthcare provision.
- *Orphaned and vulnerable children (OVCs) who do not have parents or legal guardians who are able to consent.* This is discussed further below.

It is worth noting that in all of the above examples, the children are likely to be considerably more vulnerable and at risk of ill health than their peers, and research and consequent evidence-based intervention with these groups is particularly pertinent (Table 1).

Prohibiting independent consent from minors is also problematic, in that it conflicts with the consent provisions in the Children's Act,[11] which recognises the evolving capacity of children, and allows them to consent to a range of health interventions before the age of 18.[31] Furthermore, this regulation in the NHA is diametrically opposed to those in the NHREC ethical guidelines, which, for example, allow for independent consent by children in certain circumstances.[26]

Limiting the authority to provide proxy consent to parents or legal guardians

Section 71 of the NHA limits the authority to provide proxy consent to either parents or legal guardians. Generally, parents are the biological

Table 1. Examples of completed studies that would in the future be difficult to undertake with the requirement of parental/legal guardian consent

Name of study	Ethically approved consent requirements	Reasons why obtaining parental consent would be difficult or impossible	Health benefits to children
HIV-related knowledge, attitudes and behaviour among SA street youth: reflections on power, sexuality and the autonomous self[28]	Independent consent	The child research participants were street children living away from adult supervision	Better understanding of the HIV risk of children living on the street
A systemic approach to the experiences of adolescents, with regard to terminating their pregnancies[29]	Participants aged 13 - 22 years; independent consent obtained	The study explored experiences of pregnancy termination. In many cases, participants may not have disclosed their pregnancy or their decision to terminate to their parent/legal guardian. Only 5 of 19 participants had disclosed their pregnancy to their mothers	Better understanding of adolescent experiences could inform policy and practice – particularly regarding school support and processes, as well as health and community services
Persisting mental-health problems among HIV-orphaned children in SA[30]	The mean age of the participants was 13.4 years at the study outset; consent was obtained from participants and caregivers	By definition, participants did not have a parent to provide consent, and it was unlikely that all caregivers would have been designated as legal guardians	The identification of the impact of psychological distress due to AIDS orphanhood over time in comparison with other groups, and highlighting the need for a focus on addressing the specific psychosocial needs of children

SA = South Africa.

or adoptive parents of a child, while a guardian is a 'person with guardianship of a child'.[11] Unmarried, biological mothers over the age of 18 are automatically the guardian of their child, and in certain circumstances an unmarried father will be a co-guardian. If the biological parents are married, they will be joint guardians. A guardian may also be appointed by the High Court or nominated by a parent in a will.[11] Persons caring for children but not falling into any of the categories above will, in the future, not be able to provide consent for children to participate in health research. This will affect a significant number of children, given that it is estimated that by 2015, ~5 700 000 children would have lost one or both parents to AIDS.[32]

In essence, this means that future studies with children who do not have parents or legal guardians will no longer be possible. Furthermore, such children may not volunteer for health research, as they do not have an adult with the legal authority to provide proxy consent. This principle will also apply to mothers under the age of 18 who have lost parental support but who are at particular risk of both HIV acquisition and transmission. There are also far-reaching implications for research on child-headed households, OVCs and undocumented migrant children. OVCs are increasingly recognised as a special population in terms of HIV risk and transmission, yet they will not be able to inform research.[33] OVC and child-headed households present unique and contemporary issues that must be responded to.

Limiting the authority to provide proxy consent to parents and legal guardians is also out of step with the Children's Act, which recognises that caregivers may consent to certain health interventions such as medical treatment and HIV testing on behalf of children.[34]

Conclusions

Given the principled nature of many of the concerns set out above, we call on the Minister of Health and the Parliamentary Health Portfolio Committee to address the need for law reform as a matter of urgency. If research institutions are required to comply with these regulations, child research in SA will grind to a halt, and this will ultimately harm the population it purports to protect. Ensuring and supporting rigorous and equitable review by RECs, and promoting clear communication to children and their caregivers during consent and study processes, should be the emphasis of developments in this field rather than restrictive legislation that reduces access to research participation. The nature and form of consent should be driven by the research itself, its benefits, risks, costs and consequences, rather than a blanket one-size-fits-all approach.[25]

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ORIGINAL ARTICLE

Which clinical parameters predict a CSF diagnosis of meningitis in a population with high HIV prevalence?

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Background. The HIV epidemic has changed the aetiology of meningitis in sub-Saharan Africa, and frontline clinicians are faced with a variety of meningitic presentations. Doctors working in resource-limited settings have the challenge of appropriately selecting patients for lumbar puncture (LP), a potentially risky procedure that requires laboratory analysis.
Methods. In a rural South African hospital, the practice of performing LPs was audited against local guidelines. Data were collected retrospectively between February and June 2013. Symptoms and signs of meningitis, HIV status, investigations performed prior to LP and cerebrospinal fluid (CSF) results were recorded. With the aim of determining statistically significant clinical predictors of meningitis, parameters were explored using univariate and multivariate logistic regression analyses.
Results. A total of 107 patients were included, of whom 43% had an abnormal CSF result. The majority (76%) of patients were HIV-positive ($CD4^+$ cell count <200 cells/µl in 46%). Cryptococcal meningitis (CCM) was the most prevalent microbiological diagnosis, confirmed in 10 out of 12 patients. Of the non-microbiological diagnoses, lymphocytic predominance was the most common abnormality, present in 17 out of 33 patients. Confusion (p=0.011) was the most statistically significant predictor of an abnormal CSF result. Headache (p=0.355), fever (p=0.660) and photophobia (p=0.634) were not statistically predictive.
Conclusion. The high incidence of CCM correlates with previous data from sub-Saharan Africa. In populations with high HIV prevalence, the classic meningitic symptoms of headache, fever and photophobia, while common presenting symptoms, are significantly less predictive of a meningitis diagnosis than confusion.

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Meningitis contributes significantly to mortality in countries with high HIV prevalence.[1] Cryptococcal meningitis (CCM) has become a significant opportunistic infection, accounting for 12 - 44% of early mortality in HIV-infected cohorts in resource-limited countries.[2,3] It carries an estimated annual global mortality of 500 000, with a case fatality rate of 35 - 65% in sub-Saharan Africa.[1,4] Lumbar puncture (LP) and cerebrospinal fluid (CSF) analysis remain the cornerstones of diagnosis. Clinicians must be efficient in targeting patients to undergo this time-consuming and potentially risky procedure. Furthermore, laboratory CSF analysis is a cost consideration in resource-limited settings.

Studies exploring the presentation of meningitis in populations with high HIV prevalence generally lack statistical analysis of clinical predictors of an abnormal CSF result. Studies that analyse presenting features of meningitis are generally only from cohorts of patients with confirmed meningitis, without comparison with similar cohorts with normal CSF results.[5,6] The accuracy of variables to predict abnormal CSF results is not possible without this comparison. In addition, in studies determining clinical presentation of meningitis, there is a lack of logistic regression analysis; confounding features are not removed.[5,6] There is therefore little evidence that demonstrates statistically significant predictors of a positive meningitis diagnosis in populations with high HIV prevalence. Consequently, clinicians working in this setting have a paucity of evidence to use when trying to select patients for LP appropriately. Given the dangers associated with LP and the potential cost implications of CSF analysis, such literature is essential for guiding frontline clinicians in resource-limited settings as to when to suspect meningitis and when an LP is justified. Alongside this, clinicians must be aware of the contra-indications to LP and must make appropriate use of tests such as platelet count, clotting and imaging, where available, to ensure patient safety.

Objective

Our first objective was to improve departmental practice through auditing the practice of performing diagnostic LPs against local guidelines in a regional referral hospital serving a rural South African (SA) population. We established local meningitis aetiology by reviewing the results of CSF analysis. The key objective was to determine which clinical parameters

were statistically significant in predicting a positive CSF diagnosis of meningitis.

Methods

Setting

Stanger Provincial Hospital is a regional centre located in Ilembe District, in rural KwaZulu-Natal, SA. It serves a population of approximately 700 000 and has 500 inpatient beds. Referrals are received from 15 smaller hospitals and satellite clinics. HIV prevalence in Ilembe district is 35.4%, one of the highest district rates in SA.[7] The emergency department (ED) treats ~2 500 patients a month, of whom 1 500 have a medical rather than a surgical complaint. Patients presenting with suspected meningitis are seen in the ED, where an LP is performed and CSF results are analysed, with referral to the Department of Internal Medicine where appropriate.

Data collection

Audit data were collected retrospectively between February and June 2013. All patients undergoing diagnostic LPs in the ED, regardless of indication, were eligible for inclusion. Doctors working in the department were asked to make a note of patients undergoing LP by documenting their hospital number. The audit team then used a data collection tool to gather information from the patient's notes, namely indication for LP, HIV status, presenting symptoms and signs, $CD4^+$ cell count, platelet count, international normalised ratio (INR), whether a computed tomography (CT) head scan was performed before LP, and CSF result. As the data collection was primarily for audit purposes, identifying information such as name, age and sex was not recorded; unique study numbers were used to identify patients. If information from the medical notes did not provide all the key information for the data collection tool, the patient was excluded from the audit. When the audit period had finished, the results were summarised.

Clinicians did not record every patient undergoing LP. While every patient coming through the department was recorded in the ED admissions book, admitting diagnoses were not always clear in terms of whether meningitis was likely or an LP was performed. Therefore determining the audit sample size as a total number of patients undergoing LP could not be done accurately.

Patient management was audited against the Stanger Provincial Hospital Department of Internal Medicine 2010 guidelines for diagnostic LPs. According to these, relative contraindications were coagulopathy (platelets $<50 \times 10^9$/l and INR >1.2) or suspicion of raised intracranial pressure (Glasgow Coma Scale (GCS) <12, focal neurological signs or papilloedema on fundoscopy). In these circumstances, platelet and/or freeze-dried plasma infusion or head CT, respectively, are advised prior to LP.

CSF diagnostic criteria were obtained from a study based in Cape Town in which 4 971 LPs were evaluated, the largest study of diagnostic LPs in SA.[8] A positive diagnosis for meningitis was made either microbiologically with staining, antigen testing and culture, or, if these were negative, through abnormal protein, glucose or chloride levels. Microbiological CSF diagnoses were defined as CCM (positive cryptococcal latex antigen test (CLAT) and/or India ink stain) or bacterial meningitis (BM) (positive Gram stain and/or culture). In this rural setting, samples are not consistently sent for tuberculosis (TB) culture, and therefore this outcome was omitted from the study. Abnormal biochemical and microscopic findings were classified as follows: lymphocytic (lymphocytes $>6 \times 10^6$/l), pyogenic (neutrophils $>2 \times 10^6$/l), mixed or normocellular with abnormal protein and/or glucose (protein >0.5 g/dl and/or glucose <1.5 mmol/l). Abnormal CSF was a binary variable, with a positive result defined as any of the above abnormalities in staining, culture and antigen testing, as well as raised protein (>0.5 g/dL), low glucose (<1.5 mmol/L) or low chloride (<110 mEq/L).

Data analysis

Statistical analysis included descriptive statistics with proportions, medians and interquartile ranges (IQRs) calculated for non-parametric data, and means and standard deviations presented for parametric data. Potential predictors of abnormal CSF were evaluated using univariate and multivariate logistic regression analyses, namely headache, confusion, terminal neck stiffness, photophobia, focal neurological signs, seizures, HIV status and $CD4^+$ cell count. Odds ratios (ORs) were presented with 95% confidence intervals (CIs) and a statistical significance cut-off of $p \leq 0.05$.

Stata 10.0 software (StataCorp, Stata Statistical Software, release 10, 2007) was used for the statistical analysis.

Ethical approval

As this project was an audit of departmental practice, we did not apply for formal ethics committee approval. Approval to undertake the audit was granted by the ED consultants and Head of Department. In terms of consent for the LP procedure, clinicians obtained and recorded informed consent from all patients, or a relative in cases where the patient lacked capacity (e.g. low GCS). In children aged between 16 and 18 years old, consent was obtained from a parent/guardian of the child.

Results

From February to June 2013, 107 patient cases were audited. An HIV test was refused by 15 out of 107 patients (14%); among those who consented to the test, 81 out of 92 (88%) were seropositive. Many HIV-positive patients had significant immunosuppression, with 46% (*n*=37) having a $CD4^+$ cell count below 200 cells/µl and 34% (*n*=28) below 100 cells/µl.

The most common symptom was headache, present in 76% (*n*=80) of patients. Approximately half (51%) described the headache as focal, and the median duration was 4 days (IQR 2 - 7). Neck stiffness was reported by 56% (*n*=60), and 38% (*n*=41) reported photophobia. Seizures were recalled by 5.6% (*n*=6), and confusion was present in 41% (*n*=44) (Table 1).

Table 1. Patient characteristics*

Characteristic	
HIV-positive, *n* (%)	81 (75.7)
HIV-negative, *n* (%)	11 (10.3)
HIV status unknown, *n* (%)	15 (14.0)
$CD4^+$ (cells/µl), median (IQR)	285.5 (47 - 408)
Headache, *n* (%)	80 (76.0)
Photophobia, *n* (%)	41 (38.0)
Confusion, *n* (%)	44 (41.0)
Seizures, *n* (%)	6 (5.6)
Temperature, *n* (%)	34 (32.0)
Kernig's or Brudzinski's sign, *n* (%)	20 (19.0)
Terminal neck stiffness, *n* (%)	38 (36.0)
Focal neurology, *n* (%)	2 (2.1)

IQR = interquartile range.
*N=107

On examination, 32% (*n*=34) were pyrexial. Kernig's or Brudzinski's signs were present in 19% (*n*=20) and terminal neck stiffness was felt in 36% (*n*=38). There were two cases with focal neurology and one case with papilloedema, who underwent an LP. Papilloedema was not examined for in 86% (*n*=92) of patients. Fifty-nine per cent (*n*=63) had a GCS of 15, 26% (*n*=28) had a GCS of between 13 and 14, and 8.4% (*n*=16) had a GCS of ≤12 (Table 2). Of patients with a GCS <12, three had a CT prior to LP and three did not. The indication for the LP was meningitis in 83% (*n*=89), seizures in 6.2% (*n*=6) and psychosis in 11% (*n*=12).

Prior to LP, 89% (*n*=94) had platelets checked, and of these one patient had a significantly reduced count at 14×10^9/l (1%). Only 7.5% (*n*=8) of patients had their INR checked prior to LP and of these, three patients had a result of 1.3 - 1.4 units. Prior to LP, 6.5% (*n*=7) underwent a CT scan. Out of the 107 patients, 42% (*n*=45) had an abnormal CSF result. Of these, 27% (*n*=12) had confirmed microbiological diagnoses – 10 CCM and 2 BM. The remaining abnormal results were 17 lymphocytic, 10 mixed, 2 pyogenic and 4 normocellular with abnormal protein and/or glucose (Fig. 1).

Overall, polymorphs were $>2 \times 10^6$/l in 18% (*n*=19) and the lymphocytes were $>6 \times 10^6$/l in 29% (*n*=31). Chloride was <110 in 7.6% (*n*=8) of cases. CSF glucose was <1.5 mmol/l in 14% (*n*=15) and protein was >0.5 g/dl in 33% (*n*=35). Gram staining revealed Gram-positive diplococci (streptococcus pneumoniae on culture) in one case and Gram-positive cocci (failed to culture) in another case. India ink staining identified *Cryptococcal neoformans* in five patients, and all of these plus an additional four cases had a positive CLAT. (Fig. 2)

Potential clinical predictors of abnormal CSF results were evaluated using univariate and multivariate logistic regression analyses (Table 3). Seizures and papilloedema were excluded from these analyses, as numbers were too small for these variables. In the unadjusted analysis, none of the factors were significantly associated with abnormal CSF results. Terminal neck stiffness was almost statistically significant, with an OR of 2.17 (95% CI 0.97 - 4.87), as was raised temperature with an OR of 2.15 (95% CI 0.94 - 4.93). In the multivariate analysis, terminal neck stiffness remained almost statistically significant with an OR of 2.52 (95% CI 0.96 - 6.66). Confused patients were found to be significantly more likely to have abnormal CSF, with an OR of 4.91 (95% CI 1.45 - 16.67) and *p*=0.011.

Discussion

The primary objective of this study was to determine statistically significant clinical predictors of meningitis through logistic regression analysis. Previous research has generally lacked this analysis and therefore has failed to account for confounding factors. Unlike previous studies, which only analysed presenting features from a cohort of patients with abnormal CSF results, in our study patients were selected before LP, allowing for proper estimation of the accuracy of each variable predicting abnormal CSF results. The logistic regression analyses demonstrated confusion as a strong predictor, with an OR of 4.91 and *p*=0.011. Previous papers that focused on sub-populations with HIV and

Table 2. Frequency and percentage by GCS

GCS	Frequency	Percentage
Missing	7	6.5
3	1	0.9
9	3	2.8
10	2	1.9
11	1	0.9
12	2	1.9
13	3	2.8
14	25	23.4
15	63	58.9

GCS = Glasgow Coma Scale.

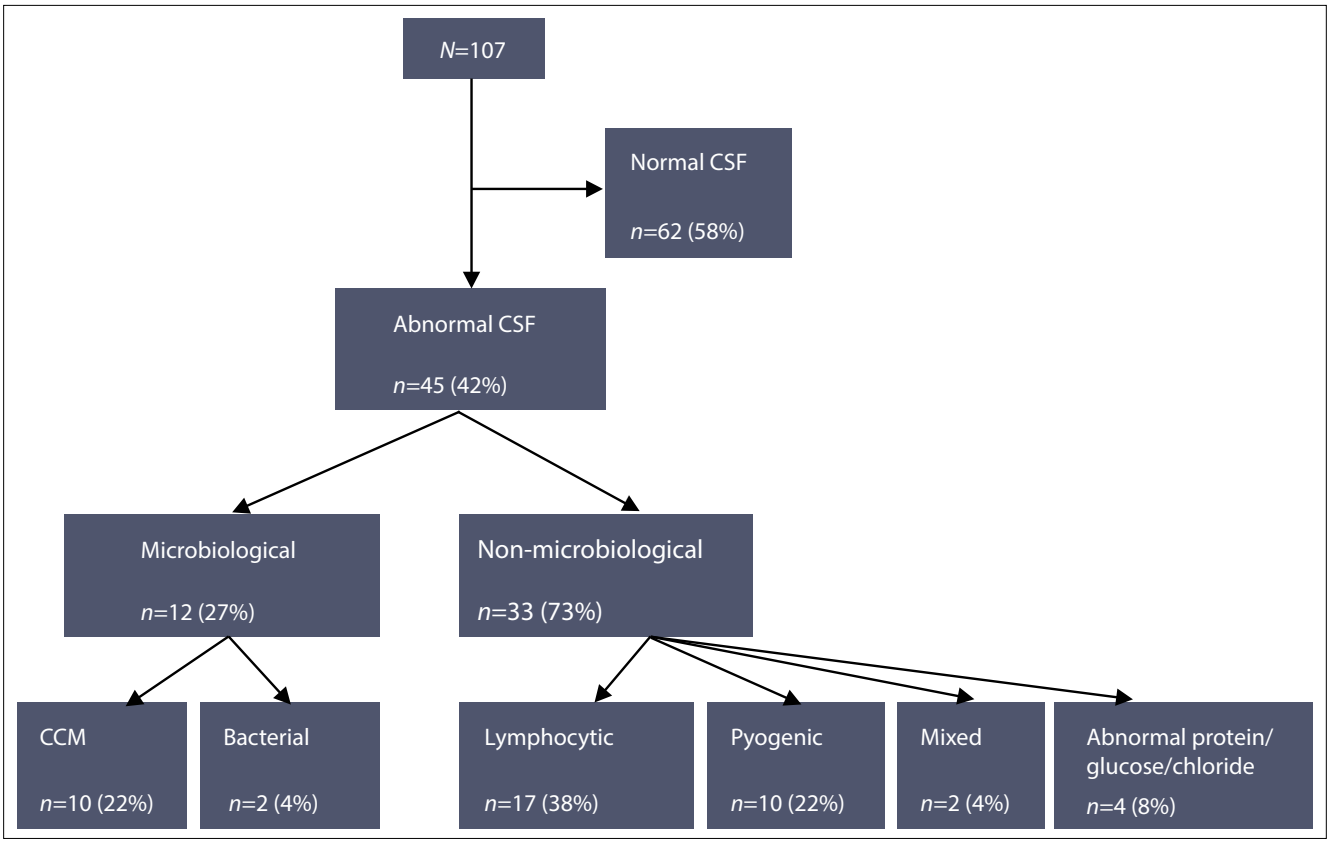

Fig. 1. Frequency of CSF results. (CSF = cerebrospinal fluid; CCM = cryptococcal meningitis.)

meningitis have found headache and fever as the most common presenting features, with a lower incidence of confusion.[5,6] For example, one study from Zambia found headache or abnormal temperature present in 91% of their HIV-positive patients with CCM, but only 13% were confused.[6] Our study has highlighted that although headache and fever were common symptoms in patients presenting for LP, they lacked specificity in predicting meningitis, as they were often present in patients with a normal CSF result (headache OR 1.39, p=0.355; fever OR 1.24, p=0.660). An explanation for this lack of specificity is that patients with HIV are at increased risk of multiple pathologies, of which headache and fever are symptoms. For example, migraines are more common in HIV-positive patients.[9] Therefore, these symptoms lack specificity in predicting meningitis. Photophobia, another symptom classically associated with meningitis and common in patients presenting for LP, was not statistically significant in predicting meningitis (OR 1.06, p=0.634). These findings highlighted that meningitis is difficult to predict in HIV-positive patients. Furthermore, the varied aetiology showed that the classic presentation of meningitis is unlikely, proven by the lack of statistical predictability of classic meningitis symptoms. The fact that the incidence of meningitis is high (42% in our cohort) reveals that clinicians should have a low threshold for undertaking LP when not contraindicated. In resource-limited settings, selecting patients with more specific features, such as confusion rather than headache, may allow for a higher identification rate of abnormal CSF results. More research is needed in similar populations of high HIV prevalence to validate these findings and support clinicians in their decision to undertake LP.

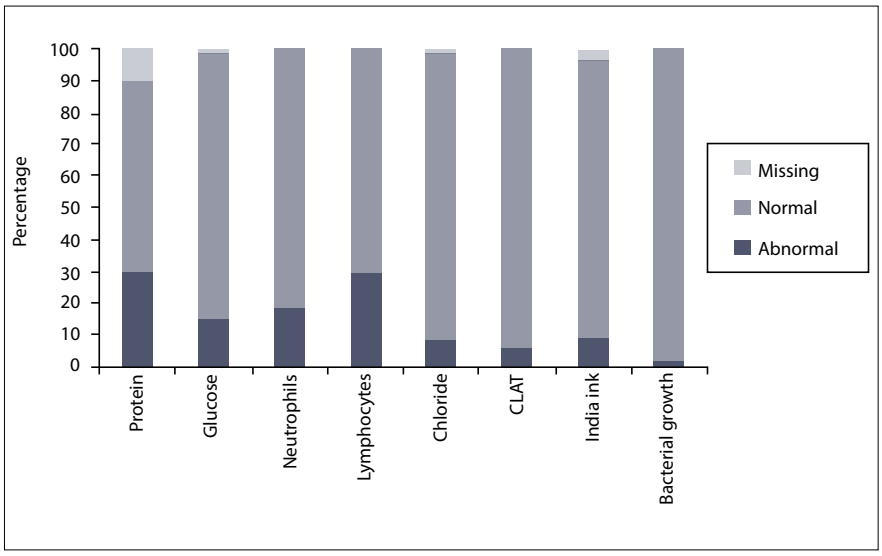

Fig. 2. Percentage of patients with abnormal CSF. (CSF = cerebrospinal fluid; CLAT = cryptococcal latex antigen test.)

Table 3. Univariate and multivariate analyses of potential predictors of abnormal CSF results

	Unadjusted		Adjusted		
Potential predictors of abnormal CSF result	OR	CI (95%)	OR	CI (95%)	p
HIV status	1.99	0.78 - 5.10	2.20	0.71 - 6.82	0.171
Headache	1.39	0.57 - 3.41	1.79	0.52 - 6.12	0.355
Headache ≥7 days	1.06	0.43 - 2.60	-	-	-
Photophobia	1.06	0.48 - 2.33	1.26	0.48 - 3.32	0.634
Kernig's/Brudzinski's sign	0.86	0.32 - 2.31	0.50	0.16 - 1.59	0.243
Terminal neck stiffness	2.17	0.97 - 4.87	2.52	0.96 - 6.66	0.062
Confusion	1.63	0.75 - 3.54	4.91	1.45 - 16.67	0.011
GCS	0.81	0.62 - 1.06	0.84	0.64 - 1.11	0.229
Fever	2.15	0.94 - 4.93	1.25	0.46 - 3.35	0.660
$CD4^+$ (cells/μl) ≤100	1.61	0.57 - 4.56	0.84	0.26 - 2.76	0.779

CSF = cerebrospinal fluid; OR = odds ratio; CI = confidence interval; GCS = Glasgow Coma Scale.

The audit compared the practice of performing LPs with current hospital guidelines. The checking of platelets was thorough, with 89% of patients checked prior to LP. However, checking of the INR was poor, with only 7.5% checked; among those with an INR result, 37.5% of the results were >1.2 (hospital guidelines stated that these patients required freeze-dried plasma prior to LP). The aetiology of these clotting disorders is unknown but may be explained by comorbidities such as viral hepatitis. The lack of INR checking can be explained by the lack of suspicion of coagulopathy on admission. Warfarin prescription and sepsis at presentation were rare, so iatrogenic or sepsis-induced coagulopathies were not suspected clinically. While there has not been any published research examining the risk of bleeding from LP in patients with coagulopathy, it is considered best practice to correct an INR of >1.4 prior to LP.[10] In one literature review, 47% of 21 published cases of spinal haematoma following LP occurred in patients with a coagulopathy.[11] Following these audit results, new departmental protocol was to perform an INR on all patients and correct abnormalities accordingly.

In this study, local guidelines stated that patients with a GCS of <12 required a head CT prior to LP, but a CT was performed on only 50% of such patients, which shows that practice clearly falls short of local standards. However, the reasoning behind performing a CT prior to LP remains controversial. A CT may identify an alternative pathology such as a mass lesion or indications of raised intracranial pressure, which could contraindicate LP. However, a normal CT scan does not exclude raised intracranial pressure and the scanning of patients potentially delays LP and antibiotic administration.[12] Guidelines need to be set locally. Following presentation and discussion of these audit findings at a departmental meeting, it was decided to perform a CT on any patient with a fluctuating GCS or GCS <12 prior to LP.

In this rural SA setting with high HIV prevalence, 42% of patients had abnormal CSF results. The Cape Town study had a similarly high incidence of abnormal results, at 35%.[8] Of the abnormal results, 27% had definitive microbiological diagnoses, compared with 47% in Cape Town. Of the

positive microbiological diagnoses, 83% cultures grew *Cryptococcus neoformans* and 17% pyogenic bacteria (BM), in contrast to 63% and 8%, respectively, in Cape Town.[8] We did not have culture results for tuberculous meningitis (TBM), for which the Cape Town figure was 28%.[8] Recent studies from other high HIV prevalence locations in Africa have found similar high rates of CCM; it is now the most prevalent cause of meningitis in sub-Saharan Africa (25 - 47%).[1]

As TB CSF culture was excluded from this study, direct comparisons could not be made with regard to TBM and total microbiological diagnoses – this was a limitation as discussed below. However, the high levels of lymphocytic predominance in the non-microbiological diagnoses almost certainly correlated with high levels of TBM, in the context of high HIV prevalence.[11]

Limitations

A key limitation of this study was the lack of TB culture results due to the lack of TB culturing facilities at Stanger Provincial Hospital. TB meningitis is a significant cause of mortality in cohorts with high HIV prevalence and has a variety of initial CSF findings.[13] The high levels of lymphocytic predominance and raised protein would fit with high levels of TBM.[13] Therefore, our results almost certainly underestimated the prevalence of TBM in our population. While this clearly affected our aetiological results, it had less of an impact on our statistical analysis as this was based on a positive CSF diagnosis in general (microbiological and non-microbiological results), rather than specific microbiological diagnoses. The lack of TB culturing at a facility is a common issue in the developing world – clinicians often have only their clinical impression plus initial CSF results with which to make diagnostic decisions. Even if culturing facilities are available, TB culture often takes some time to grow. This is why the outcome from this study is relevant for clinicians working in this setting – it demonstrates which presenting features are predictive of the CSF results on the basis of which diagnoses are made.

Another limitation was the quality control in the clinicians' documentation. Because this was a retrospective departmental audit, we could not ensure that all information recorded in the notes was consistent. The GCS was the most common area of subjectivity, for example several patients had GCS 15 recorded in the notes while they were identified as confused in the history (therefore GCS ≤14). However, in general, the major presenting features audited were objective. One area inconsistently recorded was antiretroviral therapy use, which therefore had to be excluded from the results. This would have been a valuable set of results, comparing the presentation and aetiology of meningitis in HIV-positive patients currently on or not on ART.

Conclusion

This audit demonstrated that LP practice in the ED did not adhere to local guidelines in certain areas. Following presentation of the results and discussion at a departmental meeting, it was agreed that prior to LP, an INR should be measured routinely, and a head CT should be performed on any patient with fluctuating GCS or GCS <12. The high prevalence of CCM from our data was consistent with other studies, which demonstrated that cryptococcosis has become the leading cause of adult meningitis in sub-Saharan Africa.[1] While there were limitations to the project, the data analysis has demonstrated some valuable findings. Current literature analysing the presentation of meningitis in cohorts with high HIV prevalence generally fails to compare normal with abnormal CSF groups and lacks logistic regression analysis. Our statistical analysis demonstrated confusion to be a significant predictor of an abnormal CSF result. While headache, fever and photophobia were common presenting symptoms, they were not statistically significant predictors of meningitis. Clinicians working in populations with high HIV prevalence need to have a broad differential diagnosis for patients presenting with headache and have a low threshold for performing LP or empirically treating meningitis in confused patients.

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Timing of antenatal care and ART initiation in HIV-infected pregnant women before and after introduction of NIMART

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In this review of routinely collected data from five community health centres in the Johannesburg Health District, we assess timing of antenatal care and antiretroviral therapy (ART) initiation in HIV-infected pregnant women before and after the introduction of nurse-initiated management of ART in antenatal clinics. There are important lessons to be learnt as we reflect on the South African prevention of mother-to-child transmission of HIV programme.

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It is widely acknowledged that the highest risk of mother-to-child transmission (MTCT) of HIV is in HIV-infected women with low CD4$^+$ counts, who are eligible for antiretroviral therapy (ART).[1] Timely initiation of ART in this group is critical to decreasing paediatric HIV infection and HIV-related maternal morbidity and mortality.[2,3] ART initiation in pregnancy is also associated with better maternal immunological and virological outcomes compared with starting ART after pregnancy.[4]

Prior to the current prevention of MTCT (PMTCT) guidelines, criteria for ART initiation in pregnant women were based on a CD4$^+$ count of ≤350 cells/μl, or World Health Organization (WHO) stage 3 or 4 disease regardless of CD4$^+$ count.[5] Reflecting on the history of the PMTCT programme in South Africa, two of the main early challenges to initiating ART in pregnancy were that HIV treatment sites were physically separate from antenatal clinics and ART initiation was largely physician led.[6] This led to delays in referral and initiating treatment, and as a result, a significant proportion of ART-eligible pregnant women would go through pregnancy without starting treatment.[7] Nurse-initiated and managed ART (NIMART) in antenatal clinics was introduced to address these challenges, supported by evidence that integration of ART with antenatal care decreases time to initiation and increases the proportion of pregnant women initiated.[8-10] Pregnant women need to access antenatal care early for timeous treatment initiation.

This retrospective review of routinely collected data assesses timing of antenatal care and ART initiation in HIV-infected, eligible pregnant women presenting to five community health centres in the Johannesburg Health District.

Method

Time to initiation in pregnant women who presented prior to the introduction of NIMART was compared with those who presented after. Between October 2010 and March 2012, a total of 1 436 ART-eligible pregnant women were identified.

The study was approved by the University of Cape Town's Human Research Ethics Committee, and by the Gauteng Province office for policy, planning and research.

Results

Characteristics of the women are presented in Table 1. The mean gestational age when accessing antenatal care was 19.2 weeks (standard deviation (SD) of 6.6), and the mean gestational age at ART initiation was 24.6 weeks (SD 6.2). There was no significant reduction in time to initiation after the introduction of NIMART. Overall, the median time to initiation prior to the introduction of NIMART was 3.4 weeks (interquartile range (IQR) 2.0 - 5.9) whereas after the introduction of NIMART, it was 3.0 weeks (IQR 1.4 - 5.4). Assessing data from individual clinics, there was evidence of an increase in time to starting ART in some facilities. However, overall there was an increase in the proportion of eligible pregnant women who started ART after the introduction of NIMART, in all the facilities. Data on pregnancy outcomes were

Table 1. Baseline characteristics and timing of ART initiation

	Facilities				
	A	B	C	D	E
Baseline CD4⁺ count (cells/µl)					
Mean (±SD)	211 (±85)	223 (±86)	215 (±87)	240 (±112)	228 (±81)
Median (IQR)	214 (151 - 284)	234 (147 - 299)	225 (152 - 286)	245 (174 - 299)	235 (169 - 296)
Range	15 - 344	27 - 350	23 - 350	25 - 650	45 - 351
Gestational age at 1st antenatal visit (weeks)					
Mean (±SD)	20.7 (±6.4)	17.4 (±6.2)	19.9 (±5.9)	18.1 (±6.9)	19.5 (±6.9)
Median (IQR)	20 (16 - 24)	18 (12 - 20)	20 (16 - 24)	16 (12 - 24)	20 (15 - 24)
Range	8 - 36	4 - 30	8 - 32	4 - 36	5 - 36
Gestational age at ART initiation (weeks)					
Mean (±SD)	25.4 (±6.4)	22.0 (±5.8)	24.3 (±5.5)	24.6 (±6.6)	25.1 (±6.3)
Median (IQR)	25 (21 - 30)	21 (18 - 27)	25 (20 - 28)	24 (20 - 30)	25 (21 - 30)
Range	12 - 37	8 - 34	14 - 36	11 - 39	10 - 38
Time to initiation prior to NIMART (weeks)					
Mean (±SD)	1.6 (±0.5)	4.0 (±2.7)	5.5 (±2.5)	2.6 (±0.8)	3.3 (±1.8)
Median (IQR)	1.9 (1.3 - 2.3)	3.1 (2.0 - 5.1)	5.6 (1.7 - 7.0)	2.4 (1.9 - 3.2)	2.8 (1.9 - 5.1)
Range	1.0 - 2.3	0.9 - 10.6	1.1 - 12.6	1.6 - 4.0	1.3 - 5.9
Time to initiation after NIMART (weeks)					
Mean (±SD)	2.0 (±1.9)	4.4 (±3.3)	3.8 (±3.5)	5.7 (±3.8)	6.6 (±3.9)
Median (IQR)	1.3 (1.0 - 2.3)	3.7 (2.4 - 5.1)	2.1 (1.4 - 4.9)	4.1 (3.0 - 8.1)	6.0 (4.0 - 8.8)
Range	0 - 9.7	0 - 18.9	0.7 - 15.7	0.7 - 18.4	0.6 - 18.7

ART = antiretroviral therapy; SD = standard deviation; IQR = interquartile range; NIMART = nurse-initiated management of antiretroviral therapy.

available for 61.8% of the women, namely 881 live births, 1 neonatal death, 4 stillbirths and 2 miscarriages. Of the 881 infants tested at ~6 weeks of age, only two (0.2%) were HIV-infected.

Discussion

The data provided an interesting reflection on the PMTCT programme. While pregnant women in the review accessed antenatal care relatively late, in the second trimester, there were no lengthy delays in initiating ART in patients who reached the HIV management sites, even prior to the introduction of NIMART in antenatal clinics. Despite several challenges in the antiretroviral (ARV) roll-out in the past few years, the data showed that there were pockets of excellence. In the facilities where the intervention increased time to initiation, there are several possible reasons for this: there could have been a shortage of skilled and properly trained staff to manage ART-eligible pregnant women in the antenatal clinics; and during the early stages of the introduction of NIMART, antenatal clinics were only able to initiate treatment on select days.

Conclusion

While the updated PMTCT guidelines recommend starting all HIV-infected pregnant women on ART at the first antenatal visit, to reduce delays in initiating therapy, focus needs to shift from quantity to quality. As the number of patients on treatment increases, clinicians need to ensure that they provide high-quality services with appropriate clinical and laboratory monitoring, and long-term retention of patients in care.

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Association between symptomatic vulvovaginal candidiasis and HIV RNA levels in plasma and genital secretions among women on HAART

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Background. Genital tract (GT) inflammation plays a major role in HIV transmission. We aimed to determine the association between symptomatic vulvovaginal candidiasis (VVC) and HIV RNA levels in plasma and GTs of HIV-infected women on highly active antiretroviral therapy (HAART).

Method. Women with VVC on HAART were recruited from a primary healthcare clinic in KwaZulu-Natal Province, South Africa, between June 2011 and December 2011. VVC was diagnosed clinically, supported by Gram staining and culture of genital secretions. HIV RNA load was determined by reverse transcription polymerase chain reaction. $CD4^+$ counts were obtained from patients' medical records.

Results. Plasma HIV RNA was detected in 42 of 60 (70%) patients on HAART. The mean duration (± standard deviation) on HAART for these patients was 4.2 (±1.6) months v. 10.7 (±1.4) months for the remaining 18 patients ($p<0.0001$). Of the 42 women with detectable plasma HIV RNA, 12 (28.6%) had detectable genital HIV RNA. Plasma HIV RNA levels ranged from 2.5 (±0.8) to 4.1 (±0.8) $\log_{10}$ copies/ml. Genital HIV RNA levels ranged from 1.4 to 2.5 (±1.1) $\log_{10}$ copies/ml. The adjusted odds ratios of plasma HIV RNA levels increased for patients <35 years ($p=0.039$) and in those with VVC ($p=0.008$). Detectability of HIV in genital secretions was significantly increased in patients with a plasma HIV load ≥10 000 copies/ml ($p=0.032$) and genital absolute counts of neutrophils >10 cells/5 high microscopic fields ($p=0.007$).

Conclusion. Given that the majority of women had recently initiated HAART (allowing a high rate of detectable plasma HIV RNA), there was insufficient evidence to conclude that VVC was predictive of high plasma HIV RNA levels. It is more likely that this cohort of immunosuppressed women were prone to develop VVC. Plasma HIV loads and local genital inflammation were predictors of genital HIV detectability.

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Ulcerative and non-ulcerative lower genital tract infections (LGTIs) are thought to play a major role in early HIV pathogenesis and transmission.[1] LGTIs and plasma HIV viral load (VL) partially explain HIV shedding in the female genital tract (GT).[1,2] Some studies have suggested that changes in the normal microbial flora of the female GT contribute to viral shedding.[1-3]

Conditions associated with genital inflammation, particularly genital ulcers, have been associated with HIV shedding in the GT.[4,5] While the association between non-ulcerative LGTIs such as bacterial vaginosis (BV) and GT HIV shedding has been well established,[6,7] current studies have shown a less well-defined relationship between vulvovaginal candidiasis (VVC) and HIV shedding. In addition to BV that is not an established sexually transmitted infection (STI), vaginitis , an STI caused by *Trichomonas vaginalis*, has also been shown to increase the risk of HIV acquisition among women,[8,9] and sexual and perinatal HIV transmission.

Data on the effect of highly active antiretroviral therapy (HAART) on vaginal infections among HIV-infected women are limited. Factors such as changes in vaginal immunological cell populations, cellular activation and cytokine production have been reported to alter susceptibility or response to genital infections.[10-13] However, it remains unclear whether chemokines and cytokines interfere with the activity of polymorphonuclear neutrophil (PMN) cells and macrophages in the mucosa, or whether they facilitate the development of VVC.

Objective

Our objective was to determine the association between symptomatic VVC and HIV RNA levels in the plasma and GTs of HIV-infected women on HAART. We hypothesised that in HIV-infected women, the presence of symptomatic VVC would affect local HIV shedding, but not systemic levels.

Methods

Study population

For this cross-sectional study, consecutive adult (≥18 years) women (all HIV-infected) attending Umlazi D clinic, a primary healthcare facility in KwaZulu-Natal (KZN) Province, South Africa, between June 2011 and December 2011 were enrolled after giving informed consent. Data on current symptoms suggestive of LGTIs were obtained by means of a standard questionnaire. Patients on HAART were defined as those receiving treatment with ≥2 nucleoside reverse transcriptase inhibitors (NRTIs) and ≥1 protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI).

A physical examination was performed by the attending medical practitioner and signs of LGTIs were noted. Sixty paired plasma and cervicovaginal samples were collected from HIV-infected women receiving HAART. Patients aged <18 years and those who were menstruating or had visible blood contamination of genital samples were excluded from the study.

The study was approved by the Biomedical Research Ethics Committee of the University of KZN (Ref. BE 224/11). Consent forms were signed by all participants and confidentiality was maintained throughout the study.

Collection and processing of cervicovaginal fluid

A vaginal tampon (8 Ks), Tampax Regular® (Compak), was inserted into the vagina, left *in situ* for 3 minutes, and then placed in a sterile container containing 10 ml of phosphate-buffered saline (Oxoid Ltd Basingstoke, UK) (pH 6.9). Samples were stored at 4°C for 4 hours prior to transport to the laboratory. Vaginal fluid was expressed using an autoclaved wooden tongue depressor and filtered through 0.22 µm Costar Spin-X cellulose acetate filter membranes (Sigma). The filtered soluble fraction was aliquoted (in 1 ml cryotubes) and stored at –70°C until use.

Measurement of absolute PMN cell counts from lower GT samples

A vaginal swab (Becton Dickinson (BD)) was used to collect cells from the posterior fornix and to prepare a tissue smear on a glass slide for Gram staining. Absolute counts of neutrophils were determined by counting the total number of PMN cells in five randomly selected microscopic fields under oil immersion (×1 000 magnification) as previously described. The scores were as follows: score 1: 1 - 10 PMN cells/5 high microscopic fields (HMFs); score 2: 11 - 20 PMN cells/5 HMFs; score 3: >20 PMN cells/5 HMFs.[14-16]

Diagnostic criteria for symptomatic VVC and VVC colonisation

The diagnosis of symptomatic VVC was based on a combination of clinical and laboratory criteria (evidence level III, recommendation grade B).[17-19] Symptoms suggestive of VVC included vulval pruritus, vulval soreness, superficial dyspareunia, and/or non-offensive vaginal discharge. Signs included vulval erythema, vulval oedema, fissures, excoriation or thick curdy vaginal discharge.

A vaginal swab (BD) taken from the anterior fornix was directly plated onto Sabouraud Dextrose agar with chloramphenicol (BBL™, BD) and incubated at 29°C for 48 hours to estimate the relative vaginal fungal burden. The numbers of yeast colonies were recorded as the number of colonies per plate.[17-19]

In addition to the self-reported symptoms listed above and observation of signs suggestive of VVC on physical examination, cases of symptomatic VVC were confirmed if one of the following criteria was fulfilled (evidence level III, recommendation grade B):[17-19] (*i*) a positive Gram-stain preparation with budding yeasts, pseudohyphae, and/or hyphal forms; and (*ii*) positive culture with either moderate (10 - 99 colonies per plate) or heavy (>100 colonies per plate) *Candida* growth.

Participants without symptomatic VVC were defined as: (*i*) patients whose genital specimens were negative for yeasts, pseudohyphae, and/or hyphal forms of *Candida* on microscopy, together with negative culture; and (*ii*) patients whose genital specimens were negative for yeasts, pseudohyphae on microscopy, and/or hyphal forms of *Candida* together with light *Candida* growth (<10 colonies per plate). The latter was considered to indicate vaginal *Candida* colonisation rather than infection.

Definitions of HIV-induced immunosuppression

As part of the routine management and standard of care in the clinic, $CD4^+$ T-cell counts were measured in the blood of all HIV-infected patients. The $CD4^+$ T-cell counts used in this study were obtained from the past 3 months of measurements in patients' medical records. However, for the purpose of this research, HIV RNA was measured from the plasma and cell-free fraction of vaginal secretions using Nuclisens Easyq HIV-1 version 2.0 (BioMerieux, France) with a lowest detection limit of 20 copies/ml.[20-22]

Absolute values of $CD4^+$ T-cell counts (cells/µl) were used to determine the degree or severity of immunosuppression as per the World Health Organization immunological staging criteria: severe immunosuppression (<200/µl); advanced immunosuppression (200 - 349/µl); and mild immunosuppression (350 - 499/µl).[23] HIV VL and $CD4^+$ counts were used as markers of disease progression.[23-25] For the purpose of this study, plasma viraemia was classified as follows: <20 copies/ml (below detectable level of the test used); 20 - 9 999 copies/ml; and ≥10 000 copies/ml. We also log-transformed HIV loads for improved symmetry and included them in multivariate logistic regression models as continuous values.

Diagnosis of other LGTIs

For each study participant, clinical symptoms and signs suggestive of LGTIs were recorded and categorised as absent, mild, moderate or severe. The diagnosis of BV was made using Nugent's scores.[15,16] BD nucleic acid amplification tests (BD Probetec Assays, Sparks) were performed on all cervical swabs to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. In addition, polymerase chain reaction amplifications of DNA isolated from vaginal swabs were performed as

described previously to detect *Mycoplasma genitalium*, *Trichomonas vaginalis* and herpes simplex virus type 2.[26,27]

Statistical analyses

Categorical variables were expressed as proportions (%), and quantitative (discrete ordinal) variables were presented as mean (± standard deviation). For univariate analyses, the χ^2 test was used to compare proportions, while the R coefficient was computed to analyse the correlations between quantitative variables. Furthermore, univariate association between HIV RNA detectability (in plasma or vagina) and putative risk indicators was defined by odds ratios (ORs) with 95% confidence intervals (CIs). For multivariate analyses, linear multiple and logistic regressions were used to identify independent determinants associated with HIV RNA detectability in the vagina or plasma. Multivariate ORs (95% CI) of HIV RNA genital/plasma detectability were computed after adjusting for confounding factors. Excess risks were measured using the risk difference method.

All tests were two-sided and $p<0.05$ was considered significant. Data were analysed using SPSS® statistical software version 21.0 (SPSS Inc., Chicago, USA).

Results

The median age of the 60 women enrolled in the study was 30 years (range 18 - 46). All were black Africans.

Plasma HIV RNA was detected in 42 (70%) patients. The mean duration on HAART was 4.2 (±1.6) months for patients with detectable plasma HIV RNA (CD4⁺ T-cell count <350 cells/µl) while for the remaining 18 patients (CD4⁺ T-cell count ≥350 cells/µl) it was 10.7 (±1.4) months ($p<0.0001$).

Of the 42 women with detectable plasma HIV RNA, 12 (28.6%) had detectable genital HIV RNA. Only one patient had detectable genital HIV RNA in the absence of detectable plasma HIV RNA. Plasma HIV RNA levels ranged from 2.5 (±0.8) $\log_{10}$ copies/ml to 4.1 (±0.8) $\log_{10}$ copies/ml. Genital HIV RNA levels ranged from 1.4 $\log_{10}$ copies/ml to 2.5 (±1.1) $\log_{10}$ copies/ml (Table 1). Eight patients out of 12 (66.7%) with detectable genital HIV RNA had plasma HIV loads >10 000 copies/ml, while the remaining patients had plasma HIV loads of between 20 and 9 999 copies/ml. Fig. 1 shows the distribution of plasma HIV loads and genital HIV RNA detectability according to duration of HAART.

All patients were on HAART regimen 1, namely two NRTIs and one NNRTI. No patient received PIs.

The univariate analysis (Table 2) showed a significant association between symptomatic VVC and plasma HIV RNA detectability ($p=0.006$), and patients who complained of symptoms suggestive of symptomatic VVC had significantly increased plasma HIV RNA levels ($p=0.039$). In addition, a recent history (within the past 3 months) of STIs ($p=0.035$) and patients age <35 years ($p=0.018$) were also significantly associated with detectable HIV RNA in the plasma. Furthermore, the presence of both advanced and severe HIV-associated immunosupression was significantly associated with HIV RNA detectability in the plasma ($p<0.0001$). In contrast, no patient with a CD4⁺ T-cell count

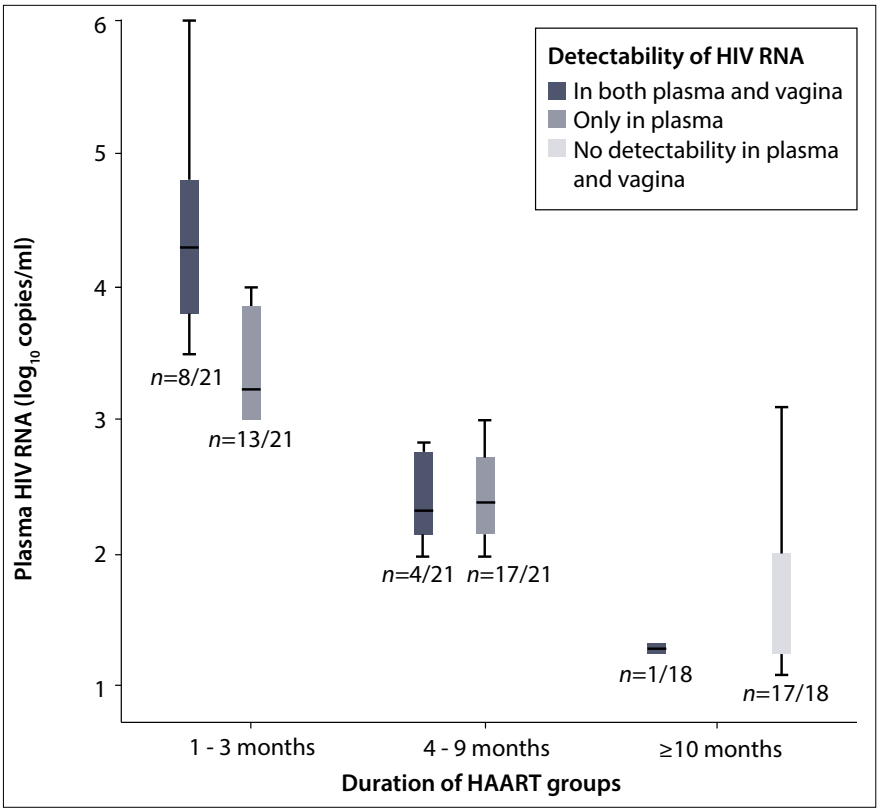

Fig. 1. Distribution of plasma HIV loads and genital HIV RNA detectability among HAART recipients (n=60). (HAART = highly active antiretroviral therapy.)

Table 1. HIV RNA levels in plasma and GT by HAART duration (*N*=60)

HAART duration (months)	Patient groups (*n*)	VL in plasma ($\log_{10}$ copies/ml), mean (±SD)*	VL in vagina ($\log_{10}$ copies/ml), mean (±SD)*
0 - 3	1A (*n*=8/21)	4.1 (±0.8)	2.5 (±1.1)
	1B (*n*=13/21)	3.3 (±1.4)	<1.3 (not detectable)
4 - 9	2A (*n*=4/21)	2.5 (±0.9)	1.6 (±0.5)
	2B (*n*=17/21)	2.9 (±1.1)	<1.3 (not detectable)
≥10	3A (*n*=1/18)	<1.3 (not detectable)	1.4
	3B (*n*=17/18)	<1.3 (not detectable)	<1.3 (not detectable)

GT = genital tract; HAART = highly active antiretroviral therapy; VL = viral load; SD = standard deviation.
*ANOVA *p*-value, $p<0.0001$.

Table 2. Univariate analysis of factors associated with plasma HIV RNA levels in HIV-positive women receiving HAART (*N*=60)

Associated factors	Detectable plasma HIV RNA, *n* (%)	*p*-value
Age groups (years)		0.018
18 - 24	11 (84.6)	
25 - 34	25 (75.8)	
≥35	6 (42.9)	
Symptomatic VVC (OR 6.1, 95% CI 1.5 - 24.1)		0.006
Yes	23 (88.5)	
No	19 (55.9)	
Presenting complaints (OR 5.7, 95% CI 0.9 - 34.7)		0.039
Group 1 (vulval itching, soreness and discharge)	40 (74.1)	
Group 2 (lower abdominal pain, vaginal discharge)	2 (33.3)	
Recent history (past 3 months) of STIs		0.035
Vaginal discharge syndrome	26 (78.8)	
Genital ulcer syndrome	6 (85.7)	
No STI diagnosed	10 (50.0)	
CD4⁺ T-cell count (cells/µl)		<0.0001
<200	22 (100)	
200 - 349	20 (100)	
≥350	0 (0)	
Genital HIV load categories (copies/ml)		0.062
≥10 000	2 (100)	
20 - 9 999	10 (90.0)	
<20	30 (65.2)	
Genital absolute neutrophil counts		0.095
>20 cells/5 HMFs	12 (60.0)	
11 - 20 cells/5 HMFs	13 (92.9)	
1 - 10 cells/5 HMFs	17 (65.4)	

HAART = highly active antiretroviral therapy; VVC = vulvovaginal candidiasis; OR = odds ratio; CI = confidence interval; STIs = sexually transmitted infections; HMFs = high microscopic fields.

≥350 cells/µl had detectable plasma HIV RNA.

In the multivariate analysis (Table 3), logistic regression was used to identify independent determinants of HIV RNA levels in plasma after adjusting for univariate confounding factors (presenting complaints and recent history of STIs). CD4⁺ T-cell counts were not included in the logistic regression model because all patients with detectable plasma HIV RNA had either advanced or severe immunosupression.

Multivariate analysis showed that a current episode of symptomatic VVC was independently (p=0.008) associated with detectable HIV RNA in the plasma. It was also found that HIV-infected women aged <35 years were more likely to have higher HIV RNA levels in their plasma than women aged ≥35 years (p=0.039) (Table 3).

Factors associated with genital HIV RNA shedding

Of the 42 women with detectable plasma HIV RNA, 12 (28.6%) had detectable levels of HIV RNA in cell-free vaginal fluid.

Table 4 shows univariate factors associated with genital HIV RNA detectability among the patients. Women with a recent history of vaginal discharge syndrome (p=0.05) and genital ulcer syndrome (p=0.038) were more likely to have detectable HIV RNA in their GTs (Table 4). However, the data showed no association between gonococcus, VVC or BV and genital HIV shedding.

Women with a neutrophil cell count score of >1 had a five-fold higher risk (OR 5.3, 95% CI 1.6 - 17.2; p=0.004) of having detectable HIV RNA in their GTs than women with a neutrophil cell count score of 1. A recent history of use of broad-spectrum antimicrobial agents was shown to be associated with

Table 3. Independent determinants of plasma HIV RNA levels in HIV-infected women receiving HAART*(*N*=60)

Independent variables	β-coefficient	Standard error	Wald χ^2	OR (95% CI)	*p*-value
Age groups (years)					0.039
18 - 24	2.328	1.043	4.978	10.3 (1.3 - 79.2)	0.026
25 - 34	1.765	0.789	5.001	5.8 (1.2 - 27.5)	0.025
≥35			Reference	1	
Symptomatic VVC					
Yes	2.082	0.790	6.943	8 (1.7 - 37.7)	0.008
No			Reference	1	
Constant	–1.236	0.719	2.954		0.086

HAART = highly active antiretroviral therapy; OR = odds ratio; CI = confidence interval; VVC = vulvovaginal candidiasis.
*Adjusted for presenting complaints and recent history of sexually transmitted infections.

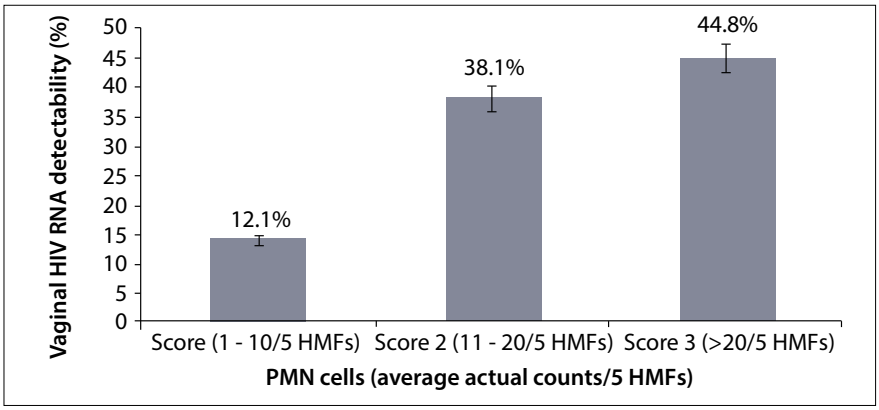

Fig. 2. Distribution of vaginal HIV RNA detectability by PMN cell groups (p=0.005). (PMN=polymorphonuclear neutrophil; HMFs = high microscopic fields.)

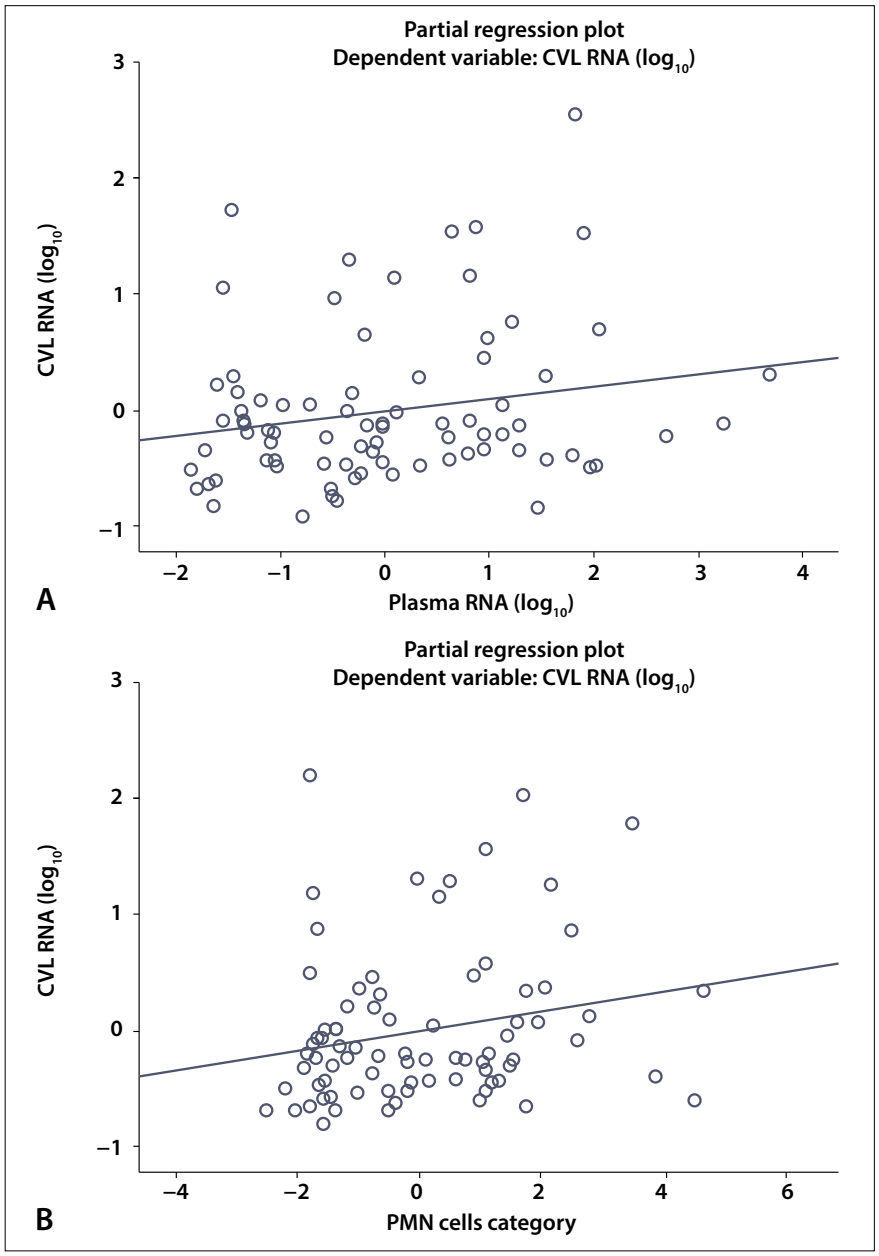

Fig. 3. Variation of genital HIV RNA levels as predicted by (A) plasma HIV loads (r=0.2, R^2=0.04; p=0.032) and (B) genital PMN cells (r=0.21, R^2=0.045; p=0.007). (CVL = cervicovaginal lavage; PMN = polymorphonuclear neutrophil.)

detectable HIV shedding in the univariate analysis (p=0.048) (Table 4).

In the multivariate analysis, after adjusting for the presence of univariate confounding factors (use of broad-spectrum antibiotics and recent history of STIs), an increase in the vaginal absolute neutrophil counts (p=0.007) and plasma HIV RNA levels (p=0.032) remained independently associated with genital HIV shedding (p=0.005) (Table 5; Fig. 3). Plasma VL (20 - 9 999 copies/ml) tended to correlate positively with genital HIV shedding, but this correlation did not reach statistical significance (p=0.166). However, plasma HIV RNA levels ≥10 000 copies/ml (p=0.032) were independently associated with genital HIV RNA detectability to a detection limit of 20 copies/ml (Table 5).

Furthermore, when using transformed $\log_{10}$ HIV RNA values, correlations between plasma HIV RNA levels and genital PMN cell counts >10 cells/5 HMFs with detectability of genital HIV RNA shedding showed significant associations (Fig. 3). Detectability and levels of mean $\log_{10}$ HIV RNA in the vaginal fluid increased with the increase of plasma HIV RNA levels ($\log_{10}$) (r=0.2, R^2=0.04; p=0.032) (Fig. 3A) and increased absolute counts of genital PMN cells (r=0.21, R^2=0.045; p=0.007) (Fig. 3B).

Discussion

Inflammation of the GT during LGTIs, particularly STIs and BV, has been reported to predict increased plasma VLs in HIV-infected women.[28,29] In this study, given that the majority of women had just initiated HAART (and therefore had a high rate of detectable plasma HIV RNA and low $CD4^+$ counts), there was insufficient evidence to demonstrate that VVC was predictive of high plasma HIV RNA levels. Although cause and effect was difficult to unravel in the current study design, it seemed more likely that the observed higher level of immunosuppression among the study participants influenced the occurrence of VVC. In addition, plasma HIV loads and local genital inflammation were predictors of genital HIV shedding, but did not predict the presence of VVC. Women aged <35 years were more likely to have increased plasma HIV RNA levels than women aged ≥35 years. A previous study of the relationship between age and plasma VL in HIV-infected individuals showed that older ages (>50 years) compared with individuals aged 18 - 39 years were associated with lower levels of HIV-1 replication.[30] This finding was independent of

antiretroviral therapy use, regimen adherence and disease stage. One possible explanation of the relationship between the plasma VL and age category would be immunological differences. It has been shown that older age was associated with an increase in peripheral CD4⁺ and CD8⁺ T lymphocytes and that thymic involution was associated with this effect, leading to lower expression of plasma HIV-1 RNA.[30]

Table 4. Univariate analysis of factors associated with genital HIV RNA detectability in HIV-infected women receiving HAART (*N*=60)

Associated factors	Detectable genital HIV RNA *n* (%)	OR (95% CI)	*p*-value
Current STIs			
Trichomonas vaginalis		0.7 (0.3 - 2.1)	0.554
Positive	6 (25.0)		
Negative	18 (31.6)		
Chlamydia trachomatis		0.8 (0.2 - 2.9)	0.781
Positive	4 (26.7)		
Negative	20 (30.3)		
Neisseria gonorrhoeae		1.2 (0.3 - 5.3)	0.796
Positive	3 (33.3)		
Negative	21 (29.2)		
Mycoplasma genitalium		0.5 (0.1 - 4.1)	0.478
Positive	1 (16.7)		
Negative	23 (30.7)		
Herpes simplex virus type 2		0.7 (0.6 - 0.8)	0.183
Positive	0 (0)		
Negative	24 (31.2)		
Other lower GT infections			
VVC		1.2 (0.5 - 3.2)	0.649
Infection	13 (32.5)		
Colonisation	12 (27.9)		
BV		1.2 (0.4 - 4.3)	0.747
Positive	21 (30.9)		
Negative	4 (26.7)		
Recent history (past 3 months) of STIs			
Vaginal discharge syndrome	14 (36.8)	3.2 (1.0 - 10.1)	0.050
Genital ulcer syndrome	6 (46.2)	4.6 (1.1 - 19.7)	0.038
No STI	5 (15.6)	1	
Genital absolute neutrophil counts		5.3 (1.6 - 17.2)	0.004
Score >1	21 (42.0)		
Score = 1	4 (12.1)		
Plasma HIV RNA levels			<0.0001
≥10 000 copies/ml (≥4 $\log_{10}$)	8 (100)		
20 - 9 999 copies/ml (1.3 $\log_{10}$ - 3.99 $\log_{10}$)	4 (11.8)		
<20 copies/ml (<1.3 $\log_{10}$)	1 (5.6)		
Antibiotic use		3.2 (1.01 - 10.60)	0.048
Yes	21 (36.8)		
No	4 (15.4)		

HAART = highly active antiretroviral therapy; OR = odds ratio; CI = confidence interval; STIs = sexually transmitted infections; GT = genital tract; VVC = vulvovaginal candidiasis; BV = bacterial vaginosis.

Studies of the association between HIV VL in the female GT and in the plasma have shown conflicting results. In this study, 28.6% of the patients shed HIV in their genital secretions. This finding is consistent with previous reports of HIV-1 DNA and/or RNA being detected in the GT of 28 - 60% of HIV-1-seropositive women.[29,31-33] These previous studies also demonstrated that genital HIV-1 was more likely to be detected from either the cell-associated fraction of the cervicovaginal fluid or whole genital fluid than from cell-free vaginal fluid.[29,31-33] Although within the published range, our finding of a relatively low frequency of viraemia in the GT of our study population may be attributed to the type of sample that was analysed (measurement of HIV loads in cell-free fraction alone). It is difficult to conclude that antiretroviral therapy significantly lowered HIV RNA levels in genital secretions, given the fact that the women had just started HAART.

We found that women with a recent history of vaginal discharge syndrome and genital ulcer syndrome were more likely to shed HIV in their GTs than women without a recent history of such STIs. However, after testing for the most common causes of LGTIs, none of the tested aetiological agents of vaginal discharge syndrome (VDS) showed significant association with genital HIV shedding.

The fact that HIV VL was only measured from the cell-free fraction of genital secretions may account for the lack of an association between GT viraemia and tested causes of VDS, including symptomatic VVC.

We identified two independent factors associated with HIV RNA shedding in the vagina; of these factors, one had been previously identified, namely the association between higher levels of plasma HIV RNA and the ability to detect HIV in genital secretions.[31,34] Consistent with previous studies, we found that genital HIV RNA detectability strongly correlated with plasma HIV VL, but that these correlations were weak, except when plasma HIV VL was ≥10 000 copies/ml. Other studies have suggested that compartmentalisation of the vaginal immune response from systemic immunity may account for such weak correlations.[35,36] This might also be explained by the fact that HIV VL was only measured from the cell-free fraction of the genital secretions.

Table 5. Independent determinants of HIV RNA detectability in GT of HIV-infected women receiving HAART* (*N*=60)

Independent variables	β-coefficient	Standard error	Wald χ^2	OR (95% CI)	*p*-value
Plasma HIV RNA (copies/ml)					
≥10 000	2.062	0.962	4.593	7.9 (1.2 - 51.8)	0.032
20 - 9 999	1.168	0.843	1.918	3.2 (0.6 - 16.8)	0.166
<20			Reference	1	
Vaginal PMN cells					
Score >1	1.758	0.650	7.308	5.8 (1.6 - 20.8)	0.007
Score = 1			Reference	1	
Constant	−1.123	0.518	2.980		0.005

GT = genital tract; HAART = highly active antiretroviral therapy; OR = odds ratio; CI = confidence interval; PMN = polymorphonuclear neutrophil.
*Adjusted for HAART, ATB use and previous STIs.

We also found that the absolute neutrophil count in the genital mucosa was another factor associated with genital HIV RNA shedding. Previous studies have shown the role of genital inflammation in HIV-1 shedding.[8,37] It has been reported that following HIV-1 infection, cytokines and chemokines are produced by infected leukocytes that attract more target cells for HIV-1 infection and allow further stimulation of the expression of HIV-1 via toll-like receptors.[8-10] In this study, we found that the number of neutrophils in the female GT was associated with HIV shedding. Of particular interest, our study did not show an association between symptomatic VVC and HIV shedding in the vagina. In addition to the fact that HIV was quantified only in the cell-free fraction of genital fluids, this lack of association could also be the result of strong recruitment of neutrophils in the vagina – various immunomodulators have been reported as associated with the anti-*Candida* activity of neutrophils.[35,36] Cytokines such as TNF-α and IL-8 increase the activity of neutrophils against *Candida albicans*.[35] Gumbi *et al.*[36] reported that women who were detectably shedding HIV-1 in the GT had significantly increased cervical levels of TNF-α, IL-1β, IL-6 and IL-8 compared with women who were not detectably shedding the virus. It remains unclear from our observations whether or not symptomatic VVC is more likely to occur among HIV-infected women who are not detectably shedding the virus.

Another possible reason why symptomatic VVC was not associated with genital HIV shedding could be the challenges associated with the diagnosis of VVC. A clinical definition of VVC could be problematic even when microscopic tests and cultures of genital samples are used to support the clinical diagnosis. While Gram staining has a sensitivity of 75% in supporting the clinical diagnosis of VVC, up to 30% of women with significant chronic symptoms suggestive of VVC may have negative cultures at presentation.[38]

Limitations

Limitations of this study include its cross-sectional design and small sample size. In addition, the impact of factors such as HAART regimen composition, adherence to HAART and effects of HIV disease stages were not explored.

The majority of our patients had just started HAART. Therefore, it is difficult to say that they were not successfully treated. A plausible hypothesis for why we did not find an association between VVC and genital HIV shedding could have been the fact that HIV RNA was only measured from the cell-free fraction of cervicovaginal secretions. If both HIV complementary DNA and HIV RNA had been measured, results could have been different. This might also explain why plasma HIV loads <10 000 copies/ml were not significantly associated with genital HIV shedding in the multivariate logistic analysis.

Finally, findings present insufficient evidence of the true impact of VVC on HIV RNA levels in plasma and genital secretions.

Conclusions

Identified factors associated with HIV RNA loads in plasma and female genital secretions were consistent with previously published factors from the literature. Of particular note, our study did not have enough evidence to demonstrate that symptomatic VVC was predictive of higher levels of HIV RNA in plasma and genital secretions. However, unlike STIs and BV, symptomatic VVC was less likely to occur among HIV-infected women who were detectably shedding the virus in their lower GT, possibly owing to the observed strong recruitment of neutrophils that possess anti-*Candida* activity or the challenges associated with the diagnosis of VVC. About 70% of the study population had detectable HIV RNA in their plasma; this finding was supported by the fact that all these patients had a $CD4^+$ T-cell count <350 cells/μl, which is a marker of systemic immunosuppression. It is highly likely that the observed higher rate of HIV RNA detectability was purely the result of immunosuppression and that the associated high prevalence of symptomatic VVC could have been a consequence of overall immunosuppression. Further studies are required to ascertain the impact of VVC on HIV RNA levels in plasma and genital secretions.

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Fatal nevirapine-induced Stevens-Johnson syndrome with HIV-associated mania

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Mania with psychotic features is one of the common presenting clusters of psychiatric symptoms in HIV-infected patients. Commonly, patients with HIV-associated mania receive antiretroviral treatment, mood stabilisers and antipsychotics. This case of Stevens-Johnson syndrome highlights the dilemmas and complications that may arise when prescribing multiple medications in HIV-associated psychiatric disorders.

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HIV enters the central nervous system early in the course of HIV infection and causes a range of neuropsychiatric complications, including HIV encephalopathy, depression, mania, cognitive disorders and frank dementia.[1] Mania is one of the most common psychiatric presentations in HIV-infected patients and requires antiretroviral therapy (ART), mood stabilisers and antipsychotics to increase patient quality of life and decrease mortality.[1-3] ART may also protect from further cognitive deterioration and preserve functionality.[1]

The link between systemic hypersensitivity reactions and nevirapine has been well documented.[4] Case reports have also demonstrated adverse cutaneous reactions associated with the use of sodium valproate and risperidone.[5,6] This report describes the case of an HIV-seropositive patient who presented with mania for the first time and was treated with nevirapine, sodium valproate and risperidone. He developed Stevens-Johnson syndrome (SJS), which progressed to toxic epidermal necrolysis (TEN) and death over a period of 26 days.

Background

SJS/TEN comprises cutaneous adverse reactions ranging from mild erythematous macules to extensive epidermal detachment and mucous membrane erosion. The international classification of SJS/TEN is based on the body surface area (BSA) involved: SJS involves <10% of BSA; TEN involves >30%; and there is an overlap in definitions with involvement of 10 - 30%.[7]

Mortality associated with SJS, SJS/TEN and TEN is 10%, 30% and >50%, respectively.[7] Common causes of death include septic shock, hypovolaemic shock, acute renal failure and fulminant hepatitis.[8] The most common drugs implicated are sulphonamide antibiotics (38%) and nevirapine (20%).[9] An immunological response involving $CD8^+$ T lymphocytes is the most likely explanation for the pathogenesis of SJS/TEN.[10] Other potential factors are the causative drug's inherent properties or chemical structure, patient factors such as HIV status and $CD4^+$ count, ethnic background, age and gender. Affected individuals with SJS/TEN are genetically predisposed to developing severe cutaneous reactions based on the major histocompatibility complex molecules on their leukocyte cell surface.[10] A study in Taiwan showed that 100% of Han Chinese patients who developed SJS in response to carbamazepine had an HLA B*1502 allele.[11] Comorbidity of SJS/TEN and HIV is posing a challenge in sub-Saharan Africa due to the high prevalence of HIV. In this setting, sulphonamides and nevirapine are the most commonly implicated drugs.

Case report

A 28-year-old man was referred to the mental health unit (MHU) with a 3-week history of manic symptoms, presenting for the first time. He was irritable, displayed pressure of speech, and had decreased need for sleep, increased drive, hyper-religiosity, impulsivity (reckless spending of money) and auditory hallucinations. He tested positive for HIV and syphilis (rapid plasma reagin (RPR) titre of 1:8). His $CD4^+$ count was 334 cells/µl and he was not on ART. Cerebrospinal fluid analysis was negative for neurosyphilis. Chest X-ray and GeneXpert on sputum excluded tuberculosis. Treatment initiated on admission was 3 mg risperidone daily, 500 mg sodium valproate twice daily and penicillin.

On day 3 after admission, he was prescribed 300 mg zidovudine twice daily, 150 mg lamivudine twice daily and 200 mg nevirapine daily. The nevirapine dose was to be doubled to 200 mg twice daily after 14 days. His psychiatric symptoms responded well to treatment and he was discharged on day 8 post admission. On day 7 he had complained of a mild rash on his hands and nevirapine was suspected as the cause. ART was continued, and 4 mg chlorphenamine three times daily and hydrocortisone 1% cream were prescribed. Review at follow-up on day 3 and day 6 post discharge revealed that the rash had settled and he remained physically and mentally well. The nevirapine dose was increased, as per treatment plan, on day 10 post discharge. He presented at his next review appointment on day 13 post discharge with

a severe SJS/TEN reaction (Fig. 1), which had developed on day 10 post discharge, coinciding with the increase in nevirapine. He had continued his treatment and had waited for his next follow-up appointment before reporting the rash. He was re-admitted to the intensive care unit where his condition deteriorated, with death occurring on day 6 of his second admission. Death was thought to be related to infection and renal failure.

The patient was initiated on ART while admitted to the MHU, which is common in our setting and other parts of South Africa (SA) as ART initiation may help to ameliorate HIV-associated neuropsychiatric symptoms and potentially improve cognitive function in particular. In this case, the choice of ART regimen was influenced by a number of factors, including the safety profile of both efavirenz and nevirapine, the risk of ART-induced neuropsychiatric side-effects (more commonly seen with efavirenz), the patient's wishes, informed consent given for further management of his condition, the applicable World Health Organization staging and the latest SA National Department of Health ART guidelines.[12] The fatal outcome raises questions regarding the initiation of ART in patients with similar presentations and the role played by the following factors in the development and clinical outcome of SJS/TEN: HIV infection, $CD4^+$ count, causative drug, age, gender and use of steroids during the acute stages. This case emphasises the need for further research into these factors to aid clinicians in decision-making when it comes to safe options for HIV-associated mania.

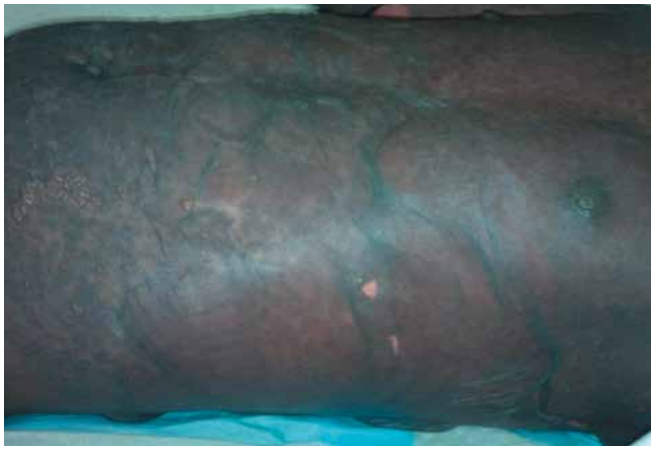

Fig. 1. Severe SJS/TEN affecting trunk and upper limbs. (SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis.)

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Needlestick injury in a pregnant inpatient in an overcrowded hospital

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Needlestick injury (NSI) is commonly reported among healthcare workers, but is not well documented in patients. We report a case of an NSI in an HIV-negative, gestational hypertensive patient admitted to a hospital for induction of labour at term. Owing to an insufficient number of hospital beds, patients were seated in an overcrowded corridor of the antenatal ward where a patient stepped on the needle of an inadvertently disconnected intravenous infusion set of another pregnant patient, who was HIV-infected. The injury occurred prior to labour induction. Antiretroviral post-exposure prophylaxis to prevent HIV infection was administered to the injured patient and her newborn. This report illustrates how hospital bed shortage may compromise patient safety and discusses measures to prevent NSI among patients and hospital overcrowding.

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Despite the availability of medical safety programmes,[1] more than 10% of patients suffer harm during hospital care.[2] The occurrence of needlestick injury (NSI) in an inpatient in particular raises serious safety concern. There are reports of NSIs sustained by healthcare workers (HCWs),[3-5] but there is no publication, to our knowledge, on such injuries occurring among inpatients. We discuss an NSI in a pregnant inpatient and outline appropriate measures to prevent such incidents.

Case report

A 25-year-old primigravida at term, who had gestational hypertension, was referred to hospital for further care. On arrival at the hospital her blood pressure was 143/93 mmHg and she was admitted to the antenatal ward for work-up and labour induction. Her antenatal care was uneventful prior to developing hypertension. Her risk factors for this condition were primiparity and obesity (body mass index of 30 kg/m^2 at booking). The clinical work-up showed no evidence of proteinuria, target organ damage or intrauterine growth restriction. During the first two days of her hospital admission, all the available beds in the antenatal ward were occupied, as the number of patients was twice the number of beds. As a result, the patient had to wait in the corridor of the antenatal ward until an empty bed became available. In this article she is referred to as the injured patient.

Another patient, referred to as the source patient, was seated on a chair in the corridor of the antenatal ward and was receiving intravenous fluids through infusion set A (Fig. 1). To this was attached a second intravenous infusion set, set B, through a needle at site X on set A, to administer medication. The needle of set B became disconnected without being discovered by the source patient or the nurses. The source patient had been diagnosed HIV-positive 3 years previously and was on antiretroviral therapy (ART) for maternal health. The injured patient, having waited for approximately 48 hours without a bed, took to walking during the day and on her way to join other patients seated on chairs in the corridor of the antenatal ward, stepped on the disconnected needle of set B and sustained an NSI on her right big toe.

The patient reported the adverse event to the medical staff on duty and the following immediate measures were taken: blood was squeezed from the injured site; and the site was washed liberally with water and cleaned with an antiseptic solution. The patient was counselled and tested HIV-negative. She was immediately initiated on a 4-week course of prophylactic ART as per hospital policy. Screening for hepatitis in both patients was negative, and a full blood count and liver and renal function tests of the injured patient were normal. Following hospital discharge of other stable pregnant women, the source and the injured patients were provided with beds for their continued medical care. A day after the incident, the injured patient was started on misoprostol to induce labour, but had a caesarean delivery due to fetal compromise and delivered a normal healthy boy (weight 2 870 g) who was started on a 6-week course of nevirapine syrup, 15 mg daily, as recommended by the neonatologist.

Fig. 1. Intravenous infusion set in use, showing an injection site for administration of supplementary medication. (X = injection site where an additional intravenous infusion set was connected.)

The injured patient and her baby were discharged in a stable condition on the 3rd day after delivery. They completed the ART prophylaxis without any side-effects. Six weeks after the incident, they remained healthy, tested HIV-negative and were scheduled for further HIV testing at 3 and 6 months.

Discussion

When the optimal patient care capacity of a healthcare system or provider is exceeded, patient safety is compromised,[6] as was seen in this case. At the time of the NSI, the number of patients in the ward was more than the available beds and the nurses who were on duty during the incident had to perform menial functions that prevented them from monitoring patients closely. The lighting was good where the injury occurred and the injured patient had no visual or physical impairment, but she may have been tired, given that she had no bed at the time of the injury.

The health facility where this incident occurred had a policy on NSI for HCWs, but owing to the rarity of NSI in patients, no policy was available for this scenario. The hospital management were aware that the number of patients in the antenatal ward usually exceeds the available beds, and had a long-term plan to construct additional wards to prevent overcrowding. Nonetheless, the adverse incident was reported to the hospital management and the interim actions taken included starting the process of converting sections of other less busy wards with extra beds to antenatal ward extensions, and deployment of additional staff to the antenatal ward.

To our knowledge, this is the first reported case where: (*i*) a pregnant inpatient had an NSI; and (*ii*) nevirapine was administered to a baby due to an NSI sustained by the mother. Nevirapine was used as it is recommended for prevention of mother-to-child transmission (PMTCT) of HIV in South Africa.[7] Owing to the urgent need to avert further NSIs in patients, particularly in overcrowded health facilities, we outline preventive measures to be taken before the injured patient and her baby complete their follow-up visits (Table 1). These consist of components relating to: health facility administration, including developing a patient NSI policy; HCWs, including safe use of needle-containing devices; and patients and their visitors adhering to the patients' rights charter and hospital policies. These interventions are based on the authors' many years of clinical experiences in different countries. Nonetheless, different settings may require other interventions and the personnel responsible for implementing a particular task may vary.

Conclusion

NSI in inpatients has not been reported previously. The lesson is that it can occur, although it is preventable using the suggested measures.

Table 1. Measures to prevent NSI in patients at different levels of the healthcare system

Administration	
Develop a policy on NSI sustained by patients	Provide appropriate staffing
Organise continuing medical education on NSI for staff and medical trainees	Provide clerks, porters and phlebotomists in the wards so that nurses/doctors can concentrate on their duties
Ensure good record keeping so that quality data are available for audit	Provide social workers, clinical psychologists, infectious disease physicians, occupational health personnel and appropriate medications to aid in managing any patient who sustains an NSI
Organise quality improvement projects on patient safety and NSI	Promote the treatment and prevention of HIV infection
Ensure that patient safety is addressed as part of HCW training	Promote vaccination against hepatitis
Ensure that staff are aware of what to do in the event of an NSI	Provide good leadership and staff motivation
Improve structural facilities of the health institution as the need arises	Promote teamwork among staff in the health facility
Provide good lighting in the hospital environment	Promote an awareness of patients' rights charter
Ensure a regular supply of clean water to the health facility	Regulate visiting hours in the health facility
Provide appropriate equipment for patient care	Provide appropriate laboratory facilities
To address patient overcrowding of a health facility: provide additional space where patients can be cared for; support referral of patients to alternative facilities; redeploy additional staff to work in the overcrowded unit; educate patients; support the establishment of a primary healthcare centre within or near the health facility for management of low-risk patients; if possible ensure that patients do not share one bed; inform district/ municipal authority in charge of overcrowding should it persist despite appropriate measures having been taken, etc.	Establish appropriate referral routes
HCWs	
Adhere to guidelines on NSIs	Avoid inappropriate prescription of parenteral therapies
Educate patients on what to do if there is an NSI	Use needle-free devices where possible, e.g. needle-free syringes and intravenous infusion sets
Show empathy, support and provide medical care to any patient who sustains an NSI	Ensure that all intravenous fluid administration sets are properly fastened to prevent inadvertent disconnection
Report NSIs	Monitor patients with intravenous/intra-arterial lines
Adhere to treatment protocols and consult senior colleagues for advice if necessary	Remove intravenous access as soon as there is no indication for their use
Manage patients at risk of injury (such as children, the mentally ill, the blind and unconscious patients, etc.) appropriately	Adhere to standard operating procedures regarding the safe use/ sterilisation of equipment and waste disposal
Counsel patients before any medical procedure	Avoid unnecessary admission of patients for inhospital care
To address patient overcrowding of a health facility: consider discharging stable patients; inform the supervisor; discuss with management, etc.	Engage in community health promotion so as to prevent illnesses
Patients and their visitors	
Ask questions when in doubt	Report concerns to HCWs (and to management if not satisfied)
Adhere to medical advice	Be aware of patient and visitor rights
Adhere to health policies such as appropriate referral routes and facility visiting hours	

NSI = needlestick injury; HCW = healthcare worker.

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Antiretroviral therapy-induced Leber's hereditary optic neuropathy

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Optic neuropathy in HIV-infected patients results from the HIV infection itself, post-infectious auto-immune disease, opportunistic infections and drugs. Nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine and stavudine have known mitochondrial toxicity and can cause mitochondrial myopathies, neuropathies, hyperlactataemia, and can induce mitochondrial genetic disorders. Individuals with the mutation for Leber's hereditary optic neuropathy (LHON), a mitochondrial disorder, are usually asymptomatic but develop visual loss when exposed to external triggers such as smoking. We report on two HIV-infected patients with LHON mutations (*m.14484T>C* and *m.11778G>A*) who developed profound visual loss with antiretroviral therapy. We postulate that the phenotypic expression of LHON in these genetically predisposed individuals was triggered by NRTI drugs lamivudine and tenofovir when used in combination, despite their relatively weak mitochondrial toxic effects.

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Optic neuropathy in HIV disease is due to the virus itself, para-infectious disease, opportunistic infections, compression, raised intracranial pressure or drug therapy.[1] Hence it is no surprise that visual loss from optic nerve disease is common in patients infected with HIV. Epidemiological studies are lacking, especially in South Africa (SA) where the prevalence of HIV is high.

The causes of HIV-associated optic neuropathies that are commonly encountered in clinical practice are listed in Table 1. The immune-mediated optic neuropathies are para-infectious and occur in the setting of seroconversion or as part of acute disseminated encephalomyelitis when $CD4^+$ counts may be relatively normal; these often respond to intravenous steroid therapy. Infectious and lymphomatous optic neuropathies occur during advanced stages of immunocompromise when $CD4^+$ counts are low; in such patients, identifying an infective cause is urgent to prevent blindness. Ethambutol-associated retrobulbar optic neuropathy is common, especially in disseminated tuberculosis where its prolonged use over 9 months is advocated.

The optic nerve is unique as it is a white matter tract that is particularly susceptible to physical, ischaemic, toxic and hypoxic insults due to: its relatively large diameter (1.2 million axons per nerve); its intra- and extra-cranial location; its myelinated and unmyelinated segments; and the high energy demand by the retinal ganglion cells.[2] The large accumulation of mitochondria within the optic nerve head is mostly responsible for its energy supply.[3] The function of these mitochondria is disturbed in the setting of Leber's hereditary optic neuropathy (LHON), where gene defects from base substitutions at *11778G>A*, *14484T>C* and *3460G>A*

Table 1. Common causes of HIV-associated optic neuropathy

Immune mediated
Isolated seroconversion optic neuritis
Acute disseminated encephalomyelitis
Infectious optic neuritis
Bacterial: syphilis, tuberculosis
Viral: cytomegalovirus, varicella zoster virus, herpes simplex virus
Parasitic: toxoplasmosis
Fungal: cryptococcal meningitis
Raised intracranial pressure
Cryptococcal-induced optic neuropathy
Infiltrative optic neuropathy
Lymphoma
Nutritional optic neuropathy
Vitamin B_{12} deficiency
Drug induced
Ethambutol
Antiretrovirals

cause optic nerve dysfunction in over 95% of patients by disrupting complex I-dependent adenosine triphosphate synthesis. The abnormal mitochondrial gene is maternally inherited with incomplete penetrance, as only 50% of males with the LHON mitochondrial mutation present with the disorder.[4] Expression of the disease occurs when triggered by environmental factors such as smoking, alcohol and acute illness. The typical presentation is sequential or bilateral, simultaneous central visual loss in the 2nd to 3rd decade of life in males carrying one of the LHON mitochondrial mis-sense mutations. Ten per cent of female carriers are symptomatic. There is initial disc swelling and circumpapillary telangiectasia, which within a few months are replaced by optic atrophy from axonal loss. Visual loss and centro-caecal scotomas progress over months to years. Idebenone, a co-enzyme Q10 derivative, has shown promise in delaying the progression of illness and in some cases substantially reversing the visual and field loss.[5]

We present two patients from our neuro-ophthalmology unit who presented with central visual loss after starting antiretroviral therapy containing tenofovir, lamivudine and efavirenz.

Patient 1

A 31-year-old HIV-infected man presented with a 4-month history of gradual and progressive loss of bilateral vision. He denied having headaches, pain on eye movements or constitutional symptoms. He was previously unemployed. Two years before, he acquired pulmonary tuberculosis and was found to be HIV-infected; he was then prescribed tenofovir, lamivudine and efavirenz. He had a four pack-year smoking history, did not consume alcohol and had no family history of visual loss.

His visual acuity was 'counting fingers' on the right and 'hand movements' on the left. Central field defects were detected bilaterally on confrontation, but were globally lost on automated Humphrey visual field (HVF) testing. Colour vision was severely impaired bilaterally (0/15 on Ishihara pseudo-isochromatic plates). Both pupils were sluggishly reactive and severe bilateral optic atrophy was present on funduscopy (Fig. 1). The visual evoked potential (VEP) P100 waveforms were obtainable bilaterally using goggles, but were markedly reduced in amplitude.

Magnetic resonance imaging (MRI) of the brain and orbits was normal. Chest radiograph and cerebrospinal fluid (CSF) examination, including neurotropic virus (cytomegalovirus, herpes simplex viruses 1 and 2, varicella zoster virus, Epstein-Barr virus and John Cunningham virus) testing were unremarkable and routine haematological investigations were normal. His vitamin B_{12}, folate and iron levels were normal. $CD4^+$ cell count was 746 cells/µl and viral load was undetectable. The antinuclear factor (ANF), antineutrophil cytoplasmic antibody (ANCA), rapid plasma reagin (RPR) and rheumatoid factor (RF) were negative, and serum angiotensin converting enzyme (SACE) was 19 U/l. Oligoclonal bands (OBs) were not detected in the CSF and aquaporin 4 antibodies were negative. He tested positive for the *14484T>C* mitochondrial mutation associated with LHON.

Patient 2

A 33-year-old HIV-infected man who worked in construction presented with bilateral visual loss over the previous 3 years. He had been diagnosed with HIV infection after voluntary testing and prescribed tenofovir, lamivudine and efavirenz 3 months before the onset of his visual symptoms. He first noticed loss of central vision in the left eye, followed by similar symptoms in the right a month later. He denied any colour desaturation and pain on eye movements, but did notice intermittent oscillopsia at the onset of his symptoms. He had no other medical history of note and denied any smoking or alcohol use, exposure to any toxins or substance abuse. He had no family history of visual loss.

His visual acuity bilaterally was 'counting fingers'. Central scotomas were noted on confrontation, but HVF showed bilateral, inferior altitudinal field defects. Colour vision was severely impaired (0/15 on Ishihara pseudo-isochromatic plates) and funduscopy revealed bilateral profound optic atrophy (Fig. 2). His pupils were reactive and a 1+ relative afferent pupillary defect was present on the right. The rest of his neurological examination was normal, and standard haematological, biochemical and CSF examination tests were normal. His $CD4^+$ count was 615 cells/µl and viral load was 27 copies/ml. MRI of the brain and orbits was normal, as was his chest radiograph. The VEP P100 wave amplitudes were reduced bilaterally. His vitamin B_{12} level was mildly reduced at 117 pmol/l (normal 133 - 675), serum folate was normal and SACE was

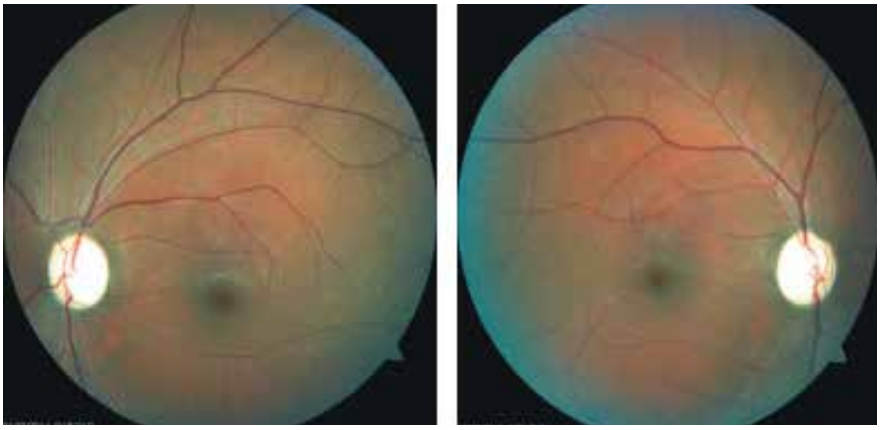

Fig. 1. Funduscopy of patient 1, showing bilateral optic atrophy and normal retinas.

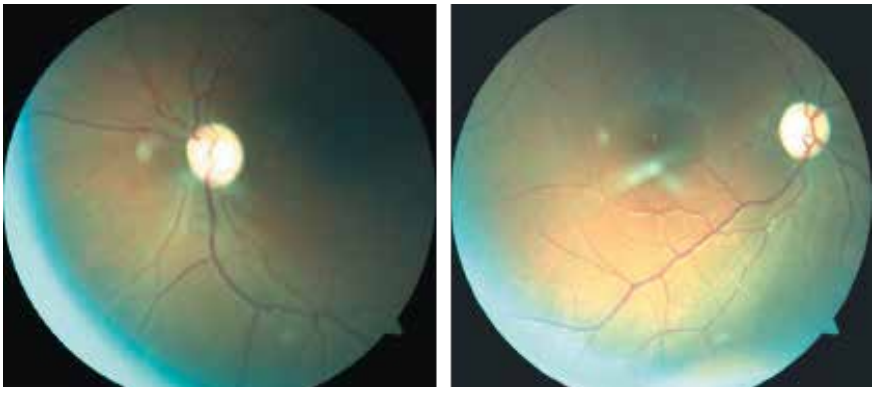

Fig. 2. Funduscopy of patient 2, showing bilateral optic atrophy and normal retinas.

27 U/l. ANF, ANCA, RF, RPR, human T lymphotropic virus 1, CSF OB and aquaporin 4 antibodies were negative. He tested positive for the *11778G>A* mitochondrial mutation associated with LHON.

Discussion

Genetic testing of LHON by polymerase chain reaction and restriction enzyme analysis is offered by the Inherited Metabolic Disease Laboratory, National Health Laboratory Service, based at Groote Schuur Hospital in Cape Town. All six LHON mutations in mitochondrial DNA are screened for, viz. *m.14484T>C*, *m.11778G>A*, *m.3460G>A*, *m.14459G>A*, *m.14482C>G*, and *m.14487T>C*. Both patients had a common mitochondrial mutation associated with LHON. Patient 1 was a smoker without a significant pack-year history and patient 2 had mild vitamin B_{12} deficiency, neither of which seemed to have contributed significantly as triggers for LHON, or to the visual loss on a toxic or nutritional basis. Both patients, however, were HIV-positive, developed their symptoms some time after starting ART and were not on other medication that could have contributed to visual impairment.

LHON following the introduction of the strongly mitochondrial toxic nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine, stavudine and zalcitabine (ddC) has previously been reported in patients with the mitochondrial mutations *11778G>A* and *14484T>C*.[6-8] No cases have been described with the weaker mitochondrial toxic NRTI drugs tenofovir and lamivudine until now. The catastrophic sequela of lactic acidosis that occurs with zidovudine and stavudine, however, is less likely to occur with the tenofovir-lamivudine combination. Tenofovir is a less toxic agent than zidovudine, didanosine, stavudine and zalcitabine, and does not usually cause lactic acidosis, myopathy and peripheral neuropathy. Its mechanism of action, like the other NRTIs, is mitochondrial DNA polymerase inhibition, and thus it is not devoid of toxic effects. Tenofovir-induced nephropathy occurs on the basis of mitochondrial toxicity within the kidneys' proximal tubular cells, which are rich in mitochondria.[9] Irregularly shaped mitochondria with fragmented cristae ensue. There are currently no data on the effects of tenofovir on the mitochondria of the optic neurons and retina. However, an analogous mechanism of toxicity at the optic nerve head, which also has abundant mitochondria, is conceivable with tenofovir. However, based on existing evidence, the role of lamivudine in mitochondrial toxicity is less clear. Hence, we postulate that in the genetically susceptible individual, tenofovir has the potential to trigger LHON.

Idebenone (co-enzyme Q10 derivative) is not available in SA. However, co-enzyme Q10 is available as an over-the-counter preparation at most pharmacies. Both patients were treated with this formulation and have not shown any deterioration or improvement after 6 months of treatment. ART was discontinued in both patients and when required, both patients will be considered for a combination of antiretrovirals with lower mitochondrial toxicities, such as protease and integrase inhibitors.

Conclusion

LHON can be triggered by NRTIs in HIV-infected patients who harbour the LHON mutations. The expression of LHON can occur regardless of the mitochondrial toxic potential of ART. The routine screening for LHON mutations on all asymptomatic male patients about to commence NRTIs is not cost effective. However, the progression of LHON is reasonably manageable with appropriate therapy; hence testing for LHON mutations in HIV-positive patients with optic neuropathy who are on ART should become standard practice.

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CPD QUESTIONNAIRE
Vol. 15, No. 2

A maximum of 3 CEUs will be awarded per correctly completed test.

Effective in 2014, the CPD programme for *SAJHIVMED* will be administered by Medical Practice Consulting:
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True or false:

Regarding consent of minors to health research:

1. Usually, health research involving children only requires parental consent for children <12 years of age.
2. New national health legislation calls for written parental consent for all minors before participating in HIV-related health research, thus making research into marginalised groups or illegal behaviours unusually difficult.

Regarding cerebrospinal fluid (CSF) findings in patients with meningitis:

3. Cryptococcal meningitis accounts for more than half of premature mortality in HIV-infected individuals.
4. Confusion on initial presentation may be a significant predictor of an abnormal CSF analysis result.
5. Coagulopathies are uncommon in HIV-infected individuals, thus checking platelets and international normalised ratio before lumbar puncture is not usually recommended.

Regarding antiretroviral therapy (ART) use in pregnancy:

6. The integration of ART services into antenatal care will always decrease the time to ART initiation during pregnancy, and increase the proportion of pregnant women initiated on ART.
7. The greatest risk of mother-to-child transmission is in HIV-infected women with low $CD4^+$ counts, who are eligible for lifelong ART.
8. The updated prevention of mother-to-child transmission (PMTCT) guidelines recommend starting all HIV-infected pregnant women on a triple-drug antiretroviral regimen at their first antenatal visit.

Regarding shedding of HIV in the female genital tract:

9. Ability to detect HIV in genital secretions is associated with higher plasma HIV viral loads.
10. Women on ART for extended periods of time are likely to have lower plasma viral loads but higher viral loads in genital tract secretions.

Regarding Stevens-Johnson syndrome:

11. Stevens-Johnson syndrome (SJS) is different from toxic epidermal necrolysis (TEN) mainly in that SJS covers more body surface area than TEN.
12. An immunological response involving $CD8^+$ T lymphocytes is the most likely explanation for the pathogenesis of SJS/TEN.
13. Sulphonamides and nevirapine are the drugs most commonly implicated in the comorbidity of SJS/TEN and HIV in sub-Saharan Africa.

Regarding needlestick injuries among hospitalised patients:

14. More than 10% of patients suffer some form of harm during hospital care.
15. Needlestick injury between patients is a well-documented form of iatrogenic injury.
16. Health systems factors, such as overcrowding, are important preventable risk factors for needlestick injuries.

Regarding optic neuropathy in HIV:

17. Among antituberculous agents, rifampicin is most widely known to cause optic neuropathies.
18. Optic neuropathy in HIV-infected patients usually results from hereditary conditions that are exacerbated by antiretroviral drugs.
19. Leber's hereditary optic neuropathy (LHON) can be triggered by nucleoside reverse transcriptase inhibitors in HIV-infected patients who harbour the LHON mutations.
20. Individuals with the mutation for LHON display a number of preceding symptoms that culminate in visual loss.

INSTRUCTIONS

1. Read the journal. All the answers will be found there.
2. Go to www.mpconsulting.co.za to answer the questions.

Accreditation number: MDB001/012/01/2014 (Clinical)