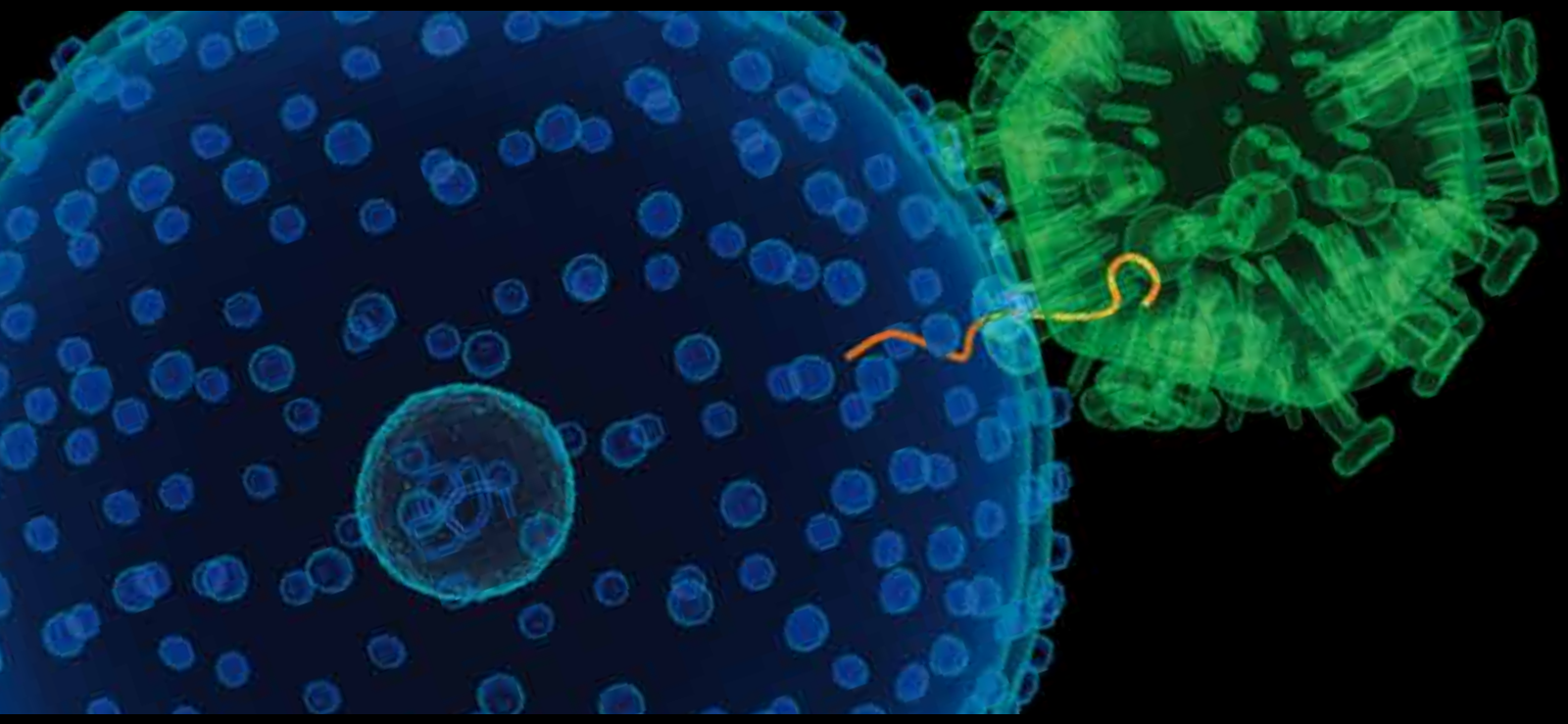
AIDS Research and Treatment

AIDS and HIV Infection after Thirty Years

Guest Editors: Giuseppe Ippolito, Jay A. Levy, Anders Sonnerborg, Ferdinand Mugusi, and Ferdinando Dianzani



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Hindawi Publishing Corporation
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http://dx.doi.org/10.1155/2013/731983

Editorial

AIDS and HIV Infection after Thirty Years

Giuseppe Ippolito,[1] Jay A. Levy,[2] Anders Sonnerborg,[3] Ferdinand Mugusi,[4] and Ferdinando Dianzani[5,6]

[1] *Department of Medicine, AIDS Research Institute, National Institute for Infectious Diseases Lazzaro Spallanzani, Via Portuense, 292-00149 Rome, Italy*
[2] *AIDS Research Institute, University of California at San Francisco, San Francisco, CA, USA*
[3] *Unit of Infectious Diseases, Department of Medicine, Karolinska Institutet, Stockholm, Sweden*
[4] *Department of Internal Medicine, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania*
[5] *Department of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani, Via Portuense, 292-00149 Rome, Italy*
[6] *Sapienza University of Rome, Piazzale Aldo Moro, 5-00185 Rome, Italy*

Correspondence should be addressed to Giuseppe Ippolito; giuseppe.ippolito@inmi.it

Received 11 December 2012; Accepted 11 December 2012

Acquired Immunodeficiency Syndrome (AIDS), caused by the Human Immunodeficiency virus (HIV), first appeared in Western countries in 1981 as a disease leading to a high death rate. It initially approached 100% after 5–10 years of the diagnosis. As evidenced from articles in this special issue, much progress has been made in the global fight against HIV/AIDS. Valuable information has been gathered on the virus and the host immune response to this new human pathogen. Successful therapies have been developed and approaches to control HIV spread have been instituted. The epidemic, however, continues to affect the Western countries and, importantly, the international community in resource-limited countries. According the United Nations Programme on HIV/AIDS (UNAIDS) estimates, published for the World AIDS Day 2012, 34 million (31.4–35.9) people are living with HIV, 2.5 (2.1–2.8) million people became new infected with HIV, and 1.7 (1.5–1.9) million died from AIDS-related illnesses. (UNAIDS Global report: UNAIDS report on the global AIDS epidemic 2012. UNAIDS/JC2417E available at http://www.unaids.org/en/resources/campaigns/20121120_globalreport2012/).

In the last 30 years since the recognition of the disease, clinicians, scientists, public health officials, policy makers, and volunteers have worked together to take care of people with HIV infection, to identify new drugs and evaluate their efficacy, to better understand the virus and to assess its pathogenicity, to find new tools to diagnose and prevent HIV infection, to assess the role of coinfections, and to mitigate the adverse events from antiretroviral treatment. Essentially, they have focussed on directions to manage patients according to a model of clinical excellence.

This large investment of resources has given impressive results and represents a welcomed scientific success. As noted in this issue, after 3 decades, AIDS has become at least for many industrialized countries a chronic disease affording patients a near-normal lifestyle and survival, if adequately treated [1, 2]. This year, UNAIDS reported an unprecedented acceleration in the clinical response to HIV/AIDS with more than a 50% reduction in the rate of new HIV infections even in some low- and middle-income countries. Impressive results have also been reached in resource-limited countries in terms of access to treatment. According to the above-mentioned UNAIDS Global report, worldwide the number of people with access to antiretroviral therapy has increased by 63% in the last 24 months. In sub-Saharan Africa the number of people on antiretroviral treatment has increased by 59% in the last two years and the number of AIDS-related deaths has been reduced by one-third in the last six years. Nevertheless, the clinical spectrum today, even with therapy, can include cardiovascular disease [3], infectious and noninfectious cancers [4], osteopenia/osteoporosis [5], liver and renal disease [6], and neurocognitive decline [7].

Importantly, the treatments for HIV/AIDS are very expensive and difficult to afford in resource-limited countries. Even in high-income countries, drug expenditure for treatment of HIV/AIDS today represent, according to recent estimates, 62,4% of total cost versus a mean of 20% for other chronic diseases and the cost per person treated is 38% higher than the treatment of cancer.

Global economic value of DALYs by HIV/AIDS is 21.6% of the value for cancer and 26% of heart diseases [8]. Concern over the costs of health care is universal and experience gained from studies of HIV/AIDS could help to influence the model of access to care for other clinical conditions.

For this special issue, a policy to have papers from researchers from resource-limited countries was adopted. The articles chosen cover several areas of research in different countries. In the paper by M. Harris et al., the problem of cost-effectiveness of highly active antiretroviral therapy for Multidrug-Resistant (MDR) HIV has been evaluated. The authors consider the possibility for patients with MDR to achieve full and durable viral suppression along with the cost of different options for treatment. The proposed solution could be a basis for careful planning and strategic use of antiretroviral drugs.

In A. E. Horace review, the evolution of the pharmacists' role in treatment of HIV-infected patients is presented. Facilitation of access to treatment of HIV-infected patients is discussed with attention to increasing effectiveness and adherence to prescribed medicines. In addition, measuring the CD4+ cell count and viral load revolutionized the possibility for monitoring the evolution of infection in HIV-infected persons. CD4+ cell counts represent the first successful application on a large scale of this technique in western and African countries. In A. Akinbami et al. paper, the authors present an evaluation, based on CD4+ cell count, of the percentage of HIV-infected people who require antiretroviral therapy at enrollment in an HIV treatment and care center in Nigeria.

Neurological manifestations, including HIV-dementia and HIV-associated neurocognitive disorder (HAND), are among the most threatening complications of HIV infection [7]. Although their incidence has decreased among people who have access to combination antiretroviral treatments, new neurological problems are being observed in countries where treatments are available. They are related to both changes in the natural history of old diseases as well as emergence of novel entities.

Two contributions on this topic are presented in this issue. In O. O. Oshinaike et al. report, the performance of the minimental state examination (MMSE) and international HIV dementia scale (IHDS) in assessing neurocognitive function in treated HIV/AIDS has been compared in Nigeria. In the second paper, M. L. Giancola et al. investigate the effect of antiretroviral therapy and single antiretroviral drugs on the cerebrospinal fluid HIV-RNA levels in HIV-infected patients affected by neurological disorders enrolled in a large multicentric Italian cohort.

Oral lesions and other oral manifestations are frequent in persons with HIV infection. Candidiasis is a common finding and lesions of the oral cavity occur in about one-third of patients with AIDS-associated Kaposi's Sarcoma (KS). Two clinical studies on this topic by the same group in South Africa appear in this issue. The first characterizes the features of oral HIV-KS and describes the patterns of evolution of the disease [9]. The second compares the prevalence and the rate of progression of chronic periodontitis in HIV-positive and -negative subjects [10].

Today the efficacy of antiretroviral drugs and the impressive changes in the progression of the HIV disease allow mothers perinatally infected to have almost a universal prevention in transmitting HIV infection to their offspring [11]. In this regard, M. L. Badell and M. Lindsay review various aspects related to maternal, obstetrical, and neonatal risks associated with pregnancy in perinatally HIV-infected mothers.

The determinants of the different time course for progression of HIV infection is still not clearly defined. J. C. Gaardbo et al. describe immunological features of controllers and long-term nonprogressors where knowledge on their ability to control HIV infection could lead to the development of immune therapies or a therapeutic vaccine.

With the introduction of antiviral therapy, deaths in HIV-infected people have been dramatically reduced with an improved quality of life for treated patients [12]. In this regard, A. Stewart et al. investigated the changes that occur in the care of persons living with HIV in a hospice.

These papers published in this special issue present exciting, insightful observations on the state of HIV/AIDS and the emergence of future topics of research for investigators. In this rapidly developing field of study, interdisciplinary and challenging research, performed in both industrialized and resource-limited countries, can bring critically important information for the life and social sciences, for public health, and for overall health care. Development of new preventive and therapeutic strategies is being encouraged as well as new models of health care. The aim of this special issue is to contribute to the development of knowledge on HIV/AIDS, to recognize the great progress many facets of medicine have made towards its complete control, and to recognize the challenges that still remain.

Acknowledgments

Unfortunately Ferdinando Dianzani, who initiated the effort for this special issue, passed away on 12 April 2012 before finishing this work. During his lifetime, he witnessed the progress made in the past thirty years of activities for fighting AIDS. His remarkable career as a virologist spanned almost 5 decades. Ferdinando Dianzani will be fondly remembered as a respected scientist, a strong leader, skilled virologist, athlete, excellent teacher and proud Italian. Drawing on years of experience spent in the field, Ferdinando Dianzani became a world-renowned expert in virology, with a remarkable role in his research on the measles virus, on the role of interferons in infectious diseases and on the pathogenesis of HIV/AIDS. His contributions to virology will survive in the thousands of students trained by him across his long academic

career, as well as his colleagues and friends throughout the world.

Giuseppe Ippolito
Jay A. Levy
Anders Sonnerborg
Ferdinand Mugusi
Ferdinando Dianzani

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 595762, 8 pages
doi:10.1155/2012/595762

Review Article

Cost-Effectiveness of Antiretroviral Therapy for Multidrug-Resistant HIV: Past, Present, and Future

Marianne Harris,[1,2] Bohdan Nosyk,[3] Richard Harrigan,[3,4] Viviane Dias Lima,[3,4] Calvin Cohen,[5] and Julio Montaner[3,4]

[1] *AIDS Research Program, Providence Health Care Institute, B518 1081 Burrard Street, Vancouver, BC, Canada V6Z 1Y6*
[2] *Department of Family Practice, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada V6T 1Z3*
[3] *British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada V6Z 1Y6*
[4] *Division of AIDS, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada V6T 1Z3*
[5] *Community Research Initiative of New England, Boston, MA 02111, USA*

Correspondence should be addressed to Marianne Harris, mharris@cfenet.ubc.ca

Received 17 January 2012; Revised 1 October 2012; Accepted 2 October 2012

Academic Editor: Giuseppe Ippolito

In the early years of the highly active antiretroviral therapy (HAART) era, HIV with resistance to two or more agents in different antiretroviral classes posed a significant clinical challenge. Multidrug-resistant (MDR) HIV was an important cause of treatment failure, morbidity, and mortality. Treatment options at the time were limited; multiple drug regimens with or without enfuvirtide were used with some success but proved to be difficult to sustain for reasons of tolerability, toxicity, and cost. Starting in 2006, data began to emerge supporting the use of new drugs from the original antiretroviral classes (tipranavir, darunavir, and etravirine) and drugs from new classes (raltegravir and maraviroc) for the treatment of MDR HIV. Their availability has enabled patients with MDR HIV to achieve full and durable viral suppression with more compact and cost-effective regimens including at least two and often three fully active agents. The emergence of drug-resistant HIV is expected to continue to become less frequent in the future, driven by improvements in the convenience, tolerability, efficacy, and durability of first-line HAART regimens. To continue this trend, the optimal rollout of HAART in both rich and resource-limited settings will require careful planning and strategic use of antiretroviral drugs and monitoring technologies.

1. Introduction

In the early years of the highly active antiretroviral therapy (HAART) era starting in 1996, HIV with resistance to two or more agents in different antiretroviral classes posed a significant clinical challenge. Multidrug-resistant (MDR) HIV was an important cause of treatment failure and consequent morbidity and mortality [1]. In 1998, a large drug resistance survey among viremic HIV patients in the United States showed that 13% harbored three-class-resistant virus and 48% had two-class resistance [2]. With improvements in understanding of viral dynamics and the efficacy of first-line regimens, MDR HIV has become less common but has not disappeared entirely, as demonstrated in a Canadian cohort of HAART-treated individuals followed until 2007 [3]. While three-class antiretroviral drug resistance is now very unusual (2%), two-class resistance was observed in 17% of the cohort. Fortunately, treatment options for patients with MDR HIV have improved substantially in terms of effectiveness, toxicity, and tolerability, while remaining cost-effective in most cases.

2. Past (1996–2005)

2.1. The HAART Era. The HAART era began in 1996, with the availability of triple drug regimens and clinical trial data demonstrating their efficacy [4, 5]. Around the same time, the availability of viral load testing improved both the understanding of viral dynamics in response

to treatment and the ability to closely monitor treatment efficacy. The consequences of exposure to sequential drug regimens in the absence of full viral suppression were not fully appreciated until the advent of widespread HIV drug resistance testing around 2000. Most of the HIV-infected patients who had previously received less effective single and dual drug regimens had already developed drug-resistant HIV by this time. Furthermore, some early triple therapy regimens were less than optimally effective, due to relatively low potency of individual drugs and adherence challenges related to complex dosing with numerous pills and poor tolerability. As a result, during this time a significant proportion of treatment-exposed HIV-infected patients harbored MDR strains [2].

2.2. Treatment Strategies for Multidrug-Resistant HIV. In the first decade of HAART, treatment options for MDR HIV were very limited. Before 2003, all available antiretroviral agents belonged to one of the original three drug classes and considerable cross-resistance existed within each class. Given the limited effective drug options available at the time, various strategies were tried. Regimens including two protease inhibitors (PIs), saquinavir and ritonavir both in therapeutic doses, achieved good results in treatment-experienced patients who had not previously been exposed to PIs [6]; however, results of this dual PI-based regimen were variable in patients who had experienced indinavir or nelfinavir failure previously [7, 8]. Another strategy was to promote reemergence of drug-susceptible virus using structured treatment interruptions and thereby to enhance virologic response to subsequent antiretroviral therapy [9]. This strategy was abandoned when it was proven to be ineffective in promoting sustained virologic suppression or disease control and, more alarmingly, was associated with protracted CD4 declines [10]. A more successful treatment strategy was the use of multiple drug rescue therapy, also called mega-HAART or giga-HAART, whereby patients were treated with as many partially active agents as possible, generally six to eight [11–13]. This strategy proved to be effective for at least some of the patients, but adherence was a significant challenge because of regimen complexity and poor tolerability. Long-term sustainability was limited by toxicity and cost issues.

2.3. Enfuvirtide. In 2003, 24-week results of two large randomized controlled studies (T-20 versus optimized background regimen only (TORO) 1 and 2) were published that demonstrated the efficacy of enfuvirtide, an HIV fusion inhibitor, for the treatment of patients with drug-resistant virus [14, 15] (Table 1). In the combined TORO 1 and 2 studies, the 48-week rates of virologic suppression to <50 copies/mL were 18.3% for enfuvirtide plus an optimized background regimen versus 7.8% for the optimized background regimen alone [16]. The drug received regulatory approval in Canada, the United States (US), and Europe in the same year (Table 2). Enfuvirtide represented an important breakthrough in that it was the first approved antiretroviral agent belonging to a new drug class, and hence cross-resistance with previous agents was not a problem. By the middle of the decade, enfuvirtide was considered a cornerstone of treatment for patients harboring MDR virus [17].

However, the complexity of synthesis and limited supply led to pricing of enfuvirtide in the US and Europe at $18,500 per person per year (calculated in 2001 US dollars, from $20,000 dollars in 2003 using the medical care component of the consumer price index), which was nearly twice as costly as any of the other approved single agents for treatment of HIV in use at the time [18–22]. In addition, enfuvirtide had to be used in combination with multiple other active antiretroviral agents [16, 23]. As a result, the annual cost of a combination antiretroviral regimen containing enfuvirtide was typically between $35,000 and $43,000 per person per year [18, 24].

Extrapolating from the 24- and 48-week TORO results, the cost-effectiveness of enfuvirtide in combination antiretroviral regimens was evaluated by Sax et al. in 2005 [25] and by Hornberger et al. in 2006 [26]. Using different methods of analysis, these two papers estimated the incremental cost-effectiveness ratio of enfuvirtide plus an optimized background regimen compared with an optimized background regimen alone to be $69,500 and $24,604, respectively, per quality-adjusted life-year (QALY) gained. (To calculate the incremental cost-effectiveness of an intervention, analyses must consider the added efficacy (generally measured in quality-adjusted life years (QALY)) and the additional costs of the intervention. The cost-effectiveness ratio is then calculated with incremental costs in the numerator and incremental benefits in the denominator ($/QALY). An intervention may be considered cost-effective if the additional benefit provided by the treatment is considered "worth" the additional cost. The World Health Organization (WHO) Commission on Macroeconomics and Health suggested that interventions may be considered very cost-effective when the cost-effectiveness ratio ($/QALY) is less than 1 times the per-capita gross domestic product (GDP) for an individual country and cost-effective when the ratio is less than 3 times the per-capita GDP [27]. As a point of reference, the estimated GDP per capita in Canada in 2010 is CDN$39,057 [28].)

The results of these studies suggested that enfuvirtide-based regimens could represent a cost-effective option for treating individuals with MDR HIV and advanced disease at the time. The projected survival benefit of enfuvirtide plus an optimized background regimen becomes more apparent with longer-term followup [26]; however, long-term sustainability of enfuvirtide therapy was hampered by the need for twice daily subcutaneous injections and bothersome injection site reactions [29, 30]. Because of these issues and the availability of newer, more convenient oral agents, enfuvirtide is no longer widely used; however, there is no doubt that this agent saved the lives of many MDR-HIV-infected patients who would otherwise not have survived until other more sustainable options became available.

Table 1: Publications of pivotal studies of drugs for multidrug-resistant HIV.

Drug	Abbreviated study title and duration	Study treatment (N) and comparator (N) arms	Journal and date of publication
Enfuvirtide (ENF)	TORO 1 (24 wks)	ENF + 3–5 drug OBT (328) versus 3–5 drug OBT (167)	NEJM, May 2003 [14]
	TORO 2 (24 wks)	ENF + 3–5 drug OBT (335) versus 3–5 drug OBT (169)	NEJM, May 2003 [15]
	TORO 1 and 2		
	48 wk efficacy	ENF + OBT (661) versus OBT (334)	JAIDS, December 2005 [16]
	48 wk safety	ENF + OBT (663) versus OBT (334)	JAIDS, December 2005 [29]
Tipranavir (TPV)	RESIST (48 wks)	TPV/r + OBT (746) versus CPI/r + OBT (737)	Lancet, August 2006 [31]
Darunavir (DRV)	POWER 1 (24 wks)	DRV/r in 1 of 4 doses + OBT (255) versus CPI/r + OBT (63)	AIDS, February 2007 [37]
	POWER 2 (24 wks)	DRV/r in 1 of 4 doses + OBT (225) versus CPI/r + OBT (53)	AIDS, March 2007 [38]
	POWER 1 and 2 (48 wks)	DRV/r 600/100 mg BID + OBT (110) versus CPI/r + OBT (120)	Lancet, April 2007 [32]
Etravirine (ETR)	DUET 1 (24 wks)	ETR + DRV/r + OBT (304) versus DRV/r + OBT (308)	Lancet, July 2007 [33]
	DUET 2 (24 wks)	ETR + DRV/r + OBT (295) versus DRV/r + OBT (296)	Lancet, July 2007 [34]
	DUET 1 and 2 (48 wks)	ETR + DRV/r + OBT (599) versus DRV/r + OBT (604)	AIDS, November 2009 [39]
Raltegravir (RAL)	BENCHMRK 1 and 2 (48 wks)	RAL + OBT (462) + OBT (237)	NEJM, July 2008 [36]
Maraviroc (MVC)	MOTIVATE 1 and 2 (48 wks)	MVC QD + OBT (414) versus MVC BID + OBT (426) versus OBT (209)	NEJM, October 2008 [35]

OBT: optimized background therapy; CPI: comparator protease inhibitor; r: ritonavir; QD: once daily; BID: twice daily; NEJM: The New England Journal of Medicine; JAIDS: Journal of Acquired Immune Deficiency Syndromes.

Table 2: HIV drug approval/authorization dates.

Drug	Canada (Health Canada)	US (FDA)	Europe (EMEA)
Enfuvirtide	July 14, 2003	March 13, 2003	May 5, 2003
Tipranavir	November 21, 2005	June 22, 2005	October 25, 2005
Darunavir	July 28, 2006	June 23, 2006	February 12, 2007
Maraviroc	September 21, 2007	August 6, 2007	September 18, 2007
Raltegravir	November 27, 2007	October 12, 2007	December 20, 2007
Etravirine	August 23, 2008	January 18, 2008	August 28, 2008

US (FDA): United States Food and Drug Administration; EMEA: European Medicines Agency.

3. Present (2006–2011)

Starting in 2006, results of a number of clinical trials involving new antiretroviral agents were published in rapid succession (Table 1). Taken together, these studies represented a significant step forward in the treatment of MDR HIV: tipranavir (RESIST) [31], darunavir (POWER) [32], etravirine (with darunavir in DUET) [33, 34], maraviroc (MOTIVATE) [35], and raltegravir (BENCHMRK) [36]. The regulatory approval of these drugs in Canada, the US, and Europe between 2005 and 2008 enabled prescribers to effectively treat MDR HIV with more compact regimens including at least two and often three fully active agents, with a remarkable increase in the efficacy rates (Table 2). Full and durable viral suppression once again became a realistic goal in the treatment of these patients.

3.1. Tipranavir. Tipranavir was the first of a new generation of ritonavir- (r-) boosted PIs with efficacy against HIV strains that had reduced susceptibility to older PIs, including strains with multiple PI resistance-associated mutations [31]. Using 48-week data from the RESIST studies, Hubben et al. [40] and Simpson et al. [41] demonstrated that regimens including tipranavir/r could provide longer-term benefits in terms of reductions in AIDS events and corresponding QALY gains and life years saved, as compared to regimens based on older ritonavir-boosted PIs. These analyses found similar cost-effectiveness ratios for tipranavir/r versus comparator PI/r of €42,500 [40] and $56,517 [41] per QALY gained. Excluding patients also treated with enfuvirtide reduced the incremental cost-effectiveness ratio to $46,147 per QALY [41]. However, the use of tipranavir was limited by important tolerability and toxicity issues, including relatively uncommon but potentially fatal hepatotoxicity and intracranial hemorrhage [42].

3.2. Darunavir. Darunavir, another ritonavir-boosted PI that was also developed to treat PI-resistant HIV, is effective

and generally safe and well-tolerated. The phase IIb POWER (performance of TMC-114/r when evaluated in treatment-experienced patients with PI resistance) trials [32, 37, 38] and the phase III TITAN (TMC114/r in treatment-experienced patients naïve to lopinavir) trial [43] demonstrated the efficacy of darunavir/r 600/100 mg twice daily among treatment-experienced HIV-infected adults. Subjects in the comparator arms received single (74%) or dual (23%) boosted PIs (mainly lopinavir, saquinavir, and/or amprenavir/fosamprenavir) in POWER and lopinavir/r in TITAN. A recent systematic review summarized the results of a number of cost-utility analyses conducted alongside these trials and demonstrated that the use of darunavir/r in this setting was cost-effective and, in some cases, cost saving [44].

As a result of complex PI resistance profiles, during this period there was some clinical use of two or more ritonavir-boosted PIs in a regimen [45]. A study comparing single to dual unboosted PIs showed modest benefit from the addition of the second PI [46]. The paucity of antiretrovirals from new classes led to clinical use of such dual PI regimens as one way to attempt to reestablish virologic suppression especially in patients with PI resistance. Given the results of the POWER studies, the ability of boosted darunavir to be used in place of a dual boosted PI regimen was explored in two similar randomized controlled trials of immediate substitution of ritonavir-boosted PIs with darunavir/r versus deferred substitution after 24 weeks [47, 48]. Together these two pilot-sized studies randomized 48 subjects (24 to each arm) who had undetectable plasma HIV RNA (<50 copies/mL) while receiving regimens including dual or triple boosted PIs. All 45 subjects who completed 24 weeks on study (23 in the immediate switch arms and 22 in the deferred switch arms) had undetectable viral load at week 24. Median CD4 cell count changes from baseline to week 24 were similar in the two arms. At week 48, virologic suppression was maintained in all but two subjects, one in each treatment arm. In this context, darunavir/r was shown to be an effective, compact, and relatively safe and tolerable option, as well as being less costly than two or three concomitant ritonavir-boosted PIs.

3.3. Etravirine, Maraviroc, and Raltegravir. The DUET, MOTIVATE, and BENCHMRK trials evaluated the efficacy of etravirine (plus darunavir/r), maraviroc, and raltegravir respectively, versus placebo, each given with an optimized background regimen of nucleoside reverse transcriptase inhibitors, PIs, and/or enfuvirtide [33–36].

Etravirine, the first available next-generation nonnucleoside reverse transcriptase inhibitor, has been successfully used for the treatment of HIV with some degree of resistance to the first-generation nonnucleoside reverse transcriptase inhibitors delavirdine, nevirapine, and efavirenz. Its main use, as supported by data from the DUET study, is in regimens also including a ritonavir-boosted PI, specifically darunavir [33, 34]. In the combined 48-week results of the DUET 1 and 2 studies among subjects with nonnucleoside- and PI-resistant HIV, virologic suppression to <50 copies/mL was observed in 61% of the etravirine group versus 40% of those randomized to placebo (both combined with an optimized background regimen that included darunavir/ritonavir) [39]. The antiviral activity of etravirine is reduced in the presence of three or more specific resistance-associated mutations [49].

Maraviroc and raltegravir, a CCR5 receptor antagonist and an integrase inhibitor, respectively, were the first available new-class options that could be given orally. While being an attractive agent in terms of its effectiveness and tolerability, maraviroc is limited to use in the treatment of patients with CCR5-tropic virus, which is an issue in treatment-experienced patients [50]. In the pooled results of the MOTIVATE 1 and 2 studies, the 48-week rates of virologic suppression to <50 copies/mL for treatment-experienced subjects with R5-tropic virus were 43% with maraviroc once daily and 46% with maraviroc twice daily versus 17% with placebo (all with an optimized background regimen) [35]. Economic evaluations of the MOTIVATE 1 and 2 studies have compared maraviroc plus optimized background therapy to optimized background alone. The incremental cost-effectiveness ratios per QALY gained were €23,457 and US$42,429 in analyses conducted in Spain and Mexico, respectively [51, 52]. The incremental cost-effectiveness ratio was found to be somewhat lower (more favorable) when maraviroc was modeled in individuals whose HIV was susceptible to two or fewer components of the background regimen and higher (less favorable) in individuals with HIV susceptible to three or more regimen components [52, 53].

Use of the integrase inhibitor raltegravir is not restricted by tropism, and the drug is effective against virus resistant to other drug classes. Given orally twice daily, it is relatively safe and well-tolerated, with minimal toxicity and drug interactions [54]. In the combined BENCHMRK 1 and 2 study results, the 48-week rates of virologic suppression to <50 copies/mL for treatment-experienced subjects with drug-resistant HIV were 62% with raltegravir versus 33% with placebo (both combined with an optimized background regimen) [36]. A pair of studies from Spain and Switzerland used data from the BENCHMRK 1 and 2 trials to assess the long-term cost-effectiveness of raltegravir plus background therapy as compared to background therapy alone. Incremental cost-effectiveness ratios for three years of treatment were calculated to be €22,908 and 42,751 Swiss francs in the two studies, respectively, and increased with longer treatment durations [55, 56]. By 2009, many patients receiving enfuvirtide in rescue therapy regimens were switched to raltegravir [57–60], with a significant improvement in patient acceptability and cost. Given the inconvenience of twice daily injections and the availability of raltegravir and other oral agents effective against MDR virus, the clinical role of enfuvirtide diminished, and it is seldom used today.

When they became approved and available, these newer agents were somewhat more expensive than the previous antiretrovirals (except enfuvirtide). The DUET, MOTIVATE, and BENCHMRK trials were conducted in treatment-experienced patients, where complex and expensive drug combinations are typically required. The average annual per

patient cost of antiretrovirals for the active plus optimized background regimen arm versus placebo plus optimized background regimen was US$ 47,324 versus 38,267 in the DUET Trials, US$ 46,633 versus 36,404 in MOTIVATE, and US$ 45,484 versus 34,585 in BENCHMRK. Of note, in the three trials, the highest treatment costs were from nucleoside analogues (29-30% of total costs) and enfuvirtide (22–25% of total costs) [61]. The ability to design an effective regimen for a patient with MDR virus using fewer drugs than the previous multiple drug rescue therapy regimens permitted more cost-effective therapy. In addition, the improved safety and tolerability of most of these newer agents resulted in lower overall health care costs for the treatment of these individuals. By 2008 it became possible to successfully treat patients with MDR virus with a regimen including three active new agents: ritonavir-boosted darunavir, etravirine, and raltegravir, with or without partially effective nucleosides [62, 63].

4. Future: 2012 and Beyond

Despite evidence of ongoing risk behavior in patients infected with drug-resistant HIV [64], the spectre of widespread transmission of multidrug-resistant HIV has not materialized. This is probably related at least in part to the reduced fitness of multiply-mutant strains [65]. As combination antiretroviral regimens have become more potent in suppressing viral replication and genotypic resistance testing prior to treatment has become standard of care, the majority of HIV drug resistance emerges in the setting of incomplete adherence. First-line HAART regimens are becoming more convenient, more forgiving to missed doses, and better tolerated, so the emergence of resistance continues to become less frequent. As well, the long-term durability of modern HAART regimens is increasing [66].

On the other hand, the significant flourishing of new antiretroviral drugs and new drug classes that has occurred over the past several years is unlikely to be duplicated in the future. Fewer new agents are being developed for treating HIV, and some of them (e.g., rilpivirine) are aimed specifically at first-line therapy of treatment-naïve patients [67]. Two new integrase inhibitors, elvitegravir and dolutegravir, may play a role in treatment of drug-resistant HIV [68, 69]; however, elvitegravir demonstrates significant cross-resistance with raltegravir and is therefore unlikely to be effective for patients who have failed a raltegravir-based regimen [70]. Given the paucity of new antiretroviral drugs in the pipeline, the agents that are currently available will need to continue to control HIV replication for many years. Furthermore, many regimens in current use include drugs with a low genetic barrier to resistance (e.g., efavirenz, raltegravir), meaning that resistant HIV mutants can emerge relatively quickly as a consequence of virologic failure [49, 71, 72]. Therefore, strategic use of the available drugs and cautious management of patients will be critical for successful HIV management in the future. Adherence assessment and counseling will need to be integrated into routine clinical visits prior to and throughout antiretroviral treatment, in order to avert the emergence of drug-resistant HIV. In addition, routine pretreatment genotypic testing to detect primary resistance, regular viral load monitoring, and early genotypic testing in cases of virologic failure will be particularly important to prevent accumulation of resistance-associated mutations.

The burden of resistance could increase if new agents are not made available or their introduction is staggered, resulting in suboptimal regimens or functional monotherapy. This issue is particularly relevant to developing countries, where options for second-line and salvage therapy are limited by scarcity of resources; the newer drugs that are effective against drug-resistant HIV are costly and often not available in generic formulations. Furthermore, in these resource-limited settings, access to viral load monitoring and resistance testing may be restricted, if available at all. The absence of these laboratory tools to monitor the ongoing effectiveness of antiretroviral therapy can lead to significant delays in the diagnosis and management of virologic failure, with devastating consequences [64, 73]. The inevitable emergence of HIV drug resistance in these settings is a particular concern at a time when it has been recognized that sustained full suppression of viral replication is crucial, both to optimize the individual benefit of HAART and to decrease HIV transmission [74–77]. The optimal rollout of HAART in both rich and resource-limited settings will require careful planning to ensure access to the best available antiretroviral regimens and to the appropriate technologies to monitor their use.

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AIDS Research and Treatment
Volume 2012, Article ID 869891, 5 pages
doi:10.1155/2012/869891

Review Article

Pharmacists' Research Contributions in the Fight against HIV/AIDs

Alexis E. Horace

College of Pharmacy, University of Louisiana at Monroe, Baton Rouge Campus, 3849 North Boulevard, Baton Rouge, LA 70808, USA

Correspondence should be addressed to Alexis E. Horace, horace@ulm.edu

Received 16 January 2012; Accepted 7 August 2012

Academic Editor: Giuseppe Ippolito

Pharmacists have made many contributions to HIV/AIDs research and are still showing their significance as members of the healthcare team through innovative clinical trials. Pharmacists are showing advances in several healthcare settings including inpatient, outpatient, and community pharmacies. Because of the complex regimens of highly active antiretroviral therapy (HAART), the increased life span of patients living with HIV, and other concomitant medications taken for comorbid disease states, there is a high risk for health-related complications and the development of adverse events. These adverse events may lead to decreased adherence to HAART, which may cause the development of HIV drug resistance. Pharmacists are providing examples through growing research on how they help combat medication-related errors and also continue to contribute as healthcare providers as a part of a holistic healthcare team.

1. Introduction

With the invention and administration of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) has transformed from an acute disease to a chronic disease. The Centers for Disease Control and Prevention estimate that between the years 1996–2003, HAART extended life expectancy of patients living with HIV from 10 to 20 years [1]. Care for patients infected with HIV has shifted from primarily requiring acute treatment in the inpatient setting to needing chronic treatment in the primary and ambulatory care settings. During this time, pharmacists have assumed larger roles as members of healthcare teams who specialize in caring for patients of this population. The responsibilities of pharmacists surpass caring for HIV patients in a community pharmacy setting and have evolved into participating in direct patient care in hospitals and outpatient clinics.

Concerns for patients with HIV extend beyond increasing CD4 cell counts and decreasing viral loads. Successful treatment for patients with this disease state depends on a holistic, patient-specific approach by a multidisciplinary team [9]. Management of all aspects of HAART is needed for successful treatment. Clinical pharmacists can provide many services such as pharmacokinetic drug monitoring, medication reconciliation, therapeutic medication recommendations, patient counseling, adherence consult services, and medication therapy management. These services have prevented medication errors, decreased medication misuse, and increased health-related outcomes [10–12]. Due to the complexity of HAART, there is a greater need to monitor adverse effects, drug-drug interactions, and resistance patterns of HIV-1 virus that can develop through patient nonadherence.

Patients may require treatment for chronic disease states such as hepatitis coinfection, or, solid organ transplant, diabetes, cardiovascular disease, or renal disease, which also use many medications for treatment further increasing the chance for drug interactions to occur.

Pharmacists have demonstrated their usefulness in the clinical setting through providing cost savings by making valuable therapeutic interventions [13–15]. Kopp and colleagues showed pharmacists' interventions over 4.5 months of service provided a cost avoidance of $205,919–$280,421

[16]. Not only do pharmacists play a vital role as a part of the healthcare team providing optimal care for patients with HIV, but also they are a cost-effective resource.

The role of pharmacists treating patients with HIV is constantly evolving. The American Society of Health System Pharmacists (ASHP) released a statement in 2003 that supports the use of clinical pharmacists in caring for patients with this disease state [17]. ASHP suggests that possible areas for implementation of pharmacists include outpatient pharmacies, ambulatory care clinics, inpatient settings, dialysis units, hospice care centers, and home health services. Since the publication of this statement, there has been a plethora of studies documenting the contributions of pharmacists in the fight against HIV. This paper reviews efforts of pharmacists and pharmacy services to aid in the management of this disease state and provides insight into areas for expanding future research.

2. Hospital Pharmacy

Patients with chronic disease states are at risk for medication-related errors upon admission to the hospital [18]. Drug interactions with HAART can significantly increase or decrease therapeutic levels of medications [19] putting patients at risk for adverse effects or the development of HIV-1 viral resistance. An adherence rate of 95% to antiretroviral (ARV) therapy is recommended in order to sustain acceptable CD4 counts and viral load levels [20]. The medication-related errors that occur during inpatient hospital stays are more detrimental than they may appear on the surface [20]. Early retrospective studies documenting the need for pharmacy intervention report clinically significant error rates in prescribing ARVs of 5.8% over a 2-year period and 26% over a 1-year period [21, 22]. Because of the impact these errors can make on the health of HIV positive patients, research has emerged showing how the participation of pharmacists decreases these risks.

In 2007, Heelon and colleagues [23] published data describing the AV prescribing errors in an inpatient hospital setting and the effects of a clinical pharmacist in decreasing these errors. This descriptive, observational study was constructed in two parts consisting of a preintervention phase and an intervention phase. They found that 21% of HIV patients had at least one HAART prescribing error during their hospital stay. Most errors consisted of incomplete regimens (45%), incorrect dosage forms (30%), and incorrect scheduling (8%). With the help of a pharmacist, the duration of errors also decreased from 3.5 days to 1 day until resolution.

That same year, another study examined the effect of clinical pharmacy on health outcomes for HIV patients [12]. This observational study reviewed 1571 patients in clinics with or without a clinical pharmacist's involvement. Patients with access to a clinical pharmacist (47%) were associated with decreases in plasma HIV-1 virus levels of 0.73 log ($P < 0.001$) within 1 year and 0.33 log ($P = 0.005$) within 2 years. There were no statistically significant changes in CD4 T-cell counts after 2 years. Outcomes varied depending on the size of the practice site. For instance, the clinical pharmacist group associated with patient population of <50 were associated with a 19% (95% CI: −40% to 8%) decrease in office visits; however, for sites with panel sizes >50 HIV patients, there was a 10% increase (95% CI: −16% to 43%). Patients who had access to a clinical pharmacist required fewer clinic visits (0.88 fewer, $P < 0.001$). In addition, adherence rates in patients in the clinical pharmacists group improved to 7.1% at 1 year (81% versus 74%, $P = 0.04$) and 7.8% at 2 years (76.7% versus 68.9%, $P = 0.02$).

In the years that followed, other research on the impact of clinical pharmacists in the hospital setting emerged. A small study published by Mok and colleagues supports previous data reporting high rates of incomplete regimens for HIV patients admitted to the hospital (36% of patients, $n = 83$) and 86% of patients had at least one medication error involving HAART [24]. Also, a 3-month descriptive, prospective study conveyed similar results reporting rates of incomplete regimens in hospitalized patients as high as 42% ($n = 50$) and, with the aid of clinical pharmacists' recommendations, all patients admitted were started on appropriate regimens ($n = 34$) [25]. Another study reflected similar data [26]. However, the authors rated the risk level of each error. In a population of 68 HIV patients hospitalized over a 4-month period, 56% had at least one error that caused moderate-to-severe discomfort. Another interesting aspect of this study was the authors ability to associate an approximately 2-fold increase in the risk of experiencing HIV medication errors with the hospital pharmacy's inability to provide correct substitutions for nonformulary medications (RR = 1.95; 95% CI 1.25 to 3.4; $P = 0.02$). Not only did this study provide insight on the severity of errors detected and the importance of a pharmacist's intervention on these errors, but also it provided information on improvements the department of pharmacy could implement when dispensing AVs.

A larger study emerged in 2011 by Carcelero and colleagues identifying common prescribing errors involving AVs, and also, evaluating the level of acceptance of pharmacy-provided recommendations [27]. This observational, prospective study was conducted over 1 year and included 189 HIV-infected patients. Similar rates of medication-related errors were found in comparison to previous studies (21.7% of patients had at least one error). The pharmacist made an intervention for all detected errors with 91.7% of recommendations being accepted. The data presented supports the need for the incorporation of clinical pharmacist in the care for patients hospitalized with HIV/AIDS. Services provided by clinical pharmacists should be taken into consideration in this practicing setting.

3. Outpatient Clinics and Community Pharmacies

Care for patients with HIV/AIDs extends beyond the inpatient setting to outpatient settings as rates for developing opportunistic infections continue to decrease [28]. There are

Table 1: Pharmacist impact on adherence in patients with HIV/AIDs.

Authors	Year	Analysis type	Objective	No. of patients	Results	Statistical value
Cantwell-McNelis and James [2]	2002	Retrospective	Evaluation of a pharmacist run adherence program	80	(i) Increase in refill rates by patients in contact with a pharmacist (31 versus 50 days)	$P < 0.05$
					(ii) Significant decrease in viral load (values not reported)	$P < 0.05$
Foisy and Akai [3]	2004	Observational, prospective	Describe the implementation of a pharmacy driven direct-observation therapy service	57	(i) 149 drug-related problems identified with 95% acceptance of recommendations (ii) 13.4% drug-related problems included adherence	
Castillo et al. [4]	2004	Retrospective, observational	Compare the impact of different levels of pharmacy care on adherence and time to viral suppression	489	(i) AIDS-tertiary pharmacies had highest rates of adherence compared to outside pharmacies and physician clinics	$P = 0.000$
					(ii) Probability of HIV-1 RNA suppression by 12 months was 74.6% for the AIDS tertiary pharmacies, 59.4% for off site pharmacies, and 60% for physician offices	$P = 0.001$
Hirsch et al. [5]	2009	Cohort	Investigate the impact of pharmacy established MTM services	7,018	(i) 56.3% adherence in pilot pharmacy compared to 38.1% in comparison group	$P < 0.001$
					(ii) Difference in excess refills (19.7% versus 44.8%, pilot pharmacy versus other pharmacies)	$P < 0.001$
Ma et al. [6]	2010	Retrospective, cohort	Investigate clinical outcomes of an HIV clinical pharmacist interventions	75	(i) Prescribed daily pill quantities reduced from a mean of 7.2 ± 3.9 to 5.4 ± 2.8 pills per day	$P < 0.001$
					(ii) 25% increase in CD4+ cell count	$P < 0.001$
					(iii) 33% increase in patients with undetectable viral load	$P < 0.0001$
Henderson et al. [7]	2011	Prospective, cohort	Evaluating antiretroviral adherence and impact of pharmacy interventions	28	(i) Overall 19% increase in adherence rates	$P < 0.00001$
					(ii) Increase in the trend toward undetectable viral load (58–73%, baseline and postintervention)	$P = 1.0$
Hirsch et al. [8]	2011	Cohort	Evaluation of pharmacy driven MTM services	2,234	Increased adherence in the pilot pharmacy than nonpilot pharmacy by 22.1%	$P < 0.001$

MTM: medication therapy management, AIDS: acquired immunodeficiency syndrome, HIV: human immunodeficiency virus.

now opportunities for pharmacists to participate in the continuity of care for these patients as they transition from one area of healthcare to another. One study reports discrepancy rates as high as 53% when comparing community pharmacy and outpatient clinic medication records for patients on AVs [29]. In addition to the monitoring patient tolerance of these complex ARV regimens and identifying drug-drug interactions between ARVs and medications taken for other chronic disease states, pharmacists also have opportunities to assist in adherence counseling and help to optimize drug therapy. Taking AVs is a long-term commitment and requires excellent adherence. The level of patient adherence is crucial in optimizing therapeutic outcomes [20]. Length of therapy, psychological comorbidities, larger pill burden, increased frequency of administration, and baseline viral loads with associated resistance patterns are determinants of adherence [30]. In a retrospective study ($n = 80$) that reviews adherence by use of refill data, more patients receiving counseling from a pharmacist refilled their prescriptions in a timely manner than those who did not ($P < 0.05$) [31].

March and colleagues published a study in 2007 that showed data on the effects of pharmacists' interventions on patient outcomes in an HIV clinic [32]. Patients recruited to this study had an extensive history with AV therapy. In this observational trial which lasted approximately 4 months, 68% of the patients (23 out of 34) had more than one medication problem that required therapeutic recommendations from the pharmacist. During this time, the clinic's primary care providers accepted 100% of recommendations. When observing CD4+ cells, they found counts increased

from baseline levels by 54± cells/mm^3 ($P < 0.0002$) over the course of the study. The mean reduction in viral load was 1.02 $\log_{10}$ copies/mL ($P < 0.004$). Though this study is small, it shows that the help of a clinical pharmacist has some benefits. This study also highlights an area for further research for pharmacists hoping to make further impacts on the fight against HIV.

There are several other studies published that provide additional support to the use of pharmacy when caring for patients with HIV (Table 1). Not only are clinical pharmacists vital in the clinical setting, but also the use of specialty pharmacies for HIV patients or pharmacist-run medication therapy management (MTM) clinics have made a great impact. Opportunities for improvement lie in continuing to document pharmacy-related services to show health outcomes and cost savings in relation to pharmacists' interventions. Hopefully the continuation of research in this area will further establish clinical settings where pharmacists are needed.

4. Conclusion

Currently there are many effective medications used to treat HIV, however these medications may result in significant harm (increased toxicity) or viral resistance when they are not prescribed, administered, or taken correctly [20, 33, 34]. Pharmacists are taking a larger responsibility in caring for patients with HIV, and studies revealing our efforts are increasing. Nonetheless, many of these efforts go undocumented. There are clinical trials published supporting the use of pharmacists in both inpatient and outpatient clinical settings; however, knowledge of the usefulness of these services is often overlooked. Pharmacists are knowledgeable about medications and their management, providing a unique advantage when caring for patients with this disease state. Most studies published showing the effectiveness of pharmacists in treating patients with HIV are limited by their small study size and short duration. Some studies reported outcomes for patients with improvements in CD4+ cells and viral suppression, which can be attributed to the influence of pharmacist recommendations and collaborative efforts. These studies should spur efforts to increase research in using pharmacists as accessible and valuable resources for helping to manage patients with HIV. Another possible area of research could include using pharmacists as resources to enhance continuity of care from the hospital setting to the outpatient clinic setting, acting as guides to provide complete medication reconciliation and possibly decrease drug-related errors. There is also a lack of data researching pharmacy services in areas such as hospice and dialysis centers. These areas are also new realms for research regarding the influence of pharmacists in practice. Pharmacy as a profession is continuously evolving and pharmacists are increasingly seeking opportunities to become more involved in direct patient care. Though there are still many opportunities for research into pharmacists' involvement in care of patients with HIV, pharmacists are currently making vast improvements in the level of patient-centered care they provide for patients living with this complex disease state.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 352753, 6 pages
doi:10.1155/2012/352753

Research Article

CD4 Count Pattern and Demographic Distribution of Treatment-Naïve HIV Patients in Lagos, Nigeria

Akinsegun Akinbami,[1] Adedoyin Dosunmu,[1] Adewumi Adediran,[2] Sarah Ajibola,[3] Olajumoke Oshinaike,[4] Kikelomo Wright,[5] and Olanrewaju Arogundade[1]

[1] *Department of Haematology and Blood Transfusion, College of Medicine, Lagos State University, Ikeja, PMB 21266, Lagos, Nigeria*
[2] *Department of Haematology and Blood Transfusion, Faculty of Clinical Sciences, College of Medicine, University of Lagos, PMB 12003, Idiaraba, Lagos, Nigeria*
[3] *Department of Haematology and Blood Transfusion, Lagos University Teaching Hospital, PMB 12003, Idiaraba, Lagos, Nigeria*
[4] *Department of Medicine, College of Medicine, Lagos State University, Ikeja, PMB 1266, Lagos, Nigeria*
[5] *Department of Community Health and Primary Health Care, College of Medicine, Lagos State University, Ikeja, PMB 21266, Lagos, Nigeria*

Correspondence should be addressed to Akinsegun Akinbami, ajoke_clinic@yahoo.co.uk

Received 12 January 2012; Revised 24 August 2012; Accepted 26 August 2012

Academic Editor: Giuseppe Ippolito

Background. CD4 count measures the degree of immunosuppression in HIV-positive patients. It is also used in deciding when to commence therapy, in staging the disease, and in determining treatment failure. Using the CD4 count, this study aimed at determining the percentage of HIV-positives who require antiretroviral therapy at enrollment in an HIV treatment and care centre. *Methods*. The Baseline CD4 count, age and gender of 4,042 HAART-naïve patients, who registered between December 2006 and June 2010, at Lagos State University Teaching Hospital, Ikeja, were retrospectively studied. Data were analyzed using SPSS version 16.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). *Results*. Patients consisted of 2507 (62%) female and 1535 (38%) males. The mean age of males was 37.73 ± 9.48 years and that of females 35.01 ± 9.34 years. Overall, the mean CD4 count was of 298.76 ± 246.93 cells/mm^3. The mean CD4 count of males was 268.05 ± 230.44 cells/mm^3 and that of females 317.55 ± 254.72 cells/mm^3. A total of 72.3% males, 64.3% females and 67.4% overall registered patients had CD4 count <350 cells/mm^3, while only 15.1% males , 20.3% females, and 18.3% overall registered patients had CD4 count >500 cells/mm^3 at registration. *Conclusion*. Females account for more than half of registered patients in HIV clinic and have a relatively higher CD4 count than males. About three-quarter of HIV positives require antiretroviral therapy at registration.

1. Introduction

Worldwide Nigeria has the second highest number of new HIV/AIDS infections reported each year [1]. About 300,000 new infections occur annually with people aged 15–24 years contributing 60% of the infections and 1.5 million people living with HIV require antiretroviral using the new WHO guidelines. Only about 30% of people living with HIV who need antiretroviral have access to it [2].

In 2009 an estimated 3.6% of 150 million Nigerians are living with HIV and AIDS [3]. Approximately 215,000 people died of HIV/AIDS in Nigeria in 2010 [4].

CD4 count measures the degree of immunosuppression in HIV-positive patients. There is an inverse relationship between CD4 count and degree of immunosuppression. CD4 count is used in monitoring disease progression, deciding when to commence therapy, staging the disease, determining treatment failure, and defining the risk for mother-to-child transmission.

Laboratory markers used in monitoring management in HIV-positive patients are HIV-RNA assay (Viral load) and CD4 count. The former is the gold standard, its use is, however, limited because of its cost and technology. Furthermore, there is a mismatch between an undetectable viral load

(<50 copies/mL) and the absence of immune reconstitution, which can be confusing to both the treatment provider and patient.

Several studies have shown that CD4 count is the strongest predictor of disease progression and survival [5, 6]. The cost of CD4 count is cheaper than viral load, it is increasingly becoming more affordable to patients in resource-poor countries [7, 8]. All HIV-positive patients in resource rich and an increasing number of patients in resource-poor countries have baseline CD4 count on enrollment [9].

The CD4 cell count is the strongest predictor for risk of death and AIDS [10] at the time of initiating therapy, initiating highly active antiretroviral therapy (HAART) at higher CD4 cell counts has been demonstrated to "the risk of death, opportunistic infections and non-HIV related comorbidities" [11, 12]. Robert et al. [13] assessed CD4 count and the risk of death in HIV-infected patients on HAART, nearly all deaths occurred in patients with fewer than 50 CD4 cells/mm^3.

A common denominator amongst all the guidelines on initiating HAART in HIV-positive patients is the use of CD4 count in deciding when to initiate ART in HIV-positive patients. While some HIV-positive patients present at registration with low CD4 count, that is, less than 200 cells/mm^3 probably due to late presentation or diagnosis and are commenced on HAART almost immediately on enrollment irrespective of clinical symptoms. Some asymptomatic HIV-positive patients do not require antiretroviral (ART) drugs on enrollment because of their high CD4 count at registration hence their inability to meet criteria for initiation of therapy laid down by various organizations like World Health Organization (WHO), Centre for Disease Control, (CDC) Atlanta, Presidential Emergency Program for AIDS relief (PEPFER), Working Group of AIDS Research Advisory Council (OARAC), and European AIDS Clinical Society Guidelines, among others.

What is controversial is the optimal time to initiate antiretroviral therapy (ART). Various guidelines exist on the optimal time to initiate ART in adult asymptomatic patients. There seems to be consensus of opinion on deferral of ART in asymptomatic HIV patients whose CD4 count is greater than 500 cells/mm^3. However, the revised 2010, the World Health Organization (WHO) [14] recommended that all adult and adolescent including pregnant women with HIV infection presenting with CD4 count ≤350 cells/mm^3, should start ART regardless of the presence and absence of clinical symptoms. Those with severe and advanced clinical disease (WHO clinical stage 3 and 4) should start ART irrespective of their CD4 cell count.

The U.S. Department of Health and Human Services (DHHS) [15] recommended that treatment of HIV infection shold be commenced, in patients with CD4 counts between 350 and 500 cells/mm^3. A randomized trial still in progress (START) [16] is randomizing people with a CD4 cell count of greater than 500 per μL to either start antiretroviral therapy (ART) immediately or defer to a CD4 cell count of 350 per μL. However, Severe et al. [17] recommended that access to antiretroviral therapy should be expanded to include all HIV-infected adults who have CD4+ T-cell counts of less than 350 cells/mm^3 in those who live in areas with limited resources.

The U.S. Centre for Disease Control (CDC) and the prevention [18] staging system used the CD4 count as a tool to stage HIV into categories A, B, and C based on whether the CD4 count is >500 cells/mm^3, between 200–499 cells/mm^3 and <200 cells/mm^3, respectively. It defines AIDS as all HIV-positive patients with CD4 count <200 cells/mm^3 or CD4% < 14%. On the contrary, the WHO staging is based on clinical findings and does not require CD4 count in order to accommodate for resource-constrained settings where CD4 count testing may not be available.

CD4 count is an important tool in determining treatment failure in HIV-positive patients. The 2010 World Health Organization (WHO) [14] revised guideline defined immunological failure as a fall of CD4 count to baseline level or below, or 50% fall from on-treatment peak value or persistent CD4 count below 100 cells/mm^3. There must, however, be absence of concomitant infection to cause transient CD4 count decrease. A patient presenting with immunological or clinical failure (new or recurrent stage 4 disease) with viral load copies >5000 copies/mL is deemed to have treatment failure and switched to second-line regimens [14].

The introduction of HAART as a modality of treatment in HIV positives has resulted in a dramatic decrease in AIDS-related morbidity and mortality and a great improvement in CD4 count of patients [19]. In order to determine the true picture of CD4 count pattern in HIV positives, HAART-experienced patients must, therefore, be excluded from the study. There is paucity of data on the pattern of CD4 count of HIV-positive, HAART-naïve patients at registration in Nigeria. The data may be used to determine the percentage of HIV-infected patients who require ART at registration. This will assist clinicians and policy makers in determining the point to begin treatment and the percentage of infected patients who require treatment at registration. Thus, this study aimed at determining the percentage of HIV positives who require treatment at enrollment using the CD4 count as a tool.

2. Materials and Methods

The records of 4,042 HAART-naïve, HIV-positive patients who registered at the HIV clinic of Lagos State University Teaching Hospital (LASUTH), Ikeja, between December 2006 and June 2010 were retrospectively reviewed.

Lagos is Nigeria most prosperous and arguably the most populous city. It has one of the highest standards of living as compared to other cities in Nigeria as well as Africa. All ethnic groups in Nigeria are well represented in Lagos because of its cosmopolitan constitution. LASUTH, the only teaching hospital owned by the State Government and one of the two in Lagos, is located in the state capital Ikeja. It serves as a referral centre to 26 other secondary (General) hospitals serving an estimated 17 million Lagosians.

Data retrieved included baseline CD4 count, age, and gender. All HAART-experienced, registered patients referred from other centers were excluded from the study.

Table 1: Gender, age, and CD4 count frequency.

Gender/Age	Male	Female	Overall
Gender	1535 (4042)	2507 (4042)	4042
	38%	62%	100%
Age (years)			
15–30	364 (1535)	954 (2507)	1318 (4042)
	23.7%	38.1%	32.6%
31–50	1035 (1535)	1379 (2507)	2414 (4042)
	67.4%	55%	59.7%
>50	136 (1535)	174 (2507)	310 (4042)
	8.9%	6.9%	7.7%
Mean age	37.73 ± 9.48	35.01 ± 9.34	36.04 ± 9.49
CD4 count cells/mm^3	268.05 ± 230.44	317.55 ± 254.72	298.76 ± 93

3. Statistical Analysis

Data were analyzed using SPSS version 16.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill); a statistical computer software. The descriptive data were given as means ± standard deviation (SD). The differences were considered to be statistically significant when the P value obtained is less than 0.05.

4. Results

Data from 4,042 registered patients were reviewed, consisting of 2507 (62%) females and 1535 (38%) males (Table 1). The overall minimum age was 15years and the maximum 85 years with a mean of 36.04 ± 9.49 years (Table 1). A majority 2414 of 4042 (59.7%) of all patients were between 31–50 years, 1318 of 4042 (32.6%) between 15–30 years and only 310 of 4042 (7.7%) were older than 50 years (Table 1).

The minimum age for male was 15 years and the maximum of 85 years with a mean of 37.73 ± 9.48 years (Table 1). A majority of males, 1035 of 1535 (67.4%) were between 31–50 years, 364 of 1535 (23.7%) between 15–30 years and 136 (8.9%) older than 50 years (Table 1).

The minimum age for female was 15 years and the maximum 76 years with a mean of 35.01 ± 9.34 years (Table 2). The majority of females, 1379 of 2507 (55%) were between 31–50 years, 954 of 2507 (38.1%) between 15–30 years and 174 of 2507 (6.9%) older than 50 years (Table 1).

Overall, the minimum CD4 count was 2 cells/mm^3 and the maximum 1868 cells/mm^3 with a mean of 298.76 ± 246.93 cells/mm^3 (Table 2). Seven hundred and forty-one of 4042 (18.3%) had CD4 count of >500 cells/mm^3 consisting of 414 (55.8%) in the age group 31–50 years, 271 (36.57%) between 15–30 years and 56 (7.55%) older than 50 years. While 3301 of 4042 (81.7%) had CD4 count of <500 cells/mm^3, consisting of 578 (14.3%) with CD4 count of between 350–500 cells/mm^3, 2723 (67.4%) with CD4 <350 cells/mm^3 and 1712 (42.4%) with CD4 count <200 cells/mm^3. Only 522 of 4042 (12.9%) had CD4 count of <50 cells/mm^3. This consists of 319 of 522 (61.1%) in the age group 31–50 years, 166 (31.8%) between 15–30 years, and 37 (7%) older than 50 years (Table 2).

The minimum CD4 count for males was 3 cells/mm^3 and the maximum 1416 cells/mm^3 with a mean of 268.05±230.44 cells/mm^3 (Table 2). A total of 233 (15.1%) of 1535 male patients had CD4 count >500 cells/mm^3 consisting of 169 of 233 (65.23%) between the age of 31–50 years, 60 (25.75%) between 15–30 years, and 21 (9%) older than 50 years. The majority 1302 of 1535 (84.9%) had CD4 count <500 cells/mm^3. A total of 193 of 1535 (12.6%) had CD4 count between 350–500 cells/mm^3, while 1109 of 1535 (72.3%) had CD4 count <350 cells/mm^3, 740 of 1535 (48.3%) with CD4 count <200 cells/mm^3. Only 245 (16%) of males had CD4 count <50 cells/mm^3 consisting of 169 (68.97%) between 31–50 years, 51(20.81%) between 15–30 years, and 25 (10.2%) older than 50 years (Table 2); $P = 0.528$.

The minimum CD4 count for females was 2 cells/mm^3 and the maximum of 1868 cells/mm^3 with a mean of 317.55± 254.72 cells/mm^3 (Table 2). A total of 508 of 2507 (20.3%) had CD4 count >500 cells/mm^3 consisting of 262 of 508 (51.57%) between 31–50 years, 211 of 508 (41.53%) between 15–30 years and 35 (6.8%) older than 50 years. A total of 1999 of 2507 (79.7%) females who had CD4 count <500 cells/mm^3 consisted of 385 of 2507 (15.4%) with CD4 count of between 350–500 cells/mm^3 1614 of 2507 (64.3%) of females with CD4 count <350 cells/mm^3, and 972 of 2507 (38.77%) with CD4 count <200 cells/mm^3. A total of 277 of 2507 (11%) had CD4 count <50 cells/mm^3 consisting of 150 of 277 (54.15%) between 31–50 years, 115 of 277 (41.51%) between 15–30 years, and 12 of 277 (4.3%) older than 50 years (Table 2); $P = 0.039$.

5. Discussion

In Nigeria, the first case of HIV/AIDS was reported in 1986. HIV prevalence declined from 6% in 2001 to 4.3% in 2005, 4.2% in 2008, and 4.1% in 2010. HIV response in Nigeria was health sector driven from 1986–1989,, but a multisectoral response commenced in 2000. Funding for the HIV response in Nigeria is obtained from both domestic (Federal Government of Nigeria, private sectors and state governments) and international sources like the U.S. Government, DFID, UN agencies, and global funds. It is pertinent to determinethe percentage of HIV-infected patients who require ART at registration vis-à-vis percentage benefiting from care services in order to appreciate progress made in reaching out to those in need of accessing care and treatment.

In Nigeria, prevalence among young women aged 15–24 years is estimated to be three times higher than among men of the same age. Females constitute 58% (about 1.72 million) of persons living with HIV in Nigeria and each year, 55% of AIDS death occurs among women and girls [2] The female:male ratio in the present study of 1.6 : 1 is similar to 1.8 : 1 obtained by Omoti et al. [20] in Benin City, Nigeria. The disparity in gender prevalence is age dependent

Table 2: CD4 counts distribution according to age and gender categories.

Age categories (Years)	Male	Female	Overall
	CD4 counts cells/mm^3		
	>500: 233 (1535) 15.2%	508 (2507) 20.3%	741 (4042) 18.3%
15–30	60 (233) 25.8%	211 (508) 41.5%	271 (741) 36.6%
31–50	169 (233) 72.5%	262 (508) 51.6%	431 (741) 58.1%
>50	21 (233) 9%	35 (508) 6.9%	53 (741) 7.2%
	<500: 1302 (1535) 84.8%	1999 (2507) 79.7%	3301 (4042) 81.7%
	CD4 count ranges incells/mm^3		
350–500	193 (1535) 12.6%	385 (2507) 15.4%	578 (3301) 17.5%
<350	1109 (1535) 72.2%	1614 (2507) 64.4%	2723 (3301) 82.5%
<200	740 (1535) 48.2%	972 (2507) 38.8%	1712 (3301) 51.9%
<50	245 (1535) 16%	277 (2507) 11%	522 (3301) 15.8%

as reported by Glynn et al. [21] who reported HIV prevalence was six times higher in women than in men amongst sexually active 15–19 years old, but it drops to three times that in men among 20–24 years old and equal to that of men among 25–49 years old. The present study did not consider age in groups in relation to gender and HIV status. However, 59.7% of the studied population was between 31–50 years, a ratio of F : M of 1.6 : 1 obtained was, therefore, similar to 1 : 1 reported by Glynn et al. in those between 25–49 years. Generally, females are more predisposed to contracting HIV because of pregnancy or use of oral contraceptive, conditions which induced cervical ectopia in which there is replacement of squamous by columnar epithelium, thus increasing the risk of HIV infection for women 5-fold. Sexual intercourse during menstruation and presence of genital ulcer also increases the risk of HIV infection in females. Pelvic inflammatory disease predisposes to microulceration of the genital tract thus increasing risk of HIV infection. Culturally, the majority of males in this part of the world are circumcised, male circumcision affords some degree of protection, perhaps due to large numbers of langerhans cells in foreskin, so that the incidence of infection is reduced 8-fold over uncircumcised men [22]. Glynn reported that, despite all the predisposing factors in females, women married younger than men, and marriage was a risk factor for HIV, women often had older partners and men rarely had partners much older than themselves.

The mean ages of 35.01 ± 9.34 years and 37.73 ± 9.48 years for females and males, respectively, obtained in this study are similar to earlier study [21] of 34.41 ± 8.87 and 38 ± 9.35 for females and males, respectively, and also similar to 38 years reported by Omoti et al. [20] in both genders. This is understandably so because the majority of patients in HIV clinic are between 31–50 years being the age when sexual activity is at its peak.

This study reported mean CD4 counts in HAART-naïve, HIV positives of 268.05 ± 230.44 and 317.55 ± 254.72 cells/mm^3, respectively, for males and females and an overall mean of 298.76 ± 246.93 cells/mm^3. This could be compared with 303.16 ± 234.32 cells/mm^3 and 308.24 ± 232.2 cells/mm^3, respectively, for males and females and an overall mean CD4 count of 306.65 ± 232.24 cells/mm^3 reported by Akinbami et al. in an earlier study [23]. In both studies, females were found to a have a higher CD4 count than males.

Oladepo et al. [24] established in healthy Nigerian adults a reference value for CD4 of 365 to 1,571 cells/μL. with a mean CD4 count of 847 cells/μL similar to the mean value of 828 cells/μL reported by Aina et al. [25] in an earlier study in Nigeria. Females were found to have significantly higher values of absolute CD4 counts in Oladepo's study in contrast to the earlier limited study by Aina et al. in Nigeria. This observation of higher CD4 count in females was also reported in several other countries among Nigerians [26], Ugandans [27], and Ethiopians [28]. A sex hormone effect is one possible explanation for the reported difference in CD4 counts between genders that has been suggested [28].

Using the 350 cells/mm^3 CD4 count as cutoff from 2010 WHO [14] revised guideline for initiation of therapy in asymptomatic HIV patients, 72.3% males, 64.3% females and 67.4% overall registered patients require treatment on enrollment, while only 15.1% males, 20.3% females, and 18.3% overall registered patients with CD4 count >500 cells/mm^3 may require antiretroviral therapy deferral at registration.

In Sub-Saharan Africa, an estimated 10 million need treatment in 2010, only 5 million received it [1]. In Nigeria as at 2009, only 31% of people living with HIV have access to care services [29], the government, through the National HIV/AIDS strategic framework for 2005–2009, set out to provide ARV to 80% of adults and children with advanced HIV infection and 80% of HIV-positive pregnant women, all by 2010 [30].

ARV treatment coverage in Nigeria remains low, the slow progress led to revising the strategic framework, and resetting treatment goals in its revised 2010–2015 framework [31]. By 2010, only a quarter of adults and 7% of children in need of treatment received it. Currently, 1.4 million adults and 262,000 children eligible for antiretroviral treatment remain without it [1].

In Africa, Botswana at the end of 2010 has the highest coverage rate around 93%, other countries that have achieved more than 80% coverage are Rwanda and Namibia [1].

Access to antiretroviral therapy in Somalia is the lowest in Africa at 3% while only 55% of those in need in South Africa are receiving it [32].

Cameroon, Cote d'Ivoire, Chad, Nigeria, and Ghana are some of the countries in Sub-Saharan Africa where between 20–39% of people requiring antiretroviral drugs are receiving them [1].

Being a retrospective study, some of the limitations of this study is the nonavailability of data on clinical manifestations of the patients, lack of data on records of distribution of the CD4 count, and percentage in need of treatment per year and lack of information on HIV risk factor, area of residence, family income, and marital status.

Extra efforts must be made by the Nigerian Government at all levels to meet the need of people living with HIV/AIDS eligible for treatment so as to reduce the spread of the pandemic.

6. Conclusion

About three-quarter of HIV positives require antiretroviral therapy at registration when 2010 WHO criteria are used for initiation of therapy, female population in HIV clinic is higher than males and the former has a relatively higher CD4 counts than the latter.

Conflict of Interests

There is no conflict of interest declared.

Authors' Contribution

A. A. Akinbami conceptualized, designed, and did data entry and analysis of the study. A. O. Dosunmu drafted the paper and revised it critically for important intellectual content. A. Adediran made substantial contributions to conception and design of the paper and reviewed it before final submission. S. O. Ajibola reviewed the paper critically for important intellectual content and gave final approval of the version to be published. K. O. Wright was involved in the drafting of the paper and reviewed it critically for important intellectual content. O. Oshinaike reviewed paper before final submission. O. Arogundade carried out the CD4 count assay.

Acknowledgments

The Institute of Human Virology Nigeria provided registration, laboratory investigations, and treatment free to all registered HIV patients used for this study. The authors are also grateful to Mr. Obadeyi Oluwaseun, the ICT (information and communication technology personnel) who retrieved the data used for the study.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 581531, 6 pages
doi:10.1155/2012/581531

Clinical Study

Comparison of the Minimental State Examination Scale and the International HIV Dementia Scale in Assessing Cognitive Function in Nigerian HIV Patients on Antiretroviral Therapy

O. Olajumoke Oshinaike,[1] A. Akinsegun Akinbami,[2] O. Oluwadamilola Ojo,[3] I. Frank Ojini,[3] U. Njideka Okubadejo,[3] and A. Mustapha Danesi[3]

[1] *Department of Medicine, College of Medicine, Lagos State University, Nigeria*
[2] *Department of Hematology and Blood Transfusion, College of Medicine, Lagos State University, Nigeria*
[3] *College of Medicine, University of Lagos, Lagos State, Idi-Araba, Nigeria*

Correspondence should be addressed to O. Olajumoke Oshinaike, olajumoke68@yahoo.com

Received 12 January 2012; Revised 24 August 2012; Accepted 26 August 2012

Academic Editor: Giuseppe Ippolito

Introduction. HIV-associated neurocognitive disorder (HAND) remains common despite the availability of antiretroviral therapy. Routine screening will improve early detections. *Objective*. To compare the performance of the minimental state examination (MMSE) and international HIV dementia scale (IHDS) in assessing neurocognitive function in HIV/AIDS patients on antiretroviral therapy. *Methods*. A case-control study of 208 HIV-positive and 121 HIV-negative individuals. Baseline demographic data were documented and cognitive function assessed using the two instruments. CD4 cell counts were recorded. *Results*. Cases comprised 137 females and 71 males. Controls were 86 females and 35 males. Mean MMSE score of cases was 27.7 ± 1.8 compared to 27.8 ± 1.3 in controls ($P = 0.54$). Mean IHDS score in cases was 8.36 ± 3.1 compared to 10.7 ± 0.9 in controls ($P < 0.001$). Using the MMSE scale, 6 cases but no controls had HAND ($P = 0.09$). Using the IHDS, 113 (54.3%) had HAND compared with 10 (8.3%) controls ($P < 0.0001$). Using IHDS, 56.5% cases with CD4 count >200 had HAND compared with 92.5% with CD4 count <200 ($P < 0.001$). *Conclusion*. These findings indicate that the IHDS detects higher rates of HAND and may identify HIV/AIDS patients who require further cognitive assessment using more robust assessment batteries.

1. Introduction

HIV-associated neurocognitive disorder is often encountered in HIV infection despite the use of potent antiretroviral therapy. The spectrum ranges from mild and asymptomatic neurocognitive impairment (ANI), minor neurocognitive disorder (MND), to the more severe HIV-associated dementia (HAD) [1]. ANI is characterized by asymptomatic or unrecognized neurocognitive impairment that may go unnoticed except specifically screened for, and individuals with ANI are more likely to progress to more severe forms of cognitive dysfunction. The essential features of MND are impaired cognitive or behavioral function in at least 2 domains (e.g., impaired attention-concentration, mental slowing, abnormal memory or other cognitive functions, slowed movements, incoordination, personality change, irritability, and emotional lability). In contrast to ANI, these abnormalities typically impair work-related function or activities of daily living, albeit mildly. MND is associated with shortened survival, reduced adherence with antiretroviral therapy, and problems with employability, and its presence is predictive of HAD. HAD represents the most severe form of cognitive dysfunction, with significant functional impairments, and is synonymous with HIV encephalopathy and AIDS dementia complex (ADC). ADC is one of the most common central nervous system complications of late HIV infection occurring in 15–20% of patients before the introduction of HAART [2, 3].

The widespread use of HAART has resulted in a sharp decline in its incidence but the prevalence has actually increased because of prolonged survival [4–6]. Similarly, the prevalence of minor cognitive deficits appears to have increased, with reported prevalence between 20 and 50% of HIV-positive patients [4, 5, 7–9]. The prevalence of cognitive impairment in the aviremic HIV-positive population was 69% in one study [10]. Risk factors for HAND in HIV include a high HIV viral setpoint, lower CD4 cell counts, anemia, low body mass index, increasing age, systemic symptoms, injection drug use, and female gender [11–14].

Screening for early deficits and careful evaluation of psychomotor function would permit the use of additional treatments [15–17] to improve cognitive functioning. The diagnosis of HAND in HIV is dependent upon a clinical history and neurological examination consistent with the criteria developed by the American Academy of Neurology [18]. Neuropsychological testing is a critical component of the diagnosis showing abnormalities in psychomotor speed, attention, frontal lobe function, and verbal and nonverbal memory. However, administration of the entire neuropsychological test battery is cumbersome in a real-world clinical scenario because it is time-consuming, language and education dependent, and manpower intensive. In most countries of sub-Saharan Africa where the vast majority of HIV cases reside, simpler but effective screening tools are required to enhance early recognition of persons with cognitive dysfunction. The ideal screening tool should emphasize motor skills and timed tasks, must be inexpensive, universally available, brief, sensitive, and reliable. The Minimental state examination scale (MMSE) is a generic instrument that was originally developed to screen for dementia and delirium and is the most widely used cognitive impairment screening instrument. Despite its ease of administration and wide recognition, the validity of the MMSE in subcortical disorders such as HIV-associated cognitive impairment has been criticized. In the pre-HAART era, the HIV dementia scale (HDS) was developed [19] and subsequently modified for use in international settings as the International HIV Dementia Scale (IHDS) [20]. The study hypothesis was that there is a significant difference in the detection of HAND when MMSE is used compared to the IHDS scale. Specifically, we predicted that the IHDS would identify more cases of HIV with HAND compared with MMSE. Our purpose was to either buttress or refute the current practice of using the MMSE instead of the IHDS scale in the setting of HIV/AIDS.

2. Methodology

2.1. Study Setting and Design. The HIV clinic at the Lagos University Teaching Hospital (LUTH) is one of several HIV follow-up and treatment centers in the country funded by the Presidents Emergency Programme for AIDS Relief in Africa (PEPFAR) and is a referral center attending to about 9,000 patients annually. We used a case-control study design involving HIV-positive adults (aged >18 years) as cases and HIV-negative age-matched adults as controls. Approval of the study protocol was obtained from the Health Research and Ethics Committee of the LUTH. Informed consent was obtained from all participants.

2.2. Participant Recruitment and Data Collection. The HIV-positive cases were recruited consecutively over a 12-month period between June 2007 and May 2008. HIV-negative controls subjects were recruited from the HIV voluntary counseling and testing section of the same hospital. Cases were matched for age and sex with the controls. All cases included had low CD4 cell counts and had been on antiretroviral therapy for at least 6 months. Exclusion criteria were major opportunistic infections of the brain in the past 3 years, any other opportunistic infection not affecting the brain in the past 12 months (very ill patients), major depression according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria, active injection/inhalational recreational drug use (e.g., IV heroin, marijuana), and pregnancy.

Baseline demographic parameters, medical history, physical and neurological examination were documented using a standardized proforma. For all HIV-positive cases, latest (within preceding 3 months) CD4 count result was extracted from the clinic electronic database or case records. Face-to-face neuropsychological testing was conducted using both the MMSE [21] and the IHDS in each participant. The interviews were done by a trained neurologist conversant with the application of both instruments (O. O. Oshinaike).

2.3. Description of Study Instruments. The IHDS is a modification of the HIV dementia scale first proposed by Power et al. (1995) [19] and recently adapted by Sacktor et al. (2005) [20]. It consists of 3 subsets: timed finger tapping which measures motor speed; timed alternating hand sequence which assesses the psychomotor speed; recall of 4 items in 2 minutes which assesses memory registration and recall. Each of these subtests is rated on a scale of 0–4. The tests were administered as follows: for assessment of the verbal recall subtest, registration (new learning) was measured by reciting 4 words to the subject (blue, dog, hat, and apple) taking 1 second to say each of the words. The subject was asked to repeat the words and recall the 4 words after the timed finger tapping, and alternating hand sequence tests were performed. The MMSE is an interviewer-administered questionnaire testing 5 domains (orientation, memory registration, attention and calculation, memory recall, and language) with a maximum score of 30 points. The cut-off values for defining cognitive impairment using the MMSE and IHDS, respectively, were 26 and 10 (based on the mean score for controls minus 1 standard deviation).

2.4. Data Analysis. Data entry and analysis were achieved using Epi Info (Epi 3.5.1 version) statistical software. Group differences in mean values of numerical data (including age, CD4 cell count, and test scores) were compared using Student's t-test, while Pearson Chi-square was used to determine statistical significance of group differences for categorical variables. Level of significance was set at P value < 0.05.

Table 1: Baseline data and cognitive scores for participants in the study.

Variables	HIV-positive (cases) $N = 208$ (%)	HIV-negative (controls) $N = 121$ (%)	P value
Gender			
Male	71 (34.1%)	35 (28.9%)	0.39
Female	137 (65.9%)	86 (71.1%)	
Mean age (years)	36.8 ± 8.3	38.0 ± 8.4	0.21
Mean CD4 count ± SD (cells/mm^3)	257.2	N/A	
Mean score MMSE ± SD	27.7 ± 1.8	27.8 ± 1.8	0.54
Mean score IHIDS ± SD	8.36 ± 3.1	10.7 ± 0.9	0.0001

N/A: not applicable.

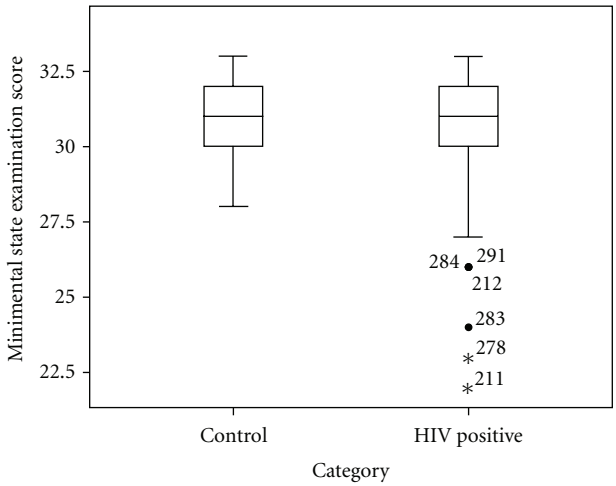

Figure 1: Comparison of MMSE scores in HIV-positive cases and HIV-negative controls. Box plot illustrating the distribution of MMSE scores in cases and controls. The mean ± SD) MMSE score of the controls (27.8 ± 1.3) and HIV-positive cases (27.7 ± 1.8) did not differ significantly (ANOVA; $P = 0.59$). Asterisked cases represent outliers within the HIV-positive group with MMSE scores below the group minimum.

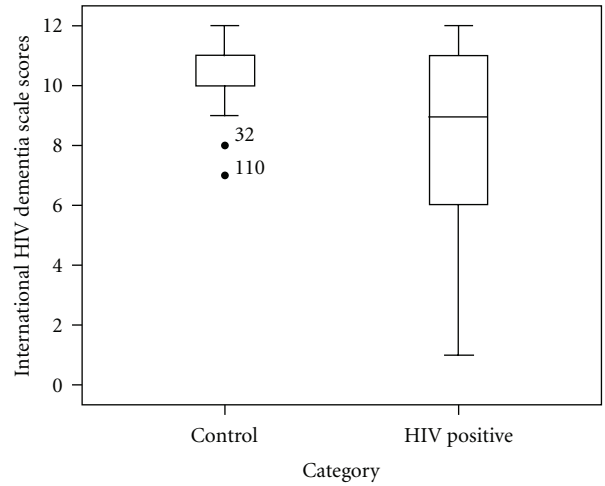

Figure 2: Comparison of IHDS scores in HIV-positive cases and HIV-negative controls. Box plot illustrating the distribution of IHDS scores in cases and controls. The mean (SD) score of the controls (10.7 ± 0.9) and HIV-positive cases (8.36 ± 3.1) differed significantly (ANOVA; $P < 0.0001$). Asterisked cases represent outliers within the control group with scores below the group minimum.

3. Results

3.1. Demographic Characteristics. The 208 cases comprised 137 (65.9%) females and 71 (34.1%) males with a mean age ± SD of 36.8 ± 8.3 years (range 19–63 years). The control group (total number = 121) was made up of 86 (71.1%) females and 35 (28.9%) males, with a mean age of 38.0 ± 8.4 years (range 22–66 years). The age and gender differences were not statistically significant ($P = 0.18$ and 0.33 resp.). The mean CD4 count of cases was 251.4 ± 171.4 cells/mm^3 (range 4–939 cells/mm^3). Data shown in Table 1.

3.2. Comparison of Cognitive Performance Using the IHDS and the MMSE Scores. The mean MMSE scores of HIV-positive cases was 27.7 ± 1.8 compared with 27.8 ± 1.3 for controls ($P = 0.54$) whilst the mean score of cases using the IHDS scale was 8.36 ± 3.1 compared with 10.7 ± 0.9 in controls ($P = 0.0001$) (Figures 1 and 2). Based on the cut-off score of 26 to define HAND, Figure 3 shows that using the MMSE scale, 6 (2.9%) HIV cases were identified to have HAND compared to none of the controls (Fisher exact $P = 0.09$). Using the IHDS scale, based on a cut-off score of 10 (<1 SD below mean score of controls of 10.66) to define HAND, 113 HIV-positive cases (54.3%) were found to have HAND compared with 10 (8.3%) among the controls ($X^2 = 69.3$; $P < 0.0001$), whereas the MMSE did not detect any significant difference between HIV cases and controls, the IHDS showed a significantly higher frequency of HAND in cases. Furthermore, HAND detection rates within the HIV-positive group were significantly higher using the IHDS (113/208 i.e., 54.3%) compared to the MMSE (6/208 i.e., 2.9%) (odds ratio 0.02; 95% confidence interval 0.01–0.06; $P < 0.0001$).

3.3. Relationship of Disease Severity with Cognitive Scores. CD4 cell count (mm^3) was categorized into two to reflect

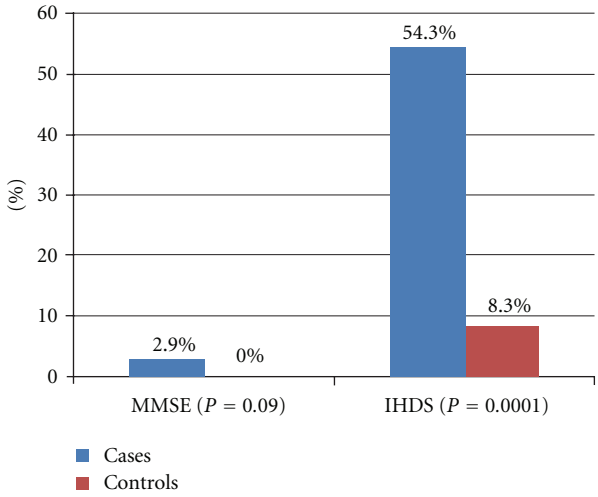

Figure 3: Frequency of cognitive impairment based on the MMSE and IHDS scores of cases (HIV-positive) and controls (HIV-negative).

disease severity (defining severe disease as CD4 count ≤200). The study comprised 115 (55.3%) with CD4 count above 200 and 93 (44.7%) ≤ 200. Using the MMSE scale, 1 (0.9%) of cases with CD4 count >200 cells/mm^3 had HAND compared with 5 (5.4%) of those with CD4 count ≤200 cells/mm^3 (Fisher's exact $P = 0.06$). Conversely, with the IHDS scale, 44/115 (38.3%) of cases with CD4 count >200 cells/mm^3 had HAND compared with 69/93 (74.2%) with CD4 count <200 cells/mm^3 ($X^2 = 26.8$; $P < 0.0001$).

3.4. Classification of HAND according to Modified Updated AAN Criteria. There was insufficient data to credibly classify the subjects using these criteria (due to absence of data relating to impact on ADL/daily functioning). However with modification of the criteria, ANI and MND were grouped together (applying only the data regarding 1SD below control values and exclusion criteria) and HAD as any with values below 2SD. A total of 25 (12%) of cases had ANI and MND whilst 88 (42.3%) had HAD. The mean CD4 count of cases with ANI/MND was 208.4 99.7 cells/mm^3 (median 188.0) whilst the mean CD4 count of cases with HAD was 173.4 1226 cells/mm^3 (median 150.5) $P = 0.0001$. A total of 13/25 cases (52%) of ANI/MND had CD4 count <200 compared with 56/88 (63.6%) of cases with HAD $P = 0.0001$.

4. Discussion

There are few studies corroborating the superiority of the International HIV Dementia Scale over the MiniMental State Examination for assessment of HAND in persons with HIV via direct comparative studies and employing a control group. The main findings from our study are that the IHDS detects a higher proportion of persons with HAND in HIV, affording an advantage for more intensive evaluation and early interventions to improve quality of life. Although extensive neuropsychological testing using a combination of tests is regarded as the "gold standard" for cognitive assessment, the IHDS offers an advantage in the "real-world" clinical setting due to the ease of administration and can thus serve as an indicator of the need for further assessment and also serve as a monitoring tool in routine practice. The MMSE scale was only able to weakly distinguish HIV cases from controls with respect to occurrence of HAND. This reinforces previous observations alluding to its lack of sensitivity to sub-cortical cognitive dysfunction. Skinner et al. compared the performance of the original HIV dementia scale (HDS), IHDS, and MMSE scales against other neurocognitive batteries in assessing cognitive dysfunction in HIV patients and also demonstrated the inferiority of MMSE in contrast to the HDS and IHDS. This may be explained by the ability of the IHDS to screen for psychomotor speed (in addition to attention/working memory, executive functioning, memory, and verbal/language), an aspect that is not included in the MMSE scale. Also, literacy level and language comprehension impair the MMSE, thus further limiting its application. The low mean test scores in the control groups may be due to bias as MMSE scores have been shown to be affected by age, sex, lower education level and sociocultural background thereby leading to improper classification of individuals [22].

This study also demonstrated a statistically significantly higher frequency of HAND in relation to disease severity, with higher rates in HIV cases with more severe immune compromise (CD4 cell count below 200). The magnitude of the difference was also higher with the IHDS. This is explicable as CD4-related inflammatory changes in the brains of presymptomatic subjects are known to be mediated by the HIV-associated breakdown of the immune system and consequent lymphocyte dysfunction, allowing brain damage to occur. Also, the evidence for viral replication in the CNS, despite the lack of symptoms, suggests that neurocognitive functioning is likely to be more affected when more systemic immune suppression appears [23]. We noted a significantly lower mean CD4 cell count and disease severity in cases with HAD compared to ANI/MND using a modified classification of the levels of HAND according to the updated AAN criteria.

Njamnshi et al. [24] reported a significant difference in mean HDS scores in HIV cases compared to HIV-negative controls. Lyon et al. [25] and Ganasen et al. [26] compared the HDS with the MMSE and found a higher sensitivity of 83% and 80%, respectively, using the HDS compared to 50% using the MMSE.

5. Conclusion

Our study has added to the body of evidence encouraging the use of the IHDS as a screening instrument in the real-world clinical scenario of HIV/AIDS management, and we reemphasize that its intrinsic ability to reveal even mild cognitive impairment amenable to earlier intervention more than compensates for the possibility of a lower specificity. Incorporation of variables to determine affectation of activities of daily living into the original design of these instruments may assist in proper classification of patients especially

in regions where more robust batteries are unavailable. With the accumulating evidence that standard HAART regimens are unable to fully reverse HAND and the unclear benefits of CNS-penetrant antiretroviral drugs even in the setting of long-term plasma viral suppression, there is a need for randomized prospective trials to explore the role of other adjuvant and neuroprotective therapies.

Limitations

We acknowledge that the study is limited by the lack of comparison to a "*gold standard*", such as a neuropsychological battery, our aim was to compare two clinically used point-of-care instruments. Intrinsic to cognitive assessment is the aspect of its determination by clinico-psychological assessment rather than structure-defining measures such as neuroimaging modalities, abnormalities of which do not necessarily correspond to impaired functioning.

Authors' Contribution

O. O. Oshinaike and A. M. Danesi designed the study. O. O. Oshinaike, A. A. Akinbami and O. O. Ojo recruited the patients and collected data. O. O. Oshinaike and U. N. Okubadejo analyzed the data. U. N. Okubadejo and F. I. Ojini reviewed the paper for intellectual content and approved the final version.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 873171, 9 pages
doi:10.1155/2012/873171

Clinical Study

Oral HIV-Associated Kaposi Sarcoma: A Clinical Study from the Ga-Rankuwa Area, South Africa

Razia A. G. Khammissa,[1] Liron Pantanowitz,[2] and Liviu Feller[1]

[1] *Department of Periodontology and Oral Medicine, University of Limpopo, Medunsa Campus, Pretoria 0204, South Africa*
[2] *Department of Pathology, University of Pittsburgh Medical Centre, Pittsburgh, PA 15260, USA*

Correspondence should be addressed to Liviu Feller, lfeller@ul.ac.za

Received 1 December 2011; Revised 31 July 2012; Accepted 31 July 2012

Academic Editor: Giuseppe Ippolito

Background. Kaposi sarcoma (KS) is one of the most common neoplasms diagnosed in HIV-seropositive subjects. Oral involvement is frequent and is associated with a poor prognosis. The aim of this study was to characterize the features of oral HIV-KS in patients from Ga-Rankuwa, South Africa. *Methods*. All cases with confirmed oral HIV-KS treated at the oral medicine clinic in Ga-Rankuwa from 2004 to 2010 were included in this retrospective study. Differences between males and females with oral HIV-KS in relation to HIV infection status, to oral KS presentation and to survival rates were statistically analysed. *Results*. Twenty (54%) of the 37 patients in the study were females and 17 (46%) were males. In 21 patients (57%), the initial presentation of HIV-KS was in the mouth. Other than the fact that females presented with larger (≥10 mm) oral KS lesions ($P = 0.0004$), there were no statistically significant gender differences. Significantly more patients presented with multiple oral HIV-KS lesions than with single lesions ($P = 0.0003$). Nine patients (24%) developed concomitant facial lymphoedema, and these patients had a significantly lower CD4+ T-cell count (28 cells/mm^3) compared to the rest of the group (130 cells/mm^3) ($P = 0.01$). The average CD4+ T-cell count of the patients who died (64 cells/mm^3) was significantly lower ($P = 0.0004$), there were no statistically significant gender differences. Significantly more patients presented with multiple oral HIV-KS lesions than with single lesions ($P = 0.016$) at the time of oral-KS presentation than of those who survived (166 cells/mm^3). Conclusions: In Ga-Rankuwa, South Africa where HIV-KS is prevalent, oral KS affects similarly males and females. A low CD4+ T-cell count at the time of oral HIV-KS diagnosis and the development of facial lymphoedema during the course of HIV-KS disease portends a poor prognosis.

1. Introduction

Kaposi sarcoma (KS) is a multicentric angioproliferative disorder of endothelial origin [1, 2]. KS predominantly affects mucocutaneous sites, but may also affect visceral organs. KS is characterized microscopically by angiogenesis, the presence of spindle-shaped tumour cells, an inflammatory cell infiltrate dominated by mononuclear cells, extravasated erythrocytes, and oedema [3, 4].

There are four clinicoepidemiological variants of KS: classic KS, endemic KS, iatrogenic KS, and HIV-associated KS (HIV-KS). These variants develop in distinct populations of subjects, and in all of them, the mouth may be affected. Human herpes virus 8 (HHV8) is a critical factor, although not on its own sufficient for the development of KS. Other cofactors including profound immune impairment, angiogenic mediators, or genetic predisposition appear to be necessary for the development of KS [5].

HIV-KS may develop at any stage of HIV infection including the stage of early HIV-seropositivity, but it is more prevalent at a lower CD4+ T-cell count [6]. It may be mild or life threatening. Aggressive HIV-KS is associated with disseminated lesions, with intraoral exophytic lesions, with facial lymphoedema, and with an increased HHV8 viral load [7, 8]. HIV-KS may sometimes present as an immune reconstitution inflammatory syndrome (IRIS) shortly after the introduction of antiretroviral therapy, in parallel with the improvement of the host immune status [9–11].

It is estimated that in 22% of HIV-seropositive subjects with KS the initial presentation of HIV-KS is in the mouth,

and that in up to 71% of subjects with HIV-KS, sooner or later the mouth will be affected [12, 13]. Oral HIV-KS lesions may be single or multifocal initially present as macules that progress to papulonodular lesions and ultimately become confluent forming large exophytic masses [14]. The mortality rate of patients with oral HIV-KS is greater than patients with only cutaneous HIV-KS. The former has a 24-month median survival rate compared to the latter that has a 72-month median survival rate [15].

HIV-KS is common in African countries where HHV8 infection is endemic, where HIV infection has reached epidemic proportions, and where antiretroviral medication is not always available [16, 17]. In South Africa, the prevalence of HIV infection is estimated to be about 30% [16, 18] and the prevalence of HHV8 infection may reach 40% in some areas [16, 17].

Worldwide, HIV-KS affects males more commonly than females [19], but in sub-Saharan Africa where HIV infection is more prevalent among young females than among young males, the frequency of HIV-KS disease among females has accordingly increased rapidly [4, 19, 20]. However, the female-to-male ratio, age distribution, and the course of oral HIV-KS in South Africa are not well defined.

The aim of this retrospective study was to characterise the clinical features and course of oral HIV-KS in patients attending the oral medicine clinic at the School of Oral Health Sciences, University of Limpopo, Medunsa campus, South Africa, and to investigate differences between females and males with oral HIV-KS with regard to their CD4+ T-cell count, to the clinical presentation of oral HIV-KS and to survival rate.

2. Materials and Methods

Approval of the study was obtained from the Medical Research and Ethics Committee of the University of Limpopo, Medunsa campus, Pretoria, South Africa (MREC 0/212/2010 : PG). All the files of patients with histologically and clinically confirmed oral HIV-KS treated in the Department of Periodontology and Oral Medicine, School of Oral Health Sciences, University of Limpopo, Medunsa campus, from January 2004 until November 2010 were retrieved.

In this retrospective study, the diagnosis of KS was confirmed by microscopic examination of incisional biopsy specimens by an oral pathologist; the HIV-serostatus of the patients was determined by enzyme-linked immunosorbent assay (ELISA) and Western blot.

Data were recorded with regard to patient age, race, and gender; the oral site affected by HIV-KS; the clinical appearance of oral HIV-KS lesions; the period of HIV-seropositivity before a KS diagnosis was rendered; the CD4+ T-cell count at the time of HIV diagnosis and when HIV-KS was diagnosed; whether patients received highly active antiretroviral therapy (HAART) at the time of oral HIV-KS diagnosis, or thereafter; any KS involvement on the skin; the presence of facial lymphoedema; the presence of an immune reconstitution inflammatory syndrome (IRIS); the treatment modality used for oral HIV-KS; the course and response to treatment of oral HIV-KS disease; the survival period of patients from the time oral HIV-KS was diagnosed until the end of the observation period; for those who died during the observation period, the time that had elapsed from oral HIV-KS diagnosis to death.

IRIS-associated oral HIV-KS was diagnosed when there was worsening of pre-existing oral HIV-KS, or when there was development of new oral HIV-KS lesions, shortly after the introduction of HAART in parallel with an improvement in the immune status.

The presence of any pertinent medical information or HIV-associated oral diseases other than oral HIV-KS was also documented.

The clinical appearance of oral HIV-KS was categorised into macular, papular, nodular, and exophytic lesions. The lesions were classified into three size groups: smaller than 10 mm; between 10 mm and 50 mm and larger than 50 mm. The lesions of oral HIV-KS were categorised as solitary or multifocal. The oral site affected and the number of lesions per site was documented. Lesions affecting the upper and lower retromolar area and the soft palate were categorised as oropharyngeal lesions.

HAART comprised nevirapine, lamivudine, and stavudine. Local cytotoxic chemotherapy consisted of intralesional bleomycin. Systemic cytotoxic chemotherapy comprised combination of low-dose intravenous vincristine, bleomycin, and daunorubicin.

2.1. Statistical Analysis. Differences between proportion were statistically tested using the Chi-squared test, two-sided $P < 0.05$.

3. Results

The study population comprised 37 patients diagnosed with oral HIV-KS, all of whom were black persons. The mean age at the time of oral HIV-KS diagnosis was 33.4 years (Table 1). Two patients were children aged 10 and 11 years. Seventeen males (46%) and 20 females (54%) were affected (M : F = 1 : 1.2). Nine patients had a history of smoking tobacco (Table 1).

In 21 patients (57%) the initial presentation of HIV-KS was in the mouth; in 6 patients the initial presentation of HIV-KS was concurrently in the mouth and on the skin; 10 patients (27%) developed cutaneous HIV-KS before the appearance of oral HIV-KS (Table 1), on average 4.5 weeks before the diagnosis of their oral HIV-KS. Three patients developed cutaneous HIV-KS after their diagnosis of oral HIV-KS on average 4.3 weeks later, and 18 patients (49%) did not develop cutaneous HIV-KS.

The CD4+ T-cell counts were obtained only for 33 patients, the mean CD4+ T-cell count being 107 cells/mm^3 (Table 1). At the time of diagnosis of oral HIV-KS, twelve patients (32%) had concomitant oral candidiasis, one had concomitant oral hairy leukoplakia, and one had concomitant necrotizing gingivitis (Table 1). Nineteen patients (51%) had concurrent infection with *Mycobacterium tuberculosis* (TB), one had gonorrhoea, and one had bronchitis.

Table 1: Clinical and laboratory features of the patients at the time of oral HIV-KS diagnosis.

	Males	Females	Total
Number of patients (%)	**17** (**46**%)	**20** (**54**%)	**37** (**100**%)
Age (years)			
Mean	**34**	**33**	**33.4**
Range	*11–55*	*19–46*	*11–55*
Standard deviation	*12.53*	*7.69*	*10.08*
Tobacco usage (%)	**7** (**41**%)	**2** (**10**%)	**9** (**24**%)
Number of patients in whom the initial presentation of HIV-KS was in the mouth	**8** (**47**%)	**13** (**65**%)	**21** (**57**%)
Number of patients in whom the initial presentation of HIV-KS was concurrently in the mouth and skin	**3** (**18**%)	**3** (**15**%)	**6** (**16**%)
Number of patients who developed cutaneous HIV-KS before oral HIV-KS diagnosis	**6** (**35**%)	**4** (**20**%)	**10** (**27**%)
Other oral lesions present			
Pseudomembranous candidiasis	**7** (**41**%)	**5** (**25**%)	**12** (**34**%)
Hairy leukoplakia		**1** (**5**%)	**1** (**2**%)
Necrotizing gingivitis	**1** (**6**%)		**1** (**2**%)
Total number of patients	**8** (**47**%)	**6** (**30**%)	**14** (**38**%)
Average CD4+ T-cell count at KS diagnosis (data available for 33 patients) [cells/mm^3]	**141**	**85**	**107**
Range	*12–409*	*13–261*	*12–409*
Standard deviation	*117.40*	*77.99*	*106.99*
Number of patients diagnosed with HIV infection and oral KS at the same time	**10** (**59**%)	**7** (**35**%)	**17** (**46**%)
Number of patients who were diagnosed with HIV infection before the diagnosis of oral KS	**7** (**41**%)	**13** (**65**%)	**20** (**54**%)
Number of patients with single oral HIV-KS lesions	**5** (**29**%)	**3** (**15**%)	**8** (**22**%)
Number of patients with multiple oral HIV-KS lesions	**12** (**71**%)	**17** (**85**%)	**29** (**78**%)
Lesion phenotype			
Number of macular lesions	**9** (**20**%)	**8** (**17**%)	**17** (**18**%)
Number of papular lesions	**10** (**22**%)	**11** (**23**%)	**21** (**23**%)
Number of nodular lesions	**16** (**36**%)	**17** (**35**%)	**33** (**35**%)
Number of exophytic lesions	**10** (**22**%)	**12** (**25**%)	**22** (**24**%)
Total number of lesions	**45** (**100**%)	**48** (**100**%)	**93** (**100**%)
Lesion size			
Number of lesions <10 mm	**15** (**33**%)	**5** (**10**%)	**20** (**22**%)
Number of lesions ≥10 mm ≤50 mm	**25** (**56**%)	**40** (**83**%)	**65** (**70**%)
Number of lesions >50 mm	**5** (**11**%)	**3** (**7**%)	**8** (**8**%)
Total number of lesions	**45** (**100**%)	**48** (**100**%)	**93** (**100**%)

Table 2: Oral sites affected by oral HIV-KS in relation to gender.

	Males	Females	Total (%)
Gingiva	**13** (**29**%)	**15** (**31**%)	**28** (**30**%)
Upper gingiva	7 (16%)	10 (21%)	17 (18%)
Lower gingiva	6 (13%)	5 (10%)	11 (10.8%)
Hard palate	**11** (**24**%)	**13** (**27**%)	**24** (**26**%)
Oropharynx	**10** (**22**%)	**12** (**25**%)	**22** (**24**%)
Alveolar mucosa	**8** (**18**%)	**6** (**13**%)	**14** (**15**%)
Upper alveolar mucosa	4 (9%)	3 (6%)	7 (7.55%)
Lower alveolar mucosa	4 (9%)	3 (6%)	7 (7.55%)
Dorsum of tongue	**4** (**9**%)	**1** (**2**%)	**5** (**5**%)
Total number of lesions	**45** (**100**%)	**48** (**100**%)	**93** (**100**%)

At the time of oral HIV-KS diagnosis, eight of the 37 patients had solitary oral lesions and 29 (78%) had multiple lesions affecting one or more oral sites (Table 1). There were significantly more patients with multiple oral HIV-KS lesions than patients with single oral HIV-KS lesions ($P = 0.0003$). All 37 patients collectively had 93 separate oral HIV-KS lesions. The clinical appearance and the size of the lesions are documented in Table 1.

Twenty-eight oral HIV-KS lesions (30%) affected the gingiva, 24 (26%) affected the hard palate, 22 (24%) affected the oropharynx (upper and lower retromolar areas, and the soft palate), 14 (15%) affected the alveolar mucosa, and five affected the dorsum of the tongue (Table 2). The oral lesions ranged in colour from pink to red and from bluish-purple to deep brown.

When the clinical features of oral HIV-KS and CD4+ T-cell counts of patients with HIV-KS at the time of oral HIV-KS diagnosis (Table 1) were compared between males and females using the Chi-squared *test*, there were no statistically significant differences identified, except for the size of the lesions. The percentage of lesions <10 mm was significantly lower in females than in males ($P = 0.007$), whereas

Table 3: CD4+ T-cell counts (cells/mm^3) of the participants.

CD4+ T-cell counts of the patients	Males	Females	Average
At the time of oral HIV-KS diagnosis (33 patients)	**141 (14)**	**85 (19)**	**107 (33)**
Standard deviation	*117.40*	*77.99*	*106.99*
Who were simultaneously diagnosed with HIV and oral KS (14 patients)	**163 (7)**	**97 (7)**	**130 (14)**
Standard deviation	*155.64*	*85.95*	*125.63*
Who were diagnosed with HIV infection before developing oral HIV-KS, at the time of HIV diagnosis (14 patients)	**210 (6)**	**129 (8)**	**164 (14)**
Standard deviation	*167.27*	*160.21*	*162.14*
Who were diagnosed with HIV infection before developing oral HIV-KS, at the time of oral HIV-KS diagnosis (19 patients)	**119 (7)**	**74 (12)**	**90 (19)**
Standard deviation	*112.92*	*75.55*	*90.75*
Receiving for some time HAART, at HIV-KS diagnosis (7 patients)	**160 (1)**	**78 (6)**	**90 (7)**
Standard deviation	*0*	*71.49*	*72.19*
Who were HAART-naïve at oral HIV-KS diagnosis (26 patients)	**140 (13)**	**87 (13)**	**114 (26)**
Standard deviation	*137.98*	*83.75*	*114.97*
Who had facial lymphoedema during their course of oral HIV-KS (8 patients)	**24 (4)**	**31 (4)**	**28 (8)**
Standard deviation	*14.66*	*15.75*	*14.61*
Who did not have lymphoedema during the course of oral HIV-KS (25 patients)	**188 (10)**	**96 (15)**	**133 (25)**
Standard deviation	*129.50*	*82.60*	*111.33*

the percentage of lesions ≥10 mm ≤50 mm was significantly higher in females than in males ($P = 0.004$).

3.1. HIV Infection and HAART. The CD4+ T-cell counts were available for only 33 of the 37 patients (Table 3). In these patients the average CD4+ T-cell count at the time of oral HIV-KS diagnosis was 107 cells/mm^3. Seventeen patients (46%) were concurrently diagnosed with HIV infection and oral KS and CD4+ T-cell counts were available for only 14 of these 17 patients. The mean CD4+ T-cell counts of these patients (130 cells/mm^3) was not statistically different ($P = 0.296$) from the 19 patients who were diagnosed with HIV infection before developing oral HIV-KS (90 cells/mm^3) (Table 3). The difference between the average CD4+ T-cell count at the time of HIV diagnosis (164 cells/mm^3) and at the time of oral HIV-KS diagnosis (90 cells/mm^3) was not statistically significant ($P = 0.11$).

Thirty patients (81%) were HAART-naïve, and seven patients had already been receiving HAART at the time of oral HIV-KS diagnosis. CD4+ T-cell counts were available for 26 of the 30 HAART-naïve patients at the time of oral HIV-KS diagnosis. Their average CD4+ T-cell count of 114 cells/mm^3 was not statistically different ($P = 0.606$) from the CD4+ T-cell counts of the seven patients who were on HAART at the time of oral HIV-KS diagnosis (90 cells/mm^3). Ten patients (27%) started HAART around the time of or soon after their oral HIV-KS diagnosis.

3.2. Facial Lymphoedema. Nine patients, five males, and four females had facial lymphoedema. Three patients presented with facial lymphoedema at the time their oral HIV-KS were diagnosed, and six patients subsequently developed facial lymphoedema on average 2.3 weeks after the diagnosis of oral HIV-KS. All patients with facial lymphoedema had multifocal exophytic oral HIV-KS lesions and their average CD4+ T-cell count at the time of oral HIV-KS diagnosis was 28 cells/mm^3 (CD4+ T-cell counts were available for eight of the nine patients) compared to 133 cells/mm^3 for those patients without such facial lymphoedema (Table 3). This difference in the average CD4+ T-cell counts was statistically significant ($P = 0.01$). All nine patients with facial lymphoedema died very soon after their oral HIV-KS occurred, on average within two weeks, regardless whether they were receiving HAART. No significant difference was observed between the average CD4+ T-cell count of females (31 cells/mm^3) and males (24 cells/mm^3) with facial lymphoedema ($P = 0.54$).

3.3. Immune Reconstitution Inflammatory Syndrome (IRIS)-Associated Oral HIV-KS. One patient had IRIS-associated HIV-KS. The CD4+ T-cell count of this female patient at the time of her HIV diagnosis was 9 cells/mm^3, and she developed IRIS-associated HIV-KS, four weeks after she started HAART.

3.4. The Course of Oral HIV-KS. Nine patients were lost to follow-up. Of the remaining 28 patients, oral HIV-KS lesions increased in number and/or in size in 21 patients (75%), remained stable or shrunk in four patients, and resolved in three patients.

Twenty-one patients died during the observation period, on average 13.6 weeks from the time of oral HIV-KS diagnosis (Table 4). Eleven of these 21 patients (52%) did not receive HAART nor any other treatment for their oral HIV-KS. The average time from oral HIV-KS diagnosis to their death was 20 weeks. Eight of the 21 patients who died were

Table 4: Mortality and survival in relation to oral HIV-KS.

	Males	Females	Total
Mortality			
Number of patients who died	11 (85%)	10 (66%)	21 (75%)
Average time of death from oral HIV-KS diagnosis	15 weeks	12.1 weeks	13.6 weeks
Average CD4+ T cell count (cells/mm^3) at oral HIV-KS diagnosis	75	54	64
Survival			
Number of patients who survived	2 (15%)	5 (33%)	7 (25%)
Average follow-up time	76 weeks	106 weeks	91 weeks
Average CD4+ T cell count (cells/mm^3) at oral HIV-KS diagnosis	258	129	166

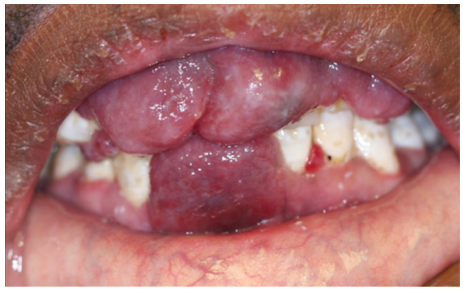

Figure 1: Exophytic oral HIV-KS lesions on the anterior maxillary and mandibular buccal gingiva of a 31-year-old male with a CD4+ T-cell count of 5 cells/mm^3.

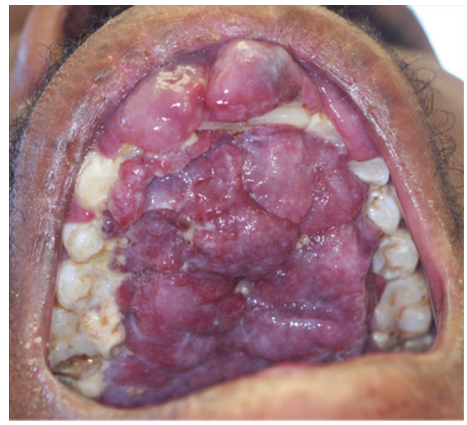

Figure 2: Exophytic confluent oral HIV-KS lesion on the hard palate in a 31-year-old male patient with a CD4+ T-cell count of 5 cells/mm^3.

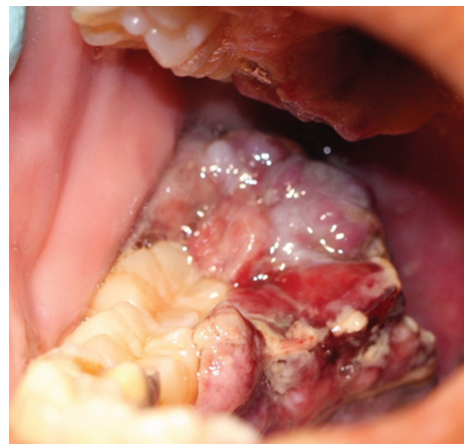

Figure 3: Exophytic oral HIV-KS lesion on the lower right retromolar area extending into the oropharynx in a 29-year-old female patient with a CD4+ T-cell count of 49 cells/mm^3. The patient died six weeks after her oral HIV-KS diagnosis.

on HAART as a sole modality of treatment, and they died on average of 4.4 weeks after their HIV-KS diagnosis. Two patients were treated with HAART in combination with local cytotoxic chemotherapy and they died on average 20.5 weeks after their HIV-KS diagnosis.

Of those seven patients who survived, in three there was resolution of the oral HIV-KS (one had IRIS-associated HIV-KS and was treated with HAART in combination with systemic cytotoxic chemotherapy and surgery; one was treated with HAART and systemic cytotoxic chemotherapy; one with HAART and surgery). In the remaining four patients, the oral HIV-KS lesions remained unchanged or shrunk. These patients were treated with HAART or with HAART in combination with local cytotoxic chemotherapy.

The average CD4+ T-cell count of the patients who were alive at the end of the study observation period was 166 cells/mm^3 at oral HIV-KS diagnosis (Table 4), while the average CD4+ T-cell count of the patients who died during the observation period was 64 cells/mm^3 at the time of oral HIV-KS diagnosis. Statistically, the difference in the CD4+ T-cell count between these two groups of patients was significant ($P = 0.016$).

4. Discussion

There was a significantly higher number of patients with multiple oral lesions at the time of oral HIV-KS diagnosis than patients who had single lesions (Table 1). With decreasing order of frequency, the gingiva, hard palate, oropharynx (upper and lower retromolar area, and soft palate), alveolar mucosa, and the dorsum of the tongue (Figures 1, 2, 3, 4, and 5) were the sites most commonly affected (Table 2), conforming to other reports in the literature [4, 21]. In none of the 37 patients included in this study was the floor of the mouth or the ventral/lateral surface of the tongue affected.

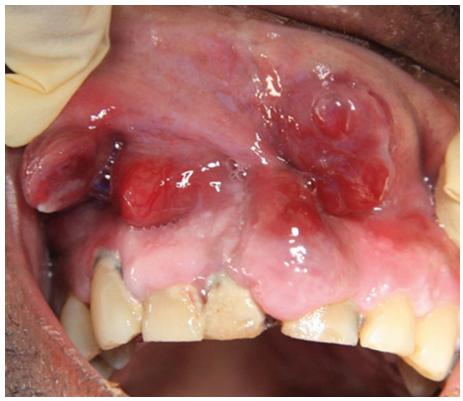

Figure 4: Exophytic oral HIV-KS lesions on the alveolar and labial mucosa in a 54-year-old male with a CD4+ T cell count of 258 cells/mm^3. The patient died 15 weeks after his oral HIV-KS diagnosis.

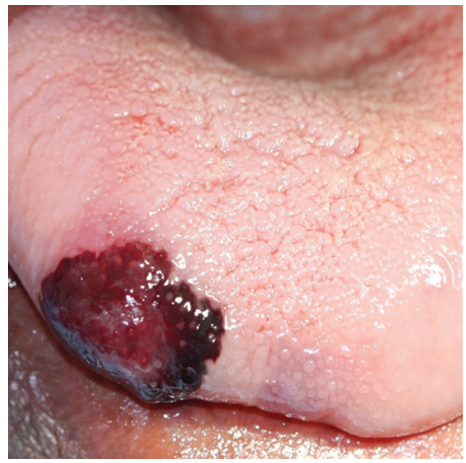

Figure 5: Macular/nodular lesion on the dorsum of the tongue in a 44-year-old female patient with a CD4+ T-cell count of 13 cells/mm^3. The patient died five weeks after the diagnosis of her oral HIV-KS.

It is unknown why HIV-KS has the tendency to affect only certain oral sites, but not others.

Forty six percent (46%) of the patients with oral HIV-KS in this study cohort did not know their HIV-serostatus at the time of oral HIV-KS diagnosis, implying that oral KS in the Ga-Rankuwa area in South Africa may serve as an indicator of HIV infection. Although the prevalence of HHV8 infection in South Africa is relatively high [22, 23], there were no recorded cases of oral KS in HIV-seronegative subjects during the study period, suggesting that endemic African KS is not frequent in the Ga-Rankuwa area of South Africa.

Seven patients were on HAART at the time of oral HIV-KS diagnosis and although at this time their average CD4+ T-cell count (90 cells/mm^3) was lower than the average CD4+ T-cell count of the HAART-naïve patients (114 cells/mm^3) (Table 3), this difference was not statistically significant. This seemingly surprising finding that the patients on HAART had a lower CD4+ T-cell count than the HAART-naïve patients could be attributed to the fact that in South Africa, HIV-seropositive persons who rely on provincial (governmental) services for their medical care have to abide by an official policy that HAART can be provided only when their CD4+ T-cell count has dropped below 200 cells/mm^3, and that some people who have medical conditions suggestive of HIV disease are reluctant to undergo serological testing for HIV and often prefer to be treated by traditional healers. As a result, HIV infection is often diagnosed and HAART is often introduced only late in the course of their HIV disease when the CD4+ T-cell counts have already fallen substantially below 200 cells/mm^3.

As a consequence of HAART being introduced only when the CD4+ T-cell count is already very low, there will be a lower level of reconstitution of the CD4+ T-cell number compared to the level of reconstitution when HAART is started at a higher CD4+ T-cell count [8, 24–28]. In addition, in provincial (governmental) medical facilities in South Africa, as in other countries in sub-Saharan Africa, monitoring the effectiveness of HAART is not always as efficient as it is in developed countries due to limited resources [28, 29]. All these factors may explain why the average CD4+ T-cell count of our study cohort was low at the time of oral HIV-KS diagnosis, why the number of HAART-naïve patients was high, and why the CD4+ T-cell counts of patients on HAART was also low.

It has been reported that in 22% of HIV-seropositive subjects with KS, the initial presentation of HIV-KS is in the mouth, and that in up to 70% of subjects with HIV-KS, the mouth will sooner or later be affected [12, 13]. As our study was designed to include only patients with oral HIV-KS regardless of whether they had extraoral KS, our findings cannot be compared to the findings of other studies reported in the literature in which the inclusion criterion was that of patients having HIV-KS, but who may or may not have had oral HIV-KS. However, our data show that in 21 patients (57%) the initial presentation of HIV-KS was in the mouth, and that in six patients the initial presentation of HIV-KS occurred concurrently in the mouth and on the skin. Ten patients (27%) developed cutaneous KS before and three after oral HIV-KS diagnosis. Eighteen patients (49%) with oral HIV-KS did not develop cutaneous HIV-KS during the observation period. These findings emphasise that in many cases the mouth may be the only body site affected by HIV-KS.

Facial lymphoedema may occur before, at the time of, or after oral HIV-KS is diagnosed. Facial lymphoedema which develops in parallel with the progression of oral HIV-KS disease is an indicator of poor prognosis [7, 8] and foretokens death [30]. In this study, the nine patients who had facial lymphedema had extensive exophytic oral lesions, severe immunosuppression, and average CD4+ T-cell count of 28 cells/mm^3 and all died very soon after the onset of facial lymphedema, regardless of HAART.

The pathogenic mechanisms that cause facial lymphoedema in association with oral HIV-KS are obscure.

However, as oral KS lesions and oral fluids of HIV-seropositive subjects carry a high HHV8 load, and as advanced exophytic oral HIV-KS lesions have a higher HHV8 load than initial maculopapular lesions [31], it is possible that in the presence of exophytic oral lesions, lymphatic obstruction secondary to HHV8-induced proliferation of endothelial cells, and/or compression of lymphatics by rapidly progressing oral HIV-KS, will bring about leakage of protein-rich fluid into the interstitial spaces, promoting the development of facial lymphoedema [32, 33]. Therefore, it is likely that treating exophytic oral HIV-KS lesions with cytotoxic chemotherapy may result in the shrinkage of oral lesions with the subsequent decrease in HHV8 load in the affected tissues, thus reducing the risk of developing facial lymphoedema. This possibility needs further investigation.

To the best of our knowledge, this is the first report in the literature documenting the prevalence of IRIS-associated oral HIV-KS in a population of patients with oral HIV-KS regardless of whether they had extraoral KS. One patient in this case series had IRIS-associated HIV-KS. This patient was successfully treated with systemic cytotoxic chemotherapy and surgical excision. A comprehensive description of the case report of this patient has been published previously [34]. At the time of writing this paper, the patient was still alive 5.5 years after the treatment of IRIS-associated oral HIV-KS and currently her CD4+ T-cell count is 383 cells/mm^3. This is in line with reports in the literature documenting that IRIS-associated oral HIV-KS responds well to conventional therapy [35, 36].

During the study observation period, 21 of the 37 patients (57%) died, on average within 13.6 weeks from the time of oral HIV-KS diagnosis; nine were lost to follow-up; 7 patients survived (average period of follow-up of 91 weeks). The average CD4+ T-cell counts of the patients who were alive at the end of the study observation period was 166 cells/mm^3 at oral HIV-KS diagnosis and the CD4+ T-cell counts of the patient who died was 64 cells/mm^3, at oral HIV-KS diagnosis. The difference in these CD4+ T-cell counts was statistically significant ($P = 0.016$). This suggests that a low CD4+ T-cell count at the time of oral HIV-KS diagnosis is a strong indicator of poor prognosis. Unfortunately, we were unable to determine the cause of death of our patients and therefore it is unknown whether they died as a direct consequence of their HIV-KS disease.

Of those who survived, in three patients oral HIV-KS completely resolved following various treatment modalities. One of these patients was treated with HAART in combination with systemic cytotoxic chemotherapy and surgery, one with HAART in combination with cytotoxic chemotherapy, and one patient with HAART and surgery. In four patients, the oral HIV-KS remained unchanged or shrunk. These patients were treated with HAART or with HAART in combination with local cytotoxic chemotherapy.

HAART is used to treat HIV infection. Even though effective HAART does not directly influence HHV8 replication, by reducing HIV load with subsequent improvement in the host-immune status, it indirectly brings about a decrease in the incidence and prevalence of HIV-KS and an improvement in the clinical manifestation of pre-existing HIV-KS disease [29]. HAART may also have a direct anti-angiogenic effect on KS. However, HAART does not ensure that HIV-KS will not develop, and despite HAART, KS remains the most frequent HIV-associated neoplasm [30].

In this study, seven patients have been on HAART at least four weeks before their oral HIV-KS diagnosis, confirming that HIV-seropositive subjects on HAART may develop KS. Eight of the 21 patients who died during the observation period were on HAART as a sole modality of treatment. These patients died on average 4.4 weeks after their oral HIV-KS diagnosis. During this period, they experienced worsening of their oral HIV-KS disease. This suggests that although introduction of HAART should be the first line of therapy for HAART-naïve HIV-seropositive subjects with oral KS, HAART by itself may not be effective in controlling oral HIV-KS disease. Two of the 21 patients who died were concurrently treated with HAART and with local cytotoxic chemotherapy.

Eleven of the 21 patients (52%) who died during the observation period received neither HAART nor any other treatment for their oral HIV-KS. The average time from oral HIV-KS diagnosis to their death was 20 weeks. The paradoxical finding that in this study HAART-naïve patients lived longer than patients on HAART might be attributed to skewed statistics associated with the small number of patients, or to ineffective HAART that was started late in the course of oral HIV-KS disease when the CD4+ T-cell count had already fallen very low.

The small number of patients who received treatment for HIV-KS in this study prevents drawing conclusions regarding what is the best treatment approach to control the progression of oral HIV-KS and to improve the prognosis of the patients. However, as reported elsewhere [37], it seems that exophytic oral HIV-KS lesions are best treated with HAART and systemic cytotoxic chemotherapy, and once the lesions have shrunk and become surgically accessible, they should be excised.

In developed countries, HIV-KS predominantly affects males. However, in many resource poor countries in sub-Saharan Africa where HIV infection is endemic and young females aged 15–24 years are more frequently infected with HIV than males, there is almost an identical incidence of HIV-KS in males and females [19, 38], and at the time of HIV-KS diagnosis females present with a more advanced disease than males [19, 39]. In our study, more females than males were affected by oral HIV-KS and although not statistically significant, females had a lower average CD4+ T-cell count (85 cells/mm^3) than males (141 cells/mm^3) at the time of oral HIV-KS diagnosis, in line with other studies documenting that females with HIV-KS have more severe immunodeficiency than males with HIV-KS [39]. However, in contrast to other studies reporting that females are younger than males at the time of HIV-KS diagnosis [39, 40], the age of the females (33 years) and males (34 years) in our cohort was very similar.

In spite the fact that the CD4+ T-cell counts of females were lower than males at oral HIV-KS diagnosis, the differences between the percentage of males and females who survived or died were not statistically significant. Other

reports in the literature also note that gender differences do not influence survival of patients with HIV-KS [19].

Two patients were children. Both were boys, aged 10 and 11 years. This supports data reported from other areas of sub-Saharan Africa, that children are not uncommonly affected by HIV-KS. In fact, in Zimbabwe, the most frequent malignancy in children between 1 and 14 years of age is HIV-KS [38]. This is probably owing to the high prevalence of HHV8 and HIV coinfection in African children in this part of the world. HIV-seropositive children with advanced oral KS have a particularly aggressive course of disease with a poor prognosis [41]. In sub-Saharan Africa, oral involvement in HIV-seropositive children with KS is common.

Thirty-eight percent (38%) of the patients in this study had common HIV-associated oral diseases which presented concurrently with oral HIV-KS (Table 1). It is probable that these diseases were associated with the low CD4+ T-cell counts of the patients, and not with the oral HIV-KS. However, one cannot exclude the possibility that oral HIV-KS-associated cytokine dysregulation may favour the development of other HIV-associated oral lesions and that some HIV-associated oral diseases may further dysregulate the cytokine milieu in the affected oral tissues, thus promoting KS tumourigenesis [42, 43].

5. Summary

In the Ga-Rankuwa area of South Africa where HIV-KS is prevalent, oral KS affects similarly males and females. In this population, a low CD4+ T-cell count at the time of oral HIV-KS diagnosis is associated with a poor prognosis. The development of facial lymphoedema during the course of HIV-KS disease portends a very poor prognosis. Owing to the small number of patients who received treatment, it was not possible to determine what the best treatment modality was for oral HIV-KS.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 620962, 7 pages
doi:10.1155/2012/620962

Clinical Study

A Comparison of Chronic Periodontitis in HIV-Seropositive Subjects and the General Population in the Ga-Rankuwa Area, South Africa

Razia Khammissa,[1] Liviu Feller,[1] Mario Altini,[2] Paul Fatti,[3] and Johan Lemmer[1]

[1] *Department of Periodontology and Oral Medicine, University of Limpopo, Medunsa Campus, Pretoria 0204, South Africa*
[2] *Division of Oral Pathology, University of the Witwatersrand, Johannesburg 2050, South Africa*
[3] *School of Statistics and Advanced Science, University of the Witwatersrand, Johannesburg 2050, South Africa*

Correspondence should be addressed to Liviu Feller, lfeller@ul.ac.za

Received 21 December 2011; Revised 19 July 2012; Accepted 19 July 2012

Academic Editor: Giuseppe Ippolito

The effect of HIV infection on the prevalence and the rate of progression of chronic periodontitis is not clear. The aim of this study was to compare parameters associated with the severity of chronic periodontitis in terms of periodontal probing depths, gingival recession, plaque indexes, and bleeding indexes of HIV-seropositive subjects and healthy age-matched control subjects, and of HIV-seropositive subjects on highly active antiretroviral therapy and those not receiving such treatment. Two cohorts of subjects with chronic periodontitis were recruited for this study over a period of six months. There were 30 HIV-seropositive subjects, and 30 control subjects. Periodontal probing depths, gingival marginal recession, plaque indexes, and bleeding indexes were compared by HIV serostatus, the use of highly active antiretroviral therapy, and CD4+ T-cell counts. All participants were black persons between the age of 18 and 45 and were of a similar socioeconomic status and age. The results of this study indicate that chronic periodontitis in HIV-seropositive subjects is similar in terms of mean periodontal probing depth, gingival marginal recession, plaque index, and bleeding index to that in healthy age-matched control subjects, and a low CD4+ T-cell count does not appear to be a risk factor for increased severity of chronic periodontitis.

1. Introduction

The relationship between chronic periodontitis and HIV infection is not clear and considerable differences of opinion exist regarding the prevalence of chronic periodontitis among HIV-seropositive subjects [1, 2]. Microbiological studies have failed to detect any major differences in the subgingival microbial flora of HIV-seropositive subjects with chronic periodontitis compared to HIV-seronegative controls [3, 4], and the humoral immune response to the periodontopathic bacteria is similar in both groups [5].

Some authors reported a higher prevalence of periodontal attachment loss and a more rapid progression of periodontal disease over time in HIV-seropositive subjects compared to HIV-seronegative controls [6–8]. A great proportion of the loss of periodontal attachment seen in HIV-seropositive subjects with chronic periodontitis is said to be owing to localized gingival marginal recession rather than to the formation of deep periodontal pockets as in HIV-seronegative subjects [7, 9–11]. However, other studies failed to document differences between the natural course of chronic periodontitis in HIV-seropositive subjects compared with the course in HIV-seronegative subjects with chronic periodontitis [12, 13].

The considerable differences of opinion about the natural course of chronic periodontitis in HIV-seropositive subjects may cause corresponding confusion with regard to their periodontal treatment. The aim of this study was to compare parameters associated with the severity of chronic periodontitis in terms of periodontal probing depths, gingival recession, plaque indexes, and bleeding indexes of HIV-seropositive subjects and control subjects and

of HIV-seropositive subjects on highly active antiretroviral therapy and those not receiving such treatment.

2. Materials and Methods

2.1. Subject Population. Approval of this study was obtained from the Ethics Committees of the Universities of Limpopo and of the Witwatersrand, Johannesburg. Two cohorts of subjects with chronic periodontitis were recruited for this study over a period of six months: thirty HIV-seropositive subjects and 30 control subjects presumed to be HIV-seronegative and apparently in good health. All these patients did not receive periodontal treatment before recruitment.

The term "apparently healthy subject" in this paper refers to someone who according to his medical history is in a state of good physical and mental well being, is not pregnant, not diabetic, and is not known to have HIV infection or any other condition of immune dysregulation or any other physical condition that is known to be associated with increased risk of periodontal disease. In addition, these apparently healthy subjects should not at the time of periodontal examination be taking any medication that may adversely affect the periodontium such as calcium channel blockers or phenytoin. After explanation of the purpose of the study, all gave their informed consent to participate. There were 34 females and 26 males, all black persons between the ages of 18 and 45 years.

Of the 30 HIV-seropositive subjects, 16 were receiving highly active antiretroviral treatment (HAART) and 14 were not receiving such treatment (HAART-naïve). The serostatus of all HIV-seropositive subjects had been confirmed by enzyme-linked immunosorbent assay (ELlSA) and western blot. CD4+ T-cell counts were performed for 13 of the 30 HIV-seropositive subjects who had given informed consent.

2.2. Periodontal Health Status. Chronic periodontitis was diagnosed by clinical and radiographic examination by a single clinician. Subjects were diagnosed with chronic periodontitis when at least three tooth sites had periodontal probing depth ≥ 5 mm and/or had measurable gingival marginal recession, and where there was radiographic evidence of loss of alveolar bone height.

Periodontal probing depths (PPD), gingival marginal recession (GR), plaque indexes (PI), and bleeding indexes (BI) were measured. PPD refers to the distance from the gingival margin to the location of the tip of a periodontal probe inserted in the pocket with moderate force [14] and gingival marginal recession refers to the distance from the cementoenamel junction to the location of the apically displaced gingival margin [15]. PPD was measured at six sites per tooth (mesiobuccally, midbuccally, distobuccally, distolingually, midlingually and mesiolingually) and GR was measured at two sites per tooth, buccal and lingual, excepting third molars and residual roots. The PPD and GR measurements were done with a periodontal probe with Williams markings.

PI and BI scored the presence or absence of plaque and bleeding on probing, respectively: presence scored 1 and absence scored 0 [14, 16]. BI was calculated as the sum of bleeding sites (any bleeding on probing around the circumference of a tooth was counted as a site) divided by the number of teeth present in the mouth, excluding third molars and residual roots, and converting the quotient into a percentage. Disclosing solution was used to display bacterial plaque and PI was calculated by dividing the sum of plaque present at four tooth sites (mesial, buccal, distal, and lingual) by the number of teeth, multiplied by four, and converting the quotient into a percentage [17, 18].

No attempt was made to differentiate sites of active periodontitis showing suppuration or bleeding on probing from inactive sites with evidence of increased PPD.

For the purpose of this study, a mean PPD value per mouth and a mean GR value per mouth were calculated to provide a straight forward comparison of periodontal status of the studied and the control group of patients.

2.3. Statistical Analysis. All data were entered into the Microsoft Excel program and analyzed using its data analysis package. ANOVA, *t*-tests, Pearson's correlation coefficients, and histograms were computed to conduct statistical hypothesis tests and to explore associations. P values of <0.05 were regarded as statistically significant.

Single factor analysis of variance was used to test for differences between the periodontal indices of the control subjects and the two HIV-seropositive subject groups. The two-sample *t*-test was used to test for differences between the periodontal indexes of the HIV-seropositive HAART-naïve subjects and of the HIV-seropositive subjects using HAART. The Pearson correlation coefficient was used to test for significant relationships between the periodontal indexes of the HIV-seropositive subjects and the log CD4+ T-cell count.

A log transformation of the CD4+ T-cell count was performed to correct for its skew distribution.

3. Results

The mean number of teeth per mouth of the group of HIV-seropositive subjects (29 teeth) and of the group of the control subjects (28 teeth) was similar. Periodontal probing depth (PPD), gingival recession (GR), plaque index (PI), and bleeding index (BI) were compared by HIV-serostatus, the use of HAART, and CD4+ T-cell counts. CD4+ T-cell counts were stratified into the following groups: CD4+ T-cell count <200 cells/mm^3, CD4+ T-cell counts 200–500 cells/mm^3, and CD4+ T-cell count >500 cells/mm^3. HAART was defined as the use of at least two nucleoside reverse transcriptase inhibitors with either a nonnucleoside reverse transcriptase inhibitor or a protease inhibitor. All participants were black persons from the Ga-Rankuwa area in South Africa and were of a similar socioeconomic status and age. CD4+ T-cell counts were available for eight HIV-seropositive subjects using HAART and for five HAART-naïve subjects. There were only eight subjects that admitted to smoking.

When all participants were evaluated, no association was found between HIV-serostatus and periodontal indexes. HIV-seropositive and control subjects with chronic

Table 1: Epidemiological features and periodontal indexes of HIV-seropositive subjects with chronic periodontitis and control subjects with chronic periodontitis.

	Presumably HIV-seronegative control subjects with chronic periodontitis	HIV-seropositive subjects with chronic periodontitis
Females	14	20
Males	16	10
Smokers	5	3
Mean periodontal probing depths	3.196 mm	3.205 mm
STDEV*	0.58	0.32
Mean gingival recession	1.53 mm	1.66 mm
STDEV*	0.89	0.71
Number of gingival recession sites	195	202
STDEV*	5.11	5.46
Mean plaque index	75.2%	75.6%
STDEV*	23.6	20.16
Mean bleeding index	50.3%	47.3%
STDEV*	19.4	23.25

*STDEV: standard deviation.

periodontitis had similar mean PPD, GR, PI, and BI (Table 1), and gender had no influence on the results (data not shown). The mean periodontal indexes for PPD, GR, PI, and BI were compared between the two groups of HIV-seropositive and the control subjects using ANOVA. None of them were significant at the $P < 0.05$ level.

When periodontal indexes of HIV-seropositive subjects using HAART were compared to the periodontal indexes of HAART-naïve subjects, using the t-test, there was no statistical differences regarding mean GR, PI, and BI.However, the mean PPD in HAART-naïve seropositive subjects was slightly greater than in HIV-seropositive subjects using HAART ($r = 0.01$), 3.36 mm and 3.07 mm, respectively (Table 2).

The Pearson correlation coefficient of mean PPD in relation to log CD4+ T-cell count in the HIV-seropositive HAART-naïve group of subjects showed a significant negative correlation ($r = -0.947$), but there was no significant correlation between the mean GR values and the log CD4+ T-cell counts in the same group. For the HIV-seropositive subjects using HAART the Pearson correlation coefficient test failed to show significant statistical relationships between log CD4+ T-cell count and mean PPD and between log CD4+ T-cell count and mean GR.

Unexpectedly, the mean CD4+ T-cell count was higher in the HAART-naïve group of subjects than in HIV-seropositive subjects using HAART ($r = 0.32$) (Table 3). When the average log CD4+ T-cell counts of HAART-naïve HIV-seropositive subjects were compared, using the t-test, to the CD4+ T-cell counts of HIV-seropositive subjects using HAART, the difference between them was not significant at the 5% level. This finding can be explained by the fact that the CD4+ T-cell counts were available only for a small number of subjects, and by the fact that HIV-seropositive subjects treated in provincial hospitals in South Africa generally receive HAART only after their CD4+ T-cell count has fallen below 200 cells/mm^3.

Evaluation of the impact of smoking on PPD, GR, PI, and BI was not an aim of this study. However, in view of the well-established association between smoking and severity of chronic periodontitis, we compared these indexes by HIV-serostatus in the non-smoking subjects of the population study (27 nonsmoking HIV-seropositive subjects and 25 non-smoking control subjects), but not in the smoking subjects as the samples were too small (3 smoking HIV-seropositive subjects and 5 smoking control subjects). There was no significant difference between the non-smoking HIV-seropositive and control subjects in any of these indexes.

4. Discussion

This study demonstrates that both HIV-seropositive and apparently healthy subjects with chronic periodontitis have similar mean PPD, GR, PI, and BI measurements and that HIV infection does not carry with it a greater risk for accelerated periodontal attachment loss [2, 19]. This conforms with other studies that documented similar clinical manifestations and natural courses of chronic periodontitis in HIV-seropositive and -seronegative subjects [12, 13, 20, 21].

The results of this study indicate that the reported general increase in the prevalence of fungal and viral microorganisms observed in periodontal pockets of HIV-seropositive subjects with chronic periodontitis compared to HIV-seronegative subjects with chronic periodontitis [22–25] has no influence on the periodontal health status, in spite of the potential of these microorganisms to exacerbate the inflammatory processes and suppress the immune responses in the periodontal tissues [26]. It is also evident that the reduction in the number of Langerhans cells in the pocket epithelium in HIV-seropositive subjects [27, 28] and the consequent local immune impairment does not play any particular role in the development and progression of chronic periodontitis.

Therefore, there would appear to be no indication for instituting different treatment modalities for chronic periodontitis in HIV-seropositive subjects and in immunocompetent subjects. If indeed some HIV-seropositive subjects demonstrate increased levels of periodontal tissue destruction, this may be the result of periodontal disease activity that took place before the onset of HIV infection. This could be determined by the study of reliable pre-HIV infection periodontal records [29].

The mean GR was similar in HIV-seropositive and healthy subjects, in HIV-seropositive subjects using HAART, and in HIV-seropositive HAART-naïve subjects, and also regardless of lower or higher CD4+ T-cell counts. This does not agree with the findings of McKaig et al. (2000) [10] and Lamster et al. (1997) [30] who found a negative correlation between CD4+ T-cell count and increased frequency of recession. McKaig et al., (2000) [10] reported that recession in HIV-seropositive subjects with chronic periodontitis is more likely to occur in association with low CD4+ T-cell

Table 2: Epidemiological features, periodontal indexes, and immunological indexes of HIV-seropositive subjects using HAART of HAART-naïve HIV-seropositive subjects and of control subjects. All subjects had chronic periodontitis.

	Presumably HIV-seronegative control subjects with chronic periodontitis	HIV-seropositive subjects using HAART with chronic periodontitis	HAART-näive HIV-seropositive subjects with chronic periodontitis
Females	14	12	8
Males	16	4	6
Smokers	5	2	1
Mean periodontal probing depths	3.196	3.069	3.359
STDEV*	0.58	0.28	0.30
Mean gingival recession	1.53	1.67	1.64
STDEV*	0.89	0.79	0.64
Number of gingival recession sites	195	100	102
STDEV*	5.11	4.14	3.56
Mean plaque index	75.2%	71.9%	79.3%
STDEV*	23.6	20.4	26.1
Mean bleeding index	47.3%	42.5%	52.1%
STDEV*	23.25	18.3	22.1
Mean CD4+ count	Not available	171.63 cell/mm^3	257.44 cell/mm^3
STDEV*		108.94	171.25

*STDEV: standard deviation.

Table 3: The categories of CD4+ T-cell levels of the HIV-seropostive subjects using HAART and HIV-seropositive HAART-naïve subjects.

	HIV-seropositive subjects using HAART with chronic periodontitis	HIV-seropositive HAART-naïve subjects with chronic periodontitis
CD4+ count <200	4	2
CD4+ count 200–500	4	2
CD4+ count >500	0	1
Mean CD4+ count	172 cell/mm^3	257 cell/mm^3

counts (<200 cells/mm^3) than with higher CD4+ T-cell counts (200–499 cells/mm^3).

As evident from the results of this study, HIV-seropositive subjects with chronic periodontitis do not have an increase either in the number of sites of gingival marginal recession or in the severity of gingival marginal recession; and a low CD4+ T-cell count is not a risk factor for increased frequency or severity of GR.

Recent studies have shown that the severity of chronic periodontitis is decreased in HIV-seropositive subjects and that there is a positive correlation between clinical attachment levels and the CD4+ T-cell counts; lower CD4+ T-cell counts are associated with reduced periodontal probing depth and with lesser degrees of recession measurements [31, 32].

The profound suppression of immunity in HIV disease does not seem to increase the risk of development of chronic periodontitis. The diminution of the CD4+ T-cell count, dysregulation of the cytokine network, and qualitative defects of macrophages, monocytes, polymorphonuclear leukocytes, dendritic cells, and T lymphocytes do not predispose HIV-seropositive subjects to periodontopathic infection [33, 34]. The profound HIV-associated immune suppression also does not appear to predispose to delayed wound healing of the oral tissues [35], to increased incidence of wound infection of periodontal or other oral surgery [36], or to plaque-induced periodontal tissue destruction [12, 13, 20].

Since the periodontal tissue destruction in chronic periodontitis is mediated mainly by host-derived cellular immune responses [37], and since these mechanisms are to a great extent suppressed in HIV infection, HIV-seropositive subjects with chronic periodontitis may be expected to show reduced rather than exaggerated periodontal tissue destruction, compared to immunocompetent subjects with chronic periodontitis. Moreover, active periodontal disease associated with periodontal attachment loss is related mainly to a Th1 cytokine profile [38, 39]. However, in advanced HIV disease, in the absence of HAART, there is a dysregulation in the cytokine network characterized by a shift from Thl predominant cytokines to a Th2 cytokine profile [40] that is less associated with periodontal tissue destruction. This reinforces the concept that HIV-related immune dysregulation may not contribute to the development of chronic periodontitis but in fact is associated with reduced periodontal tissue destruction.

Taking into consideration the immune suppression associated with HIV infection one would have expected that HIV-seropositive subjects with low CD4+ T-cell counts would show higher frequencies of infection with periodontopathic bacteria and an increased incidence of wound infection following oral surgery, compared to HIV-seropositive subjects. However, this is not the case. HIV-seropositive and

-seronegative subjects show similar types and quanta of periodontopathic bacteria in their subgingival microbiota, similar incidences of infection of surgical wound, and similar wound healing capacity [4, 29].

HIV-seropositive subjects, however, demonstrate bacterial species in their subgingival microbiota that are not usually associated with periodontal disease. These opportunistic microorganisms including *E. faecalis, A. baumannii,* and *Pseudomonas aeruginosa* are probably associated with HIV-related immunosuppression. The fact that HIV-seropositive subjects are frequently treated in a hospital environment might account for the presence of unusual opportunistic microorganisms [31].

The process of wound healing is complex, involving interaction between macrophages, dendritic cells, polymorphonuclear leukocytes, epithelial cells, fibroblasts, osteoblasts, cytokines, and growth factors [41]. While macrophages and growth factors are the driving force behind the process of wound healing [41], CD4+ T-cells do not play a critical role [42]. Consequently, in spite of some impairment in the function of macrophages and alteration in the cytokine network, HIV-seropositive subjects do not demonstrate impaired wound healing capacity [43] or increased incidence of wound infection following oral surgery [35].

There is an inverse relation between the CD4+ T-cell count and the frequency of HIV-associated oral lesions, in particular when the CD4+ T-cell count is lower than 200 cells/mm^3 regardless of the use of HAART [44]. However, HIV-seropositive subjects using HAART have a significantly lower prevalence of HIV-associated oral lesions compared to HAART-naïve HIV-seropositive subjects [44].

Chronic periodontitis is similar to other oral lesions in this respect. The use of HAART in HIV-seropositive subjects has brought about a decrease in the prevalence and severity of chronic periodontitis [11, 44]. HIV-seropositive subjects with chronic periodontitis using HAART demonstrate reduced counts of periodontopathic bacteria in their subgingival plaque, and their periodontal tissues show reduced inflammation and periodontal attachment loss, compared to HIV-seronegative subjects with chronic periodontitis [34].

Even severely immunocompromised HIV-seropositive subjects with chronic periodontitis who are using HAART and with low CD4+ T-cell counts demonstrate similar periodontopathic bacteria in their subgingival microbiota to HIV-seronegative subjects with chronic periodontitis of a comparable degree [33]. It is possible that HAART-associated reconstitution of components of the immune response is sufficient to control colonization by the periodontopathic pathogens, even though the CD4+ T-cell count remains low [33, 34]. It is also possible that one or more of the drugs in the cocktail constituting HAART have anti-inflammatory and/or antibacterial properties that act synergistically with the hosts' partially reconstituted immune mechanisms in controlling the periodontopathic bacteria [34].

This study has a few limitations. Firstly, the control group comprised subjects of unconfirmed, presumably HIV-seronegative status, and this status could not be confirmed owing to the refusal of the subjects to consent to free HIV testing. Secondly, of the HIV-seropositive cohort of 30 subjects, the CD4+ T-cell count of only 13 subjects were known. The remaining 17 subjects would not consent to free CD4+ T-cell count testing.

In the semirural community of Ga-Rankuwa, South Africa, refusal of any form of testing in relation to HIV disease, even if offered free of charge, is common and is probably related to the perceived stigma associated with HIV disease and to the possibility that HIV-seropositive subjects may be shunned by their communities. Consequently, there is reluctance to learn one's own HIV-serostatus, and when the HIV-serostatus is known, to denial.

In a study investigating necrotizing periodontal diseases (NPD) in HIV-seropositive and—seronegative subjects in the same cohort as in our study [45], 35% of subjects with NPD and unaware of their HIV-serostatus refused to test their HIV-serostatus in spite of strong recommendation and detailed explanation on the well-established association between NPD and HIV infection in the cohort. Even in research into oral disease not commonly associated with HIV infection, the offer of voluntary serostatus testing for HIV infection is invariably refused.

According to Statistics South Africa, an estimated 16.2% of South Africans between the ages of 15 and 49 years are HIV seropositive [46]. Therefore, although scientific proof of HIV-seronegativity would obviously be preferable, statistically an estimated 83.8% of control subjects in this study were likely to be HIV-seronegative. There were no statistically significant differences between the periodontal indexes of HIV-seropositive subjects all of whom had chronic periodontitis, and of control subjects all of whom had chronic periodontitis.

CD4+ T-cell counts were available for only 13 HIV-seropositive subjects (eight subjects using HAART and five HAART-naïve subjects). With such a small cohort, the statistical analysis of CD4+ T-cell counts relative to periodontal indexes could have been skewed. In this study, the correlation coefficient of PPD with CD4+ T-cell count in the HIV-seropositive HAART-naïve group of subjects was significantly negative ($P < 0.01$). This differs from other studies [32] that documented a positive correlation between CD4+ T-cell counts and PPD measurements in HIV-seropositive subjects and that HIV-seropositive subjects with healthy periodontium that are using HAART have lower CD4+ T-cell counts, while HIV-seropositive subjects with chronic periodontitis using HAART, have higher CD4+ T-cell counts [31, 33]. These differences between the results of our study and the results of other studies may be attributed to our small sample of subjects with known CD4+ T-cell counts.

In this study, the mean CD4+ T-cell counts found in HIV-seropositive HAART-naïve subjects (257 cells/mm^3) was higher than in HIV-seropositive subjects using HAART (172 cells/mm^3) (Table 3). This seemingly paradoxical finding could very well be attributed to skewed statistics associated with the small number of subjects with known CD4+ T-cell counts. However, on second consideration, this finding could be real. In South Africa, many people with oral conditions suggestive of HIV disease refuse to

undergo serological testing for HIV and often prefer to be treated by traditional healers. As a result, HIV infection is often diagnosed and HAART is often introduced late in the course of the disease when the CD4+ T-cell count has already fallen very low (200 cells/mm^3). In addition, in the South African context, HIV-seropositive subjects, who depend on provincial (governmental) services for their medical care, are subject to an official policy ruling that HAART may start only when their CD4+ T-cell count has fallen to 200 cells/mm^3 or below. Under these circumstances, in practice, HAART is initiated only when the CD4+ T-cell count has fallen substantially below 200 cells/mm^3. It is well established that starting HAART when the CD4+ T-cell count is very low will lead to a lower level of reconstitution of CD4+ T-cell numbers compared to the achieved level of reconstitution of CD4+ T-cell numbers when starting HAART at a higher CD4+ T-cell count. Hence, this seemingly paradoxical finding that the HIV-seropositive subjects using HAART in this study had a lower CD4+ T-cell count compared to the CD4+ T-cell count of HIV-seropositive HAART-naïve subjects.

In this study, the mean PPD in the HAART-naïve HIV-seropositive subjects with chronic periodontitis was slightly greater than in HIV-seropositive subjects using HAART ($r = 0.01$); the mean CD4+ T-cell count in the group of HIV-seropositive subjects using HAART was lower than in the group of HIV-seropositive HAART-naïve subjects. This conforms to other studies that report a positive correlation between PPD measurements and CD4+ T-cell counts [31, 32].

5. Conclusion

Chronic periodontitis in HIV-seropositive subjects is similar in terms of mean PPD, GR, PI, and BI to that in presumably healthy aged-matched control subjects, and a low CD4+ T-cell count does not appear to be a risk factor for increased frequency or severity of chronic periodontitis.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 418630, 6 pages
doi:10.1155/2012/418630

Review Article

Thirty Years Later: Pregnancies in Females Perinatally Infected with Human Immunodeficiency Virus-1

Martina L. Badell and Michael Lindsay

Department of Gynecology and Obstetrics, Emory University, 69 Jesse Hill Jr. Dr. S.E., Atlanta, GA 30303, USA

Correspondence should be addressed to Martina L. Badell, mbadell@emory.edu

Received 2 December 2011; Accepted 29 July 2012

Academic Editor: Giuseppe Ippolito

The first cases of mother to child transmission of human immunodeficiency virus (HIV) were described more than two decades ago and since then several thousands more have been reported in western countries. In the early 1980s the majority of perinatally acquired HIV children did not survive beyond childhood. However combined antiretroviral therapy (ART) for perinatally HIV-acquired children has prolonged their survival and in the past 2 decades, many have reached adulthood. As the perinatally HIV-infected females become sexually active, they are in turn at risk for pregnancy and of transmitting HIV infection to their children. A considerable proportion of this population appears to engage in unprotected sexual intercourse leading to teenage pregnancies, STDs, and abnormal cervical cytology despite frequent contact with HIV health care providers and clinics. Currently there is a paucity of data regarding pregnancy and neonatal outcomes in HIV perinatally infected women. As increasing number of pregnancies will occur among this population we must continue to monitor and focus on their reproductive health issues to improve perinatal and long-term maternal outcomes. This paper will summarize our current knowledge about reproductive health issues and identify areas for future inquiry.

1. Introduction

More wide scale HIV testing and counseling along with progress in HIV treatment has led to major clinical advances in HIV care and has transformed HIV/AIDS from a uniformly fatal disease to a chronic disease. According to UNAIDS program, 33 million people worldwide were estimated to be living with HIV or AIDS at the end of 2009 [1]. The majority of infected individuals are adults and reside in the developing world.

During the early 1980s when the first perinatally acquired AIDS cases were documented, infection usually progressed rapidly to death. In the United States and other developed nations through public health strategies that emphasized prenatal HIV screening and use of ART the number of perinatal HIV cases have decreased dramatically from 1,650 in 1991 to fewer than 200 in 2004 which represents an overall 92% decline [2–5]. In 2005, the estimated number of perinatally infected persons living with HIV was 6,051 for the 33 jurisdictions with HIV reporting in the United States [6]. As the perinatally infected cohort have benefited from antiretroviral therapy, there has been a significant decrease in pediatric AIDS deaths. Thus, perinatally infected children are living longer and the first wave is now approaching adolescence and young adulthood. A female is considered to have perinatally acquired HIV infection if her mother was HIV infected during pregnancy, labor or delivery according to clinical records or if she is found to be positive during infancy or early childhood without another explanation for exposure [7].

Health care providers of perinatally infected young women of reproductive age are now encountering reproductive health issues in this population with little or no evidence to guide them. Adolescents perinatally infected with HIV are often cared for in pediatric infectious disease clinics where reproductive health issues may not be routinely addressed. Several studies have examined reproductive health issues in this population [8–10]. One study found that 70% expressed intent to have children [9] and many demonstrated limited knowledge of safe sex practices [10]. In an editorial response

to the first report describing pregnancy in perinatally HIV-infected adolescents and young adults, the Centers for Disease Control recommended enhanced efforts to identify pregnancies among this population and more in-depth investigation of such pregnancies to better characterize the factors associated with these pregnancies and their outcomes [11]. Since 1998, 13 reports of pregnancies among perinatally infected adolescents have been described [7, 9, 11–21]. This paper will summarize our current knowledge about pregnancies in this special population and identify areas for future inquiry.

2. Pregnancy Outcomes

2.1. Preterm Birth. The first case report of pregnancy in a perinatally HIV infected female was described in 1998. The patient was a 14-year old who delivered an HIV negative infant at term [12]. However, perinatally HIV infected females appear to be at increased risk of preterm birth. In 2009, Williams et al. published a retrospective review of maternal and neonatal outcomes of 10 perinatally HIV-infected females [19]. They found the median age of first pregnancy was 18.5 years and the mean gestational age at the time of delivery was 38 weeks. However, premature rupture of membranes with preterm delivery occurred in 31% of the patients. Teenage pregnancy is a known risk factor for preterm birth with rates reported among adolescents between 13–18% [22, 23]. The rate in this perinatally HIV-infected cohort is significantly higher than the expected risk from adolescence alone. In addition, Thorne et al. evaluated nine viable pregnancies in perinatally HIV-infected females from Europe and found preterm delivery occurred in 44% [17]. More recently Beckerman et al. found perinatally infected females ($n = 34$) compared to sexually infected women $n = 54$ were significantly more likely to delivery prematurely with a mean gestational age of 33.7 versus 38.8 weeks $P = .03$ [21]. The etiology of the increased risk for preterm delivery in this population is unclear. Early data is conflicting as to whether receipt of combination ART during pregnancy is associated with preterm delivery [24]. A recent pooled analysis of three large studies found heterogeneity in the association between combination ART and preterm birth. However, increased rates of preterm birth (adjusted OR 1.5) were found in all three cohorts when combination ART regimens were compared with dual regimens. Additional factors found to be associated with preterm birth in all three cohorts included injection drug use and more advanced HIV disease [25]. Clinicians should be aware of a possible small increased risk of preterm birth in pregnant women receiving protease inhibitor (PI)-based combination ART; however, given the clear benefits of such regimens for both the women's health and the prevention of mother-to-child transmission (MTCT), providers should not withhold protease inhibitors for fear of altering pregnancy outcome [24].

Premature delivery in perinatally HIV infected females has an impact on the selection of ART for the newborn. Prematurity is associated with increased mortality and a number of neonatal morbidities including respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis. In premature infants medication dosing is only available for zidovudine therefore making the use of other ART more problematic. Preterm infants' immature renal and hepatic metabolism increases the risk of ART overdosing and toxicity. Postmarketing surveillance identified 10 neonates, 9 of whom were born prematurely, who received lopinavir/ritonavir and experienced life-threatening events [26]. Due to these increased adverse effects predominantly seen in preterm neonates, the Food and Drug Administration now recommends that lopinavir/ritonavir not be administered to neonates before a postmenstrual age of 42 weeks and a postnatal age of at least 14 days [24]. Finally, premature infants may be at increased risk for viral resistance secondary to a short duration and amount of ART exposure in the mother. Thus, premature delivery in this population has both immediate and long term adverse consequences and may compromise the clinician's ability to provide state of the art ART to the infant to prevent MTCT.

2.2. Cesarean Delivery. Perinatally infected females appear to be at increased risk of delivering by cesarean section. In the United States, a scheduled cesarean delivery (CD) at 38 weeks of gestation to prevent perinatal transmission of HIV is recommended for all HIV-infected women if their plasma HIV RNA remains >1,000 copies/mL near the time of delivery [24]. The rate of CD in the United States in 2007 for women under 20 was 23% [27]. In the perinatally infected cohort described by Williams et al., the rate of CD was 62% with 75% of the cases performed for HIV infection [19]. Inadequate viral suppression in that cohort reinforces the need for close prenatal followup and explicit counseling on the importance of medication adherence. In a European study of 9 viable pregnancies in perinatally HIV-infected females, 8 of the 9 (89%) were delivered by CD [17]. Six of these were described as elective and the other 2 were emergency CD. Details surrounding the decision for mode of delivery was not described, however, 2 of the 8 patients who underwent CD had a viral load >1000 copies/mL at the time of birth. Compared to vaginal birth, CD are associated with increased morbidity including excessive blood loss, infection, thromboembolism, and postoperative pain. In addition, repeat CDs have additional risks of operative injuries and of abnormal placentation including placenta previa and placenta accreta. Given the young age of these patients and potential likelihood of future pregnancies the optimal delivery would include viral suppression from adequate ART leading to opportunities for vaginal birth.

In contrast to the retrospective data from the United States, a prospective study of 30 asymptomatic perinatally HIV-infected adolescents and young adult females in India found no risk of adverse maternal or fetal outcome. The study revealed a low rate of preterm birth (3%) and cesarean delivery (3%) [13]. However the results of this study may in part be explained by selection bias as this study population included perinatally HIV-infected females who had an absence of STDs, received regular medical care, ART,

had good nutrition, and were mostly married with a strong desire for pregnancy. In addition, any clinical manifestation of HIV disease was an exclusion criterion for this study. Therefore, although these results are somewhat reassuring, they may not be generalizable to other perinatally HIV-infected female populations.

3. Perinatal HIV Transmission

Before the introduction of ART and obstetrical interventions to reduce MTCT about 1 in 4 infants born to a woman infected with HIV became infected. Among infected infants approximately 50% of transmission occurs around the time of labor or delivery, 20–25% occurs in-utero, and 25–35% occurs postnatally secondary to breastfeeding [28]. In developed countries today, MTCT rate are estimated at less than 2% with the use of ART during pregnancy and in labor, with cesarean deliveries for viral loads >1,000 (copies/mL), 6 weeks of neonatal ART prophylaxis and avoidance of breastfeeding [4, 29]. Although ART has markedly decreased the risk of MTCT in the United States among adult females infected with HIV, little is known about their effect among pregnant perinatallyinfected females.

The risk of MTCT among perinatally HIV infected females appears to be comparable to the MTCT among nonperinatally HIV infected parturients. The Pediatric AIDS Clinical Trials (PACTG) protocol 219 has enrolled and followed HIV-infected and non-HIV-infected children at clinical centers across the United States since September 2000 to study the complications of pediatric HIV infection. A subanalysis including only perinatally infected adolescent girls aged 13 or older identified a cohort of 638 adolescent girls in which there were 32 pregnancies resulting in live births. One infant was HIV infected, 29 were uninfected, and 2 had unknown infection status, for a rate of MTCT of 3.3% (95% CI = 0.1, 18.6) [7]. All adolescent girls received ART during pregnancy with 26 receiving combination therapy with at least 3 drugs including a protease inhibitor (PI); the case of perinatal transmission occurred in 1 of the 2 girls receiving a PI and a nonnucleoside reverse transcriptase inhibitor (NNRTI). In the cohort described by Williams et al., there was 1 case (10%) of perinatal HIV transmission however this was attributed to patient noncompliance [19]. At the time of publication of the European study, one of the nine infants was confirmed uninfected, seven were presumed uninfected and the most recently born was still indeterminate [17]. Although this corresponds to a 0% MTCT the 95% confidence interval is wide because of the small sample size and the upper limit is 36.7%. An earlier study from Puerto Rico identified eight cases of pregnancy in perinatally HIV-infected females. These resulted in six viable infants with no MTCT at the time of publication [11]. Most recently Millery et al. reported on 19 live births in a cohort of perinatally infected women from New York City in which there were no cases of MTCT [20]. Young people may have problems adhering to ART and pregnancy could compound this [30, 31]. Therefore, young infected pregnant women need additional counseling about proper ART use and social support to achieve maximal viral suppression which is vitally important to preventing MTCT.

4. Other Reproductive Health Concerns

4.1. Sexually Transmitted Infections. High risk sexual behavior is a concern in perinatally HIV-infected females. In the PACTG 219 cohort of perinatally infected adolescent girls, there was a six year cumulative incidence of Trichomoniasis of 6.8% (95% CI 2.4–11.5) and Chlamydia of 5.5% (95% CI 2.0–9.1) [6]. These rates are lower than (6–22%) documented in the REACH cohort a prospective cohort of 330 HIV-positive homeless and marginally housed persons recruited in San Francisco. However, that cohort included girls infected with HIV through sexual contact [32]. Also screening for genital infections was not performed routinely as part of the PACTG 219 protocol. The rate of sexually transmitted infections (80%) was significantly higher in the ten pregnant perinatally infected adolescents reviewed by Williams et al. [19]. These findings of a high STD rate among perinatally HIV-infected females underscore the importance of obtaining sexual histories, promoting safer sexual practices and screening for STDs in pregnancy in this population.

4.2. Cervical Cytological Abnormalities. According to the most recent guidelines cervical cancer screening should begin at age 21 years [33]. Women infected with HIV are at an increased risk of high-risk human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN) [34–36]. Although adolescents with HIV have a higher incidence of cervical dysplasia, the incidence of high-grade abnormalities (both high-grade squamous intraepithelial lesion and CIN 2 or CIN 3) appears to be low [37, 38]. Therefore, cytologic screening in this population is recommended twice in the first year after diagnosis and annually thereafter, with referral for colposcopy for any cytologic abnormality other than atypical squamous cells of undetermined significance (ASC-US) [39, 40]. The current guidelines do not specifically address when to begin screening in a patient with perinatally infected HIV. However, it would seem prudent to begin screening at the onset of sexual activity and certainly at the time of prenatal care in the absence of cytology screening in the previous 12 months.

In the PACTG 219 cohort, 48 of 101 girls (47.5%) with a Papanicolaou smear (Pap smear) had abnormal cervical cytology, including ASCUS ($n = 18$), low-grade squamous intraepithelial lesions (SIL) ($n = 27$), and high-grade SIL ($n = 3$) [7]. The mean age at first Pap smear was 16.7. Among the 21 adolescent girls who underwent intervention 10 (48%) persisted or progressed to more severe lesions. In the Williams et al. review of 10 pregnant patients with perinatally HIV infection 5 of 10 (50%) had abnormal cervical cytology [19]. The high proportion of abnormal cervical cytology in these adolescent girls is likely secondary to an increased susceptibility to and persistence of HPV and other genital infections as has been documented in nonperinatally HIV-infected females [41].

Finally, an equally important public health issue in perinatally HIV-infected young females is the use of the HPV vaccine. There currently are no guidelines which specifically address the use of HPV vaccine in this population. Although safety of the quadrivalent vaccine in HIV-infected children has been demonstrated, efficacy in women or girls with HIV has not yet been established [42]. HIV is not considered a contraindication to receiving the vaccine and at this time the CDC recommendations for HPV vaccination of children and adolescents should be followed similarly for both HIV-infected and non-HIV-infected populations [39, 43, 44].

5. Compliance with Treatment

Adherence to antiretroviral therapy is poorer during adolescence in HIV-infected individuals compared to younger children and adults [45]. Poor compliance with medications and care impacts significantly on virologic control. Adolescence is known to be a time of increased risk taking behaviors and the impact on adherence has been documented in other chronic health conditions such as cystic fibrosis [46]. Young people with HIV have the additional burden of stigma, secrecy, and the risk of transmitting HIV to partners and offspring. Perinatally HIV-infected females have multiple barriers to adherence including mental health/substance abuse, low expectancy for outcome of ART, and structural barriers in fitting medication into a complicated daily life [47]. In addition, many perinatally HIV-infected females have suffered bereavement losing their mothers which impacts their adult support network and possibility leaving them to care for other family members. As adolescences is a time often associated with poorer adherence to medications, adherence messages need to be repeated and particular attention should be given to the period during the transition from pediatric to adult services [48]. Counseling regarding family planning options and providing easy access to contraception is also of vital importance. The cohort of 15–25 year old perinatally infected patients in New York City identified 33 total pregnancies among 96 women of which fourteen (48%) were electively terminated [21]. In order to proactively reduce the risk of undesired pregnancy we must provide education and counseling on sexuality, reproductive health, and contraception. By focusing more efforts on this high-risk patient population at this important transition time in their lives we may achieve the goal of decreasing risk taking behaviors leading to unprotected sex, STDs, unintended pregnancies and noncompliance with ART and followup.

6. Conclusions

As the perinatally HIV-infected female population ages increasing numbers of pregnancies can be anticipated and reproductive health issues affecting this population will need to be addressed. A considerable proportion of this population appear to engage in unprotected sexual intercourse leading to teenage pregnancies, STDs, and abnormal cervical cytology despite frequent contact with HIV clinics. Although there is a paucity of published data on this population, the findings to date highlight the importance of obtaining sexual histories, counseling to prevent unintended pregnancies, screening for STDs, and performing routine Pap smears. In addition, further studies are needed to accurately assess the maternal, obstetrical, and neonatal risks associated with pregnancy in perinatally HIV-infected females. MTCT rates are now less than 2% in the United States when pregnant women with HIV infection receive ART, undergo elective cesarean delivery, and refrain from breastfeeding. Based on the current limited evidence it appears that the MTCT rate is similar in perinatally HIV-infected females compared to nonperinatally HIV-infected parturients. The rate of preterm birth and cesarean deliveries appears to be higher in perinatally HIV-infected females however the overall small sample sizes currently precludes determining an accurate relative risk. If the current treatment advances in HIV infection continue we anticipate identification of additional pregnancies in perinatally HIV infected females. Future research should focus on identifying prospective cohorts and characterizing pregnancy outcome, rate of MTCT, determinants of contraceptive choices, and HIV disease progression. We must continue to monitor and focus on the reproductive health issues in this population to better understand and improve perinatal and long-term maternal outcomes.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 708456, 7 pages
doi:10.1155/2012/708456

Research Article

Virological Response in Cerebrospinal Fluid to Antiretroviral Therapy in a Large Italian Cohort of HIV-Infected Patients with Neurological Disorders

Maria Letizia Giancola,[1] Patrizia Lorenzini,[1] Antonella Cingolani,[2] Francesco Baldini,[1] Simona Bossolasco,[3] Teresa Bini,[4] Laura Monno,[5] Giovanna Picchi,[1] Antonella d'Arminio Monforte,[4] Paola Cinque,[3] Valerio Tozzi,[1] and Andrea Antinori[1]

[1] *Clinical Department, National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS, Via Portuense, 292, 00149 Rome, Italy*
[2] *Institute of Infectious Diseases, Catholic University, 00168 Rome, Italy*
[3] *Department of Infectious Diseases, San Raffaele Scientific Institute, 20127 Milan, Italy*
[4] *Clinic of Infectious Diseases, San Paolo Hospital, University of Milan, 20142 Milan, Italy*
[5] *Clinic of Infectious Diseases, University of Bari, 70124 Bari, Italy*

Correspondence should be addressed to Maria Letizia Giancola, mletizia.giancola@inmi.it

Received 2 January 2012; Revised 29 February 2012; Accepted 11 July 2012

Academic Editor: Giuseppe Ippolito

The aim of the present study was to analyse the effect of antiretroviral (ARV) therapy and single antiretroviral drugs on cerebrospinal fluid (CSF) HIV-RNA burden in HIV-infected patients affected by neurological disorders enrolled in a multicentric Italian cohort. ARVs were considered "neuroactive" from literature reports. Three hundred sixty-three HIV-positive patients with available data from paired plasma and CSF samples, were selected. One hundred twenty patients (33.1%) were taking ARVs at diagnosis of neurological disorder. Mean CSF HIV-RNA was significantly higher in naïve than in experienced patients, and in patients not taking ARV than in those on ARV. A linear correlation between CSF HIV-RNA levels and number of neuroactive drugs included in the regimen was also found ($r = -0.44$, $P < 0.001$). Low -plasma HIV-RNA and the lack of neurocognitive impairment resulted in independently associated to undetectable HIV-RNA. Taking nevirapine or efavirenz, or regimen including NNRTI, NNRTI plus PI or boosted PI, was independently associated to an increased probability to have undetectable HIV-RNA in CSF. The inclusion of two or three neuroactive drugs in the ARV regimen was independently associated to undetectable viral load in CSF. Our data could be helpful in identifying ARV regimens able to better control HIV replication in the CNS sanctuary, and could be a historical reference for further analyses.

1. Introduction

One of the major concerns about antiretroviral (ARV) therapy is the question of whether current ARV regimens are effective in suppressing HIV-1 replication in the central nervous system (CNS) as well as in plasma. CNS is considered one of the anatomic reservoirs of HIV replication, sites in which the cellular HIV replication has a longer half-life [1, 2]. HIV dynamics in CNS and plasma can evolve independently, leading to virologic compartmentalization of HIV infection in the CNS [3]. It is well known that HIV can evolve and replicate in neurological compartment independently from plasma and the virological response in these two different compartments can be quite different [3–5]. Moreover, a residual HIV replication in CNS and persistent intrathecal immune activation can be detected also in patients on ARV [6, 7].

To assess the replication of HIV in CNS is not easy. The levels of HIV-1 RNA in cerebrospinal fluid (CSF) has been considered an indirect measure to assess active infection in brain tissue and a biological marker of HIV infection, as well as in plasma [8]. The diagnostic and prognostic role of the detection of HIV-1 RNA in CSF for the development of neuropsychological impairment has been evidenced in

HIV-infected patients [9–11]. However, in the highly active antiretroviral therapy (HAART) era the relationship between CSF HIV-RNA levels and neurocognitive impairment seems to be lost [12] and biological markers of brain damage are lacking.

The strong beneficial effect of the potent antiretroviral regimens on disease progression is clearly documented [13, 14], but the effect on the CNS and the protective role against neurologic complication of HIV infection is less evident. In the last years, a marked decline of neurologic complications has been observed [15, 16]. A decrease in the incidence of HIV-associated neurocognitive impairment has been also registered, while its prevalence has risen [17]. Cumulating evidences indicate that a relevant proportion of HIV-infected patients continue to present neurocognitive impairment despite the treatment with HAART and that currently available ARV regimens are often inadequate to treat HIV-associated neurocognitive impairment [18].

HAART is demonstrated to effectively reduce HIV-1 RNA levels in CSF [19, 20], but the neuroactive effect of antiretroviral drugs and the protective role of different drug classes in patients treated with HAART has to be conclusively defined.

The aim of the present study was to analyse the effect of antiretroviral therapy and single antiretroviral drugs on CSF HIV-1 RNA burden in a large cohort study group of HIV-infected patients affected by neurological disorders and to identify factors related to undetectable levels of CSF HIV-1 RNA in such cohort.

2. Methods

To analyse the effect of antiretroviral drugs, drug classes, and number of CNS-penetrating drugs on HIV-RNA load in CSF, a large group of HIV-infected patients affected by neurological disorders enrolled in the Italian Registry Investigative NeuroAIDS (IRINA) was studied. IRINA is a longitudinal, multicentric cohort study carried out in 45 Italian centres of infectious diseases, that since 2000 enrols HIV-infected patients affected by neurological disorders. In particular, the registry collects demographic and epidemiologic variables, natural history of HIV infection, antiretroviral therapy, clinical and radiological features, diagnostic criteria for neurological diagnosis, and virological and immunological parameters. Patients with paired CSF and plasma data available were included in the present study and were considered for the analysis. HIV-RNA levels in plasma and CSF were quantified by branched-DNA (Bayer, detection limit of 50 copies/mL, 1.69 $\log_{10}$), RT-PCR (Amplicor Roche Diagnostics, detection limit 50 copies/mL) or nucleic acid sequence-based amplification (NASBA) (Nuclisens HIV-1 QT assay Organon Teknika, detection limit of 80 (1.90 $\log_{10}$) copies/mL), depending on the assay used by each center. To account for the difference between NASBA and RT-PCR in HIV RNA quantification, values of HIV RNA by NASBA assay were divided by two. For the analysis, all HIV-RNA levels were transformed into $\log_{10}$ values. For the statistical analysis, CSF HIV-RNA were considered "undetectable" if the viral load was below the detection limit of the tool used.

The statistical analysis was performed including patients taking the drugs for which we have a larger case number of plasma-CSF paired samples.

Antiretrovirals known to have high level of penetration in CSF or to effectively suppress HIV-RNA in CSF from literature reports, were considered "neuroactive drugs." Among the antiretrovirals prescribed to the study patients, the neuroactive drugs included: zidovudine, stavudine, lamivudine, abacavir, nevirapine, efavirenz, indinavir, lopinavir [21–30]. Lopinavir was always administered associated to a boost of ritonavir at recommended doses. Since indinavir was administered with or without the boost of ritonavir, boosted indinavir was considered as a different regimen from unboosted one.

Logistic regression was used to determine predictive factors of undetectable CSF viral load. Multivariable analysis was performed fitting three different models including variables related to antiretroviral therapy: in the first model the effect of each single drug included in the antiretroviral regimen was analyzed; in the second model the effect of different drug regimens was analyzed using the following categorization criteria: unboosted Protease Inhibitors PIs-, boosted PIs-, Non-Nucleoside-reverse-trascriptase-inhibitors- (NNRTIs-), NNRTIs-plus-PIs-, only-nucleoside-reverse-trascriptase-inhibitors- (NRTIs-) based regimens, or no therapy; in the third model the effect of the number of neuroactive drugs, as defined above, was analyzed. The Student t-test was employed to compare values of CSF HIV-RNA in different groups of patients (naïve-experienced, on HAART-no HAART). Correlation between $\log_{10}$ CSF HIV-RNA and number of neuroactive drugs was calculated using Pearson correlation coefficient r. All statistical analyses were performed by SPSS (version 11.0.1) for Windows (SPSS, Chicago, Illinois, USA). P values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Results. Three hundred sixty-three HIV-positive patients affected by neurological disorders and enrolled in IRINA Study, with available data from paired plasma and CSF samples, were selected for the present analysis. General characteristics of the patients included were reported in Table 1.

Median CD4 count, plasma, and CSF HIV-1 RNA were 71 cell $\times 10^9$/L (IQR: 22–162), 4.98 $\log_{10}$c/mL (3.81–5.44) and 3.63 $\log_{10}$c/mL (2.17–4.83), respectively. In 16.5% of patients CSF HIV-RNA was undetectable. Neurologic disorders included HIV encephalopathy (28.4%), Progressive Multifocal Leucoenkephalopathy (15.4%); encephalopathies of unknown origin (10.2%); Toxoplasmic encephalitis (9.9%); cryptococcosis (9.6%); cerebral lymphoma (5%); Tuberculous meningitis (2.8%); other diseases (18.7%).

Regarding antiretroviral (ARV) therapy exposure, 182 (50.1%) patients were ARV experienced and 120 (33.1%) were taking ARV therapy at diagnosis of opportunistic or neoplastic neurological disorder. The frequency of each ARV agent included in the HAART regimen were as

Table 1: General characteristics of the 363 HIV-positive patients included in the study.

Characteristics	Patients = 363
Male gender (*n*, %)	281 (77.4%)
Age, median (years)	41 (IQR, 36–46)
HIV transmission route (*n*, %)	
(i) IVDU	157 (43.3%)
(ii) MSM	46 (12.7%)
(iii) Heterosexual	111 (30.5%)
(iv) Other/unknown	49 (13.5%)
Previous AIDS defining event (*n*, %)	109 (30.0%)
CD4 cell count, median (cell/mm^3)	71 (IQR, 22–162)
Plasma HIV-1 RNA, median ($\log_{10}$cp/mL)	4.98 (IQR, 3.81–5.44)
CSF HIV-1 RNA, median ($\log_{10}$cp/mL)	3.63 (IQR, 2.17–4.83)
Undetectable CSF HIV-RNA (*n*, %)	60 (16.5%)
Experienced to antiretroviral therapy (*n*, %)	182 (50.1%)
Experienced on ARV at neurological diagnosis (*n*, %)	120 (33.1%)
Time on HAART, median (months)	16 (IQR, 5–41)
>6 months on HAART before diagnosis (*n*, %)	118 (32.5%)
Cognitive symptoms	213 (58.7%)
Abnormal mental status	90 (24.8%)
Cerebral atrophy	137 (37.7%)
Neurological disorders	
(i) HIVE	103 (28.4%)
(ii) PML	56 (15.4%)
(iii) PCNSL	18 (5.0%)
(iv) TE	36 (9.9%)
(v) EUO	37 (10.2%)
(vi) CM/TB	45 (12.4%)
(vii) Other diseases	68 (18.7%)

IVDU: intravenous drug users, MSM: men who have sex with men, CSF: cerebrospinal fluid; HIVE: HIV encephalopathy; PML: progressive multifocal leucoencephalopathy; PCNSL: primary central nervous system lymphoma; TE: toxoplasmic encephalitis; EUO: encephalopathies of unknown origin; CM: cryptococcosis; TB: CNS tuberculosis/tubercular meningitis.

follows: zidovudine 47 patients (12.9%), didanosine 23 patients (6.3%), stavudine 60 patients (16.5%), lamivudine 93 patients (25.6%), abacavir 16 patients (4.4%), nevirapine 14 (3.9%), 26 efavirenz patients (7.2%), indinavir 15 patients (4.1%), ritonavir-boosted indinavir 8 patients (2.2%), nelfinavir 25 patients (6.9%), ritonavir-boosted lopinavir 17 patients (4.7%). Regarding drug regimens, 37 (10.2%) patients were taking unboosted PIs-, 30 (8.3%) boosted PIs-, 31 (8.5%) NNRTIs-, 8 (2.2%) NNRTIs plus PIs-, and 14 (3.9%) NRTIs-based regimens.

Eight patients (2.2%) were taking one neuroactive drug, as above defined, 45 (12.4%) were taking two neuroactive drugs, and 67 (18.5%) were taking three or four neuroactive drugs.

Mean CSF HIV-1 RNA was significantly higher in naïve (4.3 (SD: $\pm$1.3) $\log_{10}$c/mL) than in experienced (3.2 ($\pm$1.2) $\log_{10}$c/mL) patients ($P < 0.001$, Student t-test). Similarly, mean CSF HIV-1 RNA was significantly higher in patients not taking ARV therapy (4.2 ($\pm$1.2) $\log_{10}$c/mL) than in patients on ARV therapy (2.9 ($\pm$1.1) $\log_{10}$c/mL) ($P < 0.001$, t-Student test). A linear correlation between the CSF HIV-1 RNA levels and the number of neuroactive drugs included in the HAART regimen was also found ($r = -0.44$, $P < 0.001$) (Figure 1). Furthermore, analyzing the effectiveness of antiretrovirals included in the patients' regimens using the penetration score proposed by Letendre et al. [31], the significant correlation between HIV-1 RNA load in CSF and the CNS penetration-effectiveness score was confirmed ($r = -0.43$, $P < 0.001$).

Low plasma HIV-RNA and the absence of neurocognitive impairment resulted in independently associated to undetectable HIV-RNA levels in all the three models of analysis employed (Table 2). A significant correlation between HIV-1 RNA load and the evidence of neurocognitive impairment was also found ($r = 0.11$, $P < 0.041$). Regarding ARV drugs, taking nevirapine (OR: 4.46; 95% CI: 1.03–19.32, $P = 0.045$) or efavirenz (OR: 4.87; 95% CI: 1.16–20.54, $P = 0.031$) was independently associated to an increased probability to have undetectable HIV-RNA levels in CSF. Regarding ARV regimens, the use of a regimen including NNRTI (12.46 (3.28–47.41), $P < 0.01$), NNRTI plus PI (10.42 (1.59–68.46), $P = 0.015$) or boosted PI (5.64 (1.31–24.25), $P = 0.02$) was independently associated to an increased probability to have undetectable HIV-RNA levels in CSF. Similarly, the inclusion of two or three neuroactive drugs in the ARV regimen was independently associated to undetectable viral load in CSF (for two neuroactive drugs the adjusted OR was 4.11 (95% CI: 1.22–13.79), $P = 0.022$, for three neuroactive drugs the adjusted OR was 5.48 (1.94–15.48, $P = 0.001$)). Furthermore, using the CNS penetration-effectiveness rank proposed by Letendre et al. [31] was associated to a significant probability to obtain undetectable HIV-1 RNA in CSF (OR 1.20 (per 1 score higher, 95% CI 1.07–1.35, $P = 0.001$)).

No effect of neurologic disorders and of baseline CD4 cell count on HIV control in plasma and CSF was observed.

Considering the subgroup of 120 patients taking HAART at neurological diagnosis and antiretroviral classes (PI, boosted PI, NNRTI, NNRTI plus PI, only NRTI) as cofactor, NNRTI-containing regimen was the only predictive factor of CSF undetectability (OR: 5.38; 95% CI: 1.52–19.00, PI-regimen as reference).

3.2. Discussion. The goal of the long lasting therapeutic strategy in HIV-infected patients must consider the complete control of HIV replication not only in the periphery, but also in the neurological compartment. This is especially true for patients with neurological complications affecting the CNS. A lot of reasons can partially explain the particular condition of CNS compartment: first of all, the presence of the blood brain barrier (BBB) in the CNS, with the tight junctions between the endothelial cells that make peculiar the CNS from the point of an anatomic view, separating the

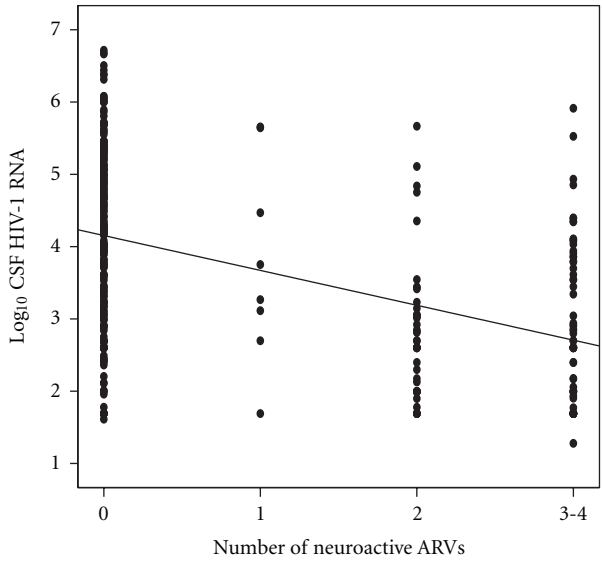

Figure 1: Correlation between the HIV-RNA load in the cerebrospinal fluid (CSF) (copies/mL) and the number of neuroactive drugs included in the HAART regimen. $r = -0.44$, $P < 0.001$. ARVs: antiretroviral drugs.

brain from the rest of the body. The CNS can be considered a sanctuary of HIV infection, where drugs penetrate in variable proportion. Some antiretrovirals penetrate less effectively, reaching sometimes inadequate concentrations. Drug penetration is based on different conditions: molecular weight, lipid solubility and protein binding for diffusion, active transport system, and drug efflux system. In presence of low concentrations of drugs in CSF the replication of HIV can continue. Some drugs are considered to have good penetration in cerebral compartment and to have efficacy on controlling HIV replication [21–30].

The issue if the use of drugs having a good penetration across the BBB is necessary to reach the control of HIV replication also in CNS is currently not clear. It is also questioned if a complete suppression of HIV viral load can be reached in CSF and if it is possible to identify an optimal antiretroviral therapy to obtain the complete control of HIV replication in CSF.

The use of nucleotide analogues has been associated to AIDS dementia decline in EUROSIDA cohort [15], but only for zidovudine a controlled trial has demonstrated a beneficial effect on dementia complex [32].

Previous studies showed a better virological decline of HIV-RNA in CSF using three or more drugs with good penetration [19] and a higher number of CSF-penetrating drugs [33–35]. The use of HAART was correlated to the decline of HIV-RNA in CSF and to a better neurocognitive performance [34, 36] in some patients, but the use of single CSF penetrating HAART versus multiple has not shown a marked benefit in psychomotor speed change in nonadvanced patients [37]. In a previous study, we failed to find a correlation between the neurocognitive performance (NPZ8 score) and the number of penetrating drugs included in the antiretroviral regimen in HIV-positive patients with a good immunological level and stable HAART [38].

A penetration score has been proposed by Letendre et al. [31] to evaluate whether the penetration of antiretrovirals in the CNS is associated to lower CSF viral load. A numeric penetration score was obtained summing the score assigned to singular drugs included in the antiretroviral regimen taken by patients, considering the published data on CSF concentrations and chemical properties. Higher penetration scores were strongly and independently associated with lower CSF viral load also after adjusting for total number of antiretrovirals and plasma viral load [31].

The data obtained from the present study, conducted on a large cohort of HIV-infected patients, confirm that antiretroviral therapy can determine a significant reduction of HIV burden in CSF as documented by the lower HIV-1 RNA load observed in antiretroviral experienced patients compared to naïve patients, and in HAART-treated patients compared to non-HAART-treated patients, also in presence of neurological disorders. Our data indicate that a higher number of CNS penetrating ARVs or an higher CNS penetration-effectiveness score using Letendre classification of ARVs correlated with lower CSF RNA levels ($r = -0.44$, $P < 0.001$, $r = -0.43$, $P < 0.001$, resp.). Moreover, the use of a higher number of CNS-penetrating drugs enhances the probability to obtain undetectable level of CSF HIV-RNA. Compared to regimens containing no CNS-penetrating ARVs, the use of two (OR = 4.11; 95% CI = 1.22–13.79) or at least three (OR = 5.48; 95% CI = 1.94–15.48) penetrating CNS ARVs markedly improved the probability of having a CSF HIV RNA level below the detection limit of 50 copies/mL.

Furthermore, we made an effort to identify antiretroviral schemes or agents that could improve HIV control in CSF. Among specific ARVs, the use of nevirapine (OR = 4.46; 95% CI = 1.03–19.32) and efavirenz (OR = 4.87; 95% CI = 1.16–20.54) showed the best correlations with the probability of having CSF HIV RNA level below the detection limit of 50 copies/mL. Among antiretroviral drug classes, the exposure to NNRTIs (OR = 12.46; 95% CI = 3.28–47.4) and boostered PI (OR = 5.64, 95% CI = 1.31–24.25) increases the probability to reach undetectable levels of HIV-RNA in CSF. Taken together these data indicate that the use of ARVs with good penetration into the CNS increases the probability of controlling HIV replication in CSF. Prospective study are needed to confirm our data.

We are aware of potential limits of our study. First, we know that the undetectable HIV load in CSF cannot fully reflect controlled replication of HIV in the brain tissue. Moreover, we have no data to support the hypotheses that controlled HIV replication in CSF translates in neurocognitive improvement in our study patients on HAART.

In recent years, a decreased frequency of HIV-related neurological disorders was observed, and a lower number of patients underwent lumbar puncture, so the number of available paired CSF-plasma samples was lower. The analysis here reported was limited to drugs for which we have a larger case number of plasma-CSF paired samples. It does not include new NRTIs, NNRTIs, PIs drugs and new classes, as fusion and entry inhibitors or integrase inhibitors, more recently introduced, that are very interesting to study, and

Table 2: Factors related to undetectable HIV-RNA levels in cerebrospinal fluid (CSF) at logistic regression model adjusted for age, gender, HIV-transmission route, time on HAART before neurological diagnosis (more or less than 6 months), abnormal mental status, cerebral atrophy, and neurological disorder.

Variables	Crude OR (95% CI)	*P*-value	Model 1		Model 2		Model 3	
			Adjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Plasma HIV-RNA (log10 cp/mL)	0.57 (0.47–0.69)	<0.01	0.70 (0.51–0.95)	0.024	0.70 (0.51–0.96)	0.027	0.68 (0.51–0.91)	0.009
CD4 at diagnosis (50 cells increase)	1.05 (0.97–1.14)	0.221	0.97 (0.86–1.10)	0.680	0.96 (0.85–1.09)	0.557	0.96 (0.86–1.08)	0.473
Drugs at diagnosis								
(i) AZT	3.60 (1.83–7.08)	<0.01	2.15 (0.51–9.04)	0.295				
(ii) DDI	2.37 (0.93–6.04)	0.071	1.67 (0.37–7.65)	0.508				
(iii) D4T	3.65 (1.95–6.83)	<0.01	1.53 (0.41–5.65)	0.527				
(iv) 3TC	4.53 (2.54–8.10)	<0.01	1.62 (0.39–6.66)	0.505				
(v) ABV	1.17 (0.32–4.25)	0.807	0.22 (0.03–1.68)	0.144				
(vi) NVP	4.10 (1.37–12.28)	0.012	4.46 (1.03–19.32)	0.045				
(vii) EFV	7.38 (3.21–16.95)	<0.01	4.87 (1.16–20.54)	0.031				
(viii) IDV	1.90 (0.58–6.17)	0.288	0.86 (0.16–4.50)	0.856				
(ix) IDV/r	3.14 (0.73–14.49)	0.125	2.06 (0.31–13.58)	0.454				
(x) NFV	1.66 (0.63–4.35)	0.302	0.68 (0.16–2.78)	0.587				
(xi) LPV/r	2.21 (0.75–6.51)	0.152	1.10 (0.22–5.64)	0.907				
Drug classes								
(i) No ARV	1.00				1.00			
(ii) NNRTIs-based regimens	14.32 (6.13–33.44)	<0.01			12.46 (3.28–47.4)	<0.001		
(iii) PIs-based regimens	3.50 (1.54–7.96)	<0.01			2.48 (0.75–8.22)	0.138		
(iv) Boosted PIs-based regimens	6.88 (2.42–19.52)	<0.01			5.64 (1.31–24.25)	0.020		
(v) Only NRTIs	3.22 (0.83–12.53)	0.092			1.25 (0.09–17.10)	0.866		
(vi) PI + NNRTIs-based regimens	7.07 (1.57–31.89)	0.01			10.42 (1.59–68.46)	0.015		
Number of neuroactive drugs								
(i) 0	1.00						1.00	
(ii) 1	1.68 (0.20–14.41)	0.634					1.67 (0.16–17.82)	0.673
(iii) 2	6.50 (3.01–14.04)	<0.01					4.11 (1.22–13.79)	0.022
(iv) 3-4	6.58 (3.32–13.05)	<0.01					5.48 (1.94–15.48)	0.001
Cognitive symptoms	0.47 (0.27–0.83)	<0.01	0.38 (0.16–0.90)	0.029	0.38 (0.16–0.88)	0.025	0.41 (0.18–0.95)	0.038

OR: odds ratio; IVDU: intravenous drug users; MSM: men who have sex with men; AZT: zidovudine; DDI: didanosine; D4T: stavudine; 3TC: lamivudine; ABV: abacavir; NVP: nevirapine; EFV: efavirenz; IDV/r: indinavir/ritonavir; IDV: indinavir; NFV: nelfinavir; LPV/r: lopinavir/ritonavir; ARV: antiretroviral therapy; NNRTI: nonnucleoside reverse trascriptase inhibitors; PI: protease inhibitors; NRTI: nucleoside reverse trascriptase inhibitors; HAART: highly active antiretroviral therapy; HIVE: HIV encephalopathy; PML: progressive multifocal leucoencephalopathy; EUO: encephalopathies of unknown origin; TE: toxoplasmic encephalitis; PCNSL: primary central nervous system cerebral lymphoma; CM: cryptococcosis; TB: CNS tuberculosis/tubercular meningitis.

it represents a limitation of the present study. Unfortunally, because of the small number available for statistics, we were not able to investigate these more recent drugs.

In conclusion, our data support the concept that the inclusion of a higher number of CNS penetrating drugs is associated with an increased probability of having undetectable CSF HIV RNA levels in HIV-infected patients affected by neurological disorders. Our data could be helpful in identifying ARV regimens able to better control HIV replication in the CNS sanctuary and could be a historical reference for further analyses regarding the "new antiretroviral drugs".

Acknowledgments

IRINA Study Scientific Commitee: A. Antinori, P. Cinque, A. Ammassari, A. d'Arminio Monforte, C. F. Perno, A. Cingolani, L. Monno, A. De Luca, A. Castagna, C. Balotta, L. Vago, L. M. Larocca, E. Girardi, P. Pezzotti, G. Rezza, G. Ippolito. Coordinating center: (INMI L.Spallanzani): P. Lorenzini, M. L. Giancola, F. Baldini, V. Tozzi, R. Libertone, S. Grisetti, G. Picchi. Partecipating centres: Azienda Ospedaliera Umberto I, Ancona (AM. Pazzi); Ospedale S. Maria Annunziata, Antella(FI) (L. Mecocci); Policlinico Universitario, Bari (L. Monno); Ospedali Riuniti, Bergamo (M. G. Finazzi); Spedali Civili, Università degli Studi, Brescia (F. Moretti); Ospedale Maggiore, Bologna (G. Fasulo); Ospedale Generale Regionale, Bolzano (O. Moling); Ospedale M.Bufalini, Cesena (S. Brighi); Ospedale Civile di Legnano, Presidio di Cuggiono, Milano (M. Mena); Azienda Ospedaliera, Ferrara (L. Sighinolfi); Ospedale Careggi, Firenze (P. Corsi); Ospedale G.B. Morgagni, Forlì (A. Mastroianni); Ospedale S. C. Gesù, Gallipoli (LE) (M. De Simone); Ospedale S. Martino, Genova (G. Mazzarello); Ospedale Felettino, La Spezia (S. Boni); Ospedale S. Maria Goretti, Latina (A. Vetica); Ospedale V. Fazi, Lecce (PP. Congedo); Ospedale Maggiore, Lodi (MI. Arcidiacono); Ospedale C.Poma, Mantova (GC. Fibbia); Ospedale SS.Giacomo e Cristoforo, Massa (P. Zannoni); Ospedale L. Sacco, Milano (T. Bini); IRCCS, Osp. S. Raffaele, Milano (S. Bossolasco); Ospedale Niguarda, Milano (B. Vigo); Università degli Studi, Modena (G. Guaraldi); Ospedale S. Gerardo, Monza (P. Fortuna, S. Foresti); Ospedale D. Cotugno, Napoli (M. Figoni); Ospedale Guadagna, Palermo (G. Rotondo); Ospedale Casa del sole, Palermo (ER. Dalle Nogare); Università degli Studi, Perugia (MS. Egidi); Ospedali Civili, Pescara (A. Agostinone); Ospedali Civili, Piacenza (A. Donisi); Ospedale di Pistoia (A. Vivarelli); Università Cattolica Sacro Cuore, Roma (A. Cingolani); Policlinico Umberto I, Roma (MR. Ciardi); Ospedale di Rovigo (F. Viviani); Ospedale S. Paolo, Savona (F. Toscanini); Ospedale SS. Annunziata, Taranto (L. Cristiano); Ospedale E. S. Macchi, Varese (F. Speranza) for the Italian Registry Investigative NeuroAIDS (IRINA) Study Group.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 161584, 14 pages
doi:10.1155/2012/161584

Review Article

Thirty Years with HIV Infection—Nonprogression Is Still Puzzling: Lessons to Be Learned from Controllers and Long-Term Nonprogressors

Julie C. Gaardbo, Hans J. Hartling, Jan Gerstoft, and Susanne D. Nielsen

Department of Infectious Diseases, Rigshospitalet, Copenhagen University Hospital, Copenhagen 2100, Denmark

Correspondence should be addressed to Susanne D. Nielsen, sdn@dadlnet.dk

Received 16 January 2012; Accepted 22 March 2012

Academic Editor: Jay A. Levy

In the early days of the HIV epidemic, it was observed that a minority of the infected patients did not progress to AIDS or death and maintained stable CD4+ cell counts. As the technique for measuring viral load became available it was evident that some of these nonprogressors in addition to preserved CD4+ cell counts had very low or even undetectable viral replication. They were therefore termed controllers, while those with viral replication were termed long-term nonprogressors (LTNPs). Genetics and virology play a role in nonprogression, but does not provide a full explanation. Therefore, host differences in the immunological response have been proposed. Moreover, the immunological response can be divided into an immune homeostasis resistant to HIV and an immune response leading to viral control. Thus, non-progression in LTNP and controllers may be due to different immunological mechanisms. Understanding the lack of disease progression and the different interactions between HIV and the immune system could ideally teach us how to develop a functional cure for HIV infection. Here we review immunological features of controllers and LTNP, highlighting differences and clinical implications.

1. Introduction

Prior to the introduction of combination antiretroviral therapy (cART) it was observed that a minority of the individuals infected with Human Immunodeficiency Virus type 1 (HIV-1, from now on referred to as HIV) did not progress to Acquired Immunodeficiency Syndrome (AIDS) or death. This minority maintained normal CD4+ cell counts in the absence of treatment for several years—in some cases for more than two decades (reviewed in [1]) and therefore the terminology Long-Term Nonprogressors (LTNP) was proposed. When the technique for measuring the viral load was introduced it became evident that some of these patients, who did not clinically progress, had low or even nondetectable viral replication. This phenomenon leads to the additive definition of the non progressor-phenotype referred to as controllers due to their ability to control viral replication in the absence of cART. Today, non-progressors are a collective name for controllers and LTNP who are clinically similar. Except for certain demands for the duration of the infection in LTNP, they are only to be differentiated according to control or not of viral replication, respectively. Understanding the mechanism for the lack of disease progression in controllers and LNTP could ideally teach us how to develop a functional cure for HIV infection, and for this reason these subpopulations of HIV-infected patients have gained immense interest.

Non-progressors are described to differ from progressors in genetics, virology, and immunology. Genetically, certain factors seem to predispose to non-progressions. Thus, it has been shown that female gender, the presence of CCR5-delta32 polymorphism, and HLA genes, in particular the HLA B57 allele, are overrepresented among non-progressors ([2–9], reviewed in [10]). Virologically, a number of studies have indeed shown that some non-progressors are infected with less virulent strains of HIV resulting in a more benign infection [11–14]. However, there are now several lines of evidence that the majority of non-progressors are infected

with replicant-competent virus [15–17]. One study has shown that CD4+ cells from controllers are less susceptible to HIV compared to CD4+ cells from progressors and healthy controls [18], while another study showed that CD4+ cells from controllers were as susceptible or even more susceptible to HIV entry and productive infection [19].

Thus, genes and viral factors play a role in non-progression, but these components do not provide a thorough explanation. For this reason the immune system is to be considered a key element in non-progression. This is supported by the recent demonstrations of better control of hepatitis C virus (HCV) in HCV-infected controllers compared to HCV-infected progressors [20, 21]. The immunological response to HIV infection can be divided into (1) an immune homeostasis resistant to HIV in LTNP and (2) the immune-mediated control of the virus in controllers. However, despite these two possible interactions between HIV and the immune system both resulting in preserved CD4+ cell counts, few studies have compared immune homeostasis in LTNP and controllers. The scope of this paper is to describe immunology in non-progressing HIV infection and to propose involved immunological mechanisms in LTNP and controllers.

2. Definitions of Nonprogressors

It is well-established that LTNP and controllers are different subpopulations [22–25], supporting the idea that different immunological mechanisms are responsible for the preserved CD4+ cell counts. LTNP and controllers are described as rare populations comprising few percentages of all HIV-infected individuals, and with little overlap between them [22–27], although the definition of the populations suffers from lack of consensus in terminology and inclusion criteria, impeding the comparison of findings.

Controllers can be further divided into elite controllers (EC) and viremic controllers (VCs), most commonly with HIV RNA <50 copies/mL and 50–2000 copies/mL, respectively, although variations with higher levels are found as well ([25], reviewed in[27, 28]). This is in particular a problem because LTNP can thereby be categorized as VC due to their often relatively low viremia. In addition, a central problem in defining the non-progressor phenotype is a complete lack of inclusion of the viral load, thereby including LTNP who fulfill controller-criteria with low or undetectable viral loads.

Patients need not necessarily be infected for a long period of time in order to be categorized as controllers. Thus, two measurements of a low viral load during one year are sometimes used as sufficient, whereas others demand duration of infection for several years. In contrast, LTNPs due to the nature of the case definition have a long duration of infection, most commonly a minimum of 7–10 years. Both groups present with a CD4+ cell count within the normal range (350–1600 cells/μL) [22, 23, 25, 29]. Due to the low prevalence of these non-progressors, it is tempting to relax the inclusion criteria. However, clinical outcomes seem to improve with the stringency of criteria, and it has been demonstrated that the clinical outcome for patients infected for 7 versus 10 years and with stable CD4+ cell counts is different. Thus, a better survival among LTNP defined by 10 years of stable infection versus 7 years is reported [25], suggesting that even 7 years of stable infection do not distinguish properly between true LTNP and progressors.

Table 1: Definitions of non-progressors.

	EC	VC	LTNP
CD4+ cell count (cells/μL)	>350	>350	>350
Viral load (copies/mL)	<50	50–200	>2000
Duration of infection (years)	*	*	>7
cART	No	No	No

* Duration of infection is not used in the definition of controllers in this review.

In this paper, unless anything else mentioned, the term "non-progressors" is used as a collective name for controllers and LTNP, while the term "controllers" is used for VC and EC. The LTNP term is only used when studied patients had substantial viremia above 2000 copies/mL and had been infected for a minimum of 7 years. Although 7 years may not be enough to exclusively distinguish LTNP from progressors, 7 years are chosen as most studies have used this definition. The duration of infection in controllers is not included in our definitions as we consider viral control at any time point to be a sufficient determinant for controller status (Table 1).

3. Immunology in Non-Progressors

The CD4+ cell count in a given patient at any time is the result of production, destruction, and traffic between blood and lymphatic tissue, and when the destruction exceeds the production the CD4+ cell count decreases. Thus, LTNP and controllers may have differences in production, destruction, or distribution of CD4+ cells compared to progressors in order to maintain a normal CD4+ cell count.

4. Production of Cells

4.1. Bone Marrow and Progenitor Cells. T cells mature in the thymus, but they originate from hematopoietic progenitor cells (HPCs) in the bone marrow (BM). Thus, a functional BM is crucial for thymopoiesis. In the hope of developing a cure for HIV infection HPC has been given greater attention, even more so after the report on eradication of HIV by transplantation of CCR5-deficient HPC in the so-called Berlin patient [30]. HIV influences BM and HPC, and impaired hematopoiesis in HIV infection is well documented [31–34]. Furthermore, several studies have shown that some HPCs express the HIV receptors CD4, CXCR4, and CCR5 making them potentially susceptible to HIV infection (reviewed in [35]). HIV infection of HPC has recently been suggested [36], although the complexities of purifying and maintaining HPC in culture make it difficult to determine if these HPCs are actually infected, as signs of infection may be due to contamination with other cell types or maturation of HPC to monocytes during in vitro culture. However, HIV proteins seem sufficient to disturb the haematopoiesis [37]. Nevertheless, in addition to T cells, natural killer cells and B cells,

Table 2: Immunological distinctions between progressors and non-progressors.

	Controller	LTNP	Progressor
Production of T cells	Thymic output preserved Haematopoiesis preserved	Thymus output? Haematopoiesis?	Thymic output ↓ Haematopoiesis exhausted
IL-7/IL-7R	IL-7 Normal IL-7R Normal	IL-7 ↑ IL-7R ↓	IL-7 ↑ IL-7R ↓
Destruction of T cells	Immune activation ↑ Turnover/apoptosis ↑ Microbial translocation ↑	Immune activation ↑↑ Turnover/apoptosis? ? Microbial translocation	Immune activation ↑↑↑ Turnover/apoptosis ↑↑ ↑↑↑Microbial translocation
Secondary lymphoid tissue	Preserved architecture	?	Damaged architecture
Pro- and anti-inflammatory cells	↓Tregs ↑Th17	? Tregs ↑Th17	↑↑Tregs ↓Th17
HIV-specific immune response	Strong	?	Blunted
Viral reservoir	Low	?	High

↑/↓: Indicates slightly, ↑↑/↓↓: moderate, ↑↑↑/↓↓↓: highly different from HIV-negative individuals.
LTNP: long-term non-progressors, Tregs: regulatory T cells.
?: Indicates unknown.

including naïve B cells, seem to be depleted during HIV infection [38]. HIV-associated lymphopenia may therefore be explained by more upstream elements of lymphocyte development than reduced thymic output.

Circulating HPCs have been found to decrease with disease progression and to be associated with CD4+ cell count [39], supporting the idea of BM and HPC as being essential in preservation of CD4+ cell count and suggesting preserved haematopoiesis in non-progressors. The haematopoiesis has only been assessed in a single study of elite controllers (Table 2). This study included progressing as well as non-progressing EC. Interestingly, the progressing EC showed signs of exhausted lymphopoiesis compared the non-progressing EC measured as CD34+ cells and lymphoid-HPC [39]. This is supportive of a sufficient haematopoiesis as a contributing factor to non-progression in controllers. Also, it indicates that the viral replication itself is not the only reason for disease progression.

4.2. Thymus and Naive Cells. The CD4+ cell count is maintained by proliferation of already existing CD4+ cells or by de novo production in the thymus. Earlier, it was believed that the thymus was only active in childhood and replaced by fatty tissue with increasing age. It is now evident that the thymus can also be active in adulthood, particularly during circumstances with lymphopenia, as is the case with HIV infection [40, 41]. Often thymic function is assessed as T-cell receptor excision circles (TRECs) or as the naive CD4+ cell count. TRECs are stable circular DNA fragments that are excised during the formation of TCR in the maturing T cell in the thymus, and TRECs are not replicated during cell division. Thus, the more immature CD4+ cells the higher the TREC content. A large thymus on CT scans has been associated with a higher CD4+ TREC frequency in HIV-infected patients [42]. Thus, TRECs and naive cells are all reasonable indirect measurements of thymic size and output.

HIV leads to a disruption in the number and function of naïve CD4+ cells in blood as well as in lymphoid tissue [43–45]. To our knowledge, no studies of naïve cells have discriminated between controllers and LTNP. In non-progressors, similar and lower numbers of naïve CD4+ cells have been found compared to progressors [8, 46, 47], suggesting that the level of naïve CD4+ cells itself is not associated with non-progression (Table 2). In contrast, increased expression of the naïve marker CCR7, higher levels of central memory cells with preserved ability to secrete interleukin 2 (IL-2), and a much higher thymic output as defined by TRECs in EC compared to progressors have been reported [46, 48], supporting preserved thymic function in non-progressors. The contribution of a well-functioning thymopoiesis to non-progression is further supported by the findings of strong correlations between TRECs and non-progression in SIV-infected rhesus macaques [49]. Also, normal levels of memory cells and preserved IL-2 secretion capacities have been shown in non-progressing SIV-infected rhesus macaques compared to progressors [50]. For this reason, it seems plausible that the thymic function is better in non-progressors compared to progressors, improving their ability to maintain a normal CD4+ cell count. However, these findings only explain the preserved CD4+ cell count in non-progressors, not the viral control in controllers. In fact, the high thymic output may indirectly be a consequence of and not a reason for the low viral replication, since lower viral replication does not lead to the exhaustion of lymphopoiesis normally seen in progressors [39]. Thus, it would be of great interest to compare the thymic output in LTNP and progressors since both populations have viral replication. It is tempting to assume that one of the main differences between these progressors and LTNP is an extraordinary capacity to produce cells. This is supported by findings of higher levels of naïve cells in a study of slow-compared to fast progressors based on the slope of their CD4+ cell loss, although this did

not reach statistical significance [51]. Likewise, a study of children with LTNP status displayed higher levels of naïve cells compared to progressors and controls [52].

Thymic output is dramatically reduced with age, and the naïve cells are increasingly generated from peripheral proliferation (reviewed in [53]). Proliferation of cells leads to a lower TREC count, and therefore naïve cells in older individuals have lower TREC counts [54, 55]. Thus, the reported loss of the non-progressor status in some individuals may be due to increasing age and thereby decreased thymic output, because the thymus is no longer able to meet the demands of a high production of cells. This is supported by the findings of an immune system in HIV-infected patients which is comparable to much older healthy individuals [39]. Also, high age is a predictor of poor immune reconstitution after initiation of cART [56].

4.3. IL7. Production of CD4+cells is influenced by Interleukin 7 (IL-7). IL-7 is crucial in the T-cell homeostasis, and the IL-7 responsiveness is determined largely by the presence or absence of the IL-7 receptor (IL-7R), which is present on most mature T cells [57]. A negative correlation between IL-7 and CD4+ cell count is described. Consequently, HIV-infected progressors have high levels of IL-7 and reduced levels of IL-7R compared to healthy controls [58, 59], consistent with the need for increased production of CD4+ cells and a down-regulation of the receptor due to high plasma levels. Thus, controllers would be expected to display a pattern of IL-7/IL-7R closer to healthy controls than to progressors. This is supported by findings of lower levels of IL7 in controllers compared to controllers who lost their controller status [60] and by findings of lower levels of IL-7R in progressors compared to non-progressors [47]. In contrast, it would make sense that LTNPs display a pattern of IL-7/IL-7R more like progressors than controllers, because the need for CD4+ cell replenishment would be expected to depend on the level of viral replication—infection and cell—turnover, which is supported by findings from our own lab (unpublished data) These results are compatible with a hypothesis of low viral replication leading to a reduced number of new CD4+ cells becoming infected. Thereby, the level of IL-7 does not increase, and the IL-7R expression stays high. However, further studies are warranted to clarify this.

5. Destruction of Cells

5.1. Immune Activation, Senescence, and Apoptosis. Immune activation (IA) is a necessary and normal acute response upon infection with any pathogen, as an effort to avoid infection. However, in HIV-infected individuals it is well established that chronic IA is linked to and predictive of disease progression, and IA has an additive or stronger prognostic value than does CD4+ cell count or viral load alone [61–68]. The influence of IA on disease progression can be partly explained by elevated levels of senescent and apoptotic cells as a consequence of IA, thereby leading to increased loss of CD4+ cells.

Elevated markers of activation are to be found in most cell compartments, but especially expression of the surface markers CD38 and HLA-DR on CD8+ cells have proven to be predictive of disease progression [64–68]. Thus, IA is a central player in disease progression, as illustrated by the development of pneumocystic pneumonia in rats solely as a consequence of IA [69]. In light of this, it is obvious to assume that IA in non-progressors is different from progressors and partly explains lack of progression. IA is one of the more well-examined features in non-progressors and has in several studies been found to be lower in EC as well as VC compared to progressors [70–74]. In support of the significance of low IA on lack of disease progression a study of EC revealed that the individuals with the highest IA presented with the lowest CD4+ cell counts [73] (Table 2). Also, the natural hosts of simian immunodeficiency virus (SIV), sooty mangabeys and African green monkeys, who do not progress despite a high viral load (and thus can be seen as a simian pendant to LTNP), do not show any signs of increased IA or T-cell turnover [75, 76]. IA is inadequately examined in LTNP. One study did not find any differences in IA between EC and LTNP, while they were both different from progressors [77], while another study did not find any differences between the three groups [78]. Interestingly, this latter study, one of the only studies to have compared controllers and LTNP, also evaluated the phenotypic and functional properties of CD56/CD16 natural killer (NK) cells, a major component of the innate immune system. Cytolytic activity against autologous CD4+ cells was found to be abrogated after treatment with an antibody to NKp44L, the cellular ligand of the natural cytotoxicity receptor NKp44, which is specifically induced on CD4+ T cells during HIV-1 infection, in LTNP and HIV progressors. In contrast, in HIV controllers and healthy donors, NKp44L expression on CD4+ cells and autologous NK lysis were both poorly detected [78]. This is strongly supportive of LTNP and controllers as being immunologically different.

Another component of the innate immune system that may be involved in non-progression is the plasmacytoid Dendritic Cells (pDCs). PDCs have been suggested as inducers of IA and CD4+ cell apoptosis as they recognize HIV single-stranded RNA (ssRNA) via Toll-like receptors (TLR) resulting in interferon (INF) α production [79–82]. Furthermore, polymorphisms in TLR7 and the interferon regulator 7 of INFα may influence disease progression and the ability of pDCs to produce INFα [83, 84]. In controllers, the number and function of pDCs are reported to be preserved [85]. In addition, pDCs from rhesus macaques produce large amounts of INFα when stimulated with SIV or HIV, while the natural hosts, sooty mangabeys, seem to have lower levels of INFα [86]. Altogether, this suggests pDCs to induce IA, and they may therefore be involved in non-progression of HIV infection.

IA is accompanied by apoptosis and immunological senescence, and HIV-infected patients present with elevated levels of both features [87, 88]. Senescent cells in EC and progressors have been examined in a single study, and comparable levels were found [8]. In contrast, in a study of non-progressors with unknown viral load the level of apoptosis was found to be similar to healthy controls and lower than in progressors [89], and others have reported lower levels

of apoptosis in VC compared to progressors [90], both supporting the idea of a lower turnover as contributing to non-progression. However, low turnover could also simply reflect a lower IA. Contrary, another study found elevated levels of apoptotic cells in non-progressors compared to progressors [91], implying that a high turnover is beneficial. This finding makes sense, if we assume that activated cells are eliminated by apoptosis. Then an increasing proportion of apoptotic cells eliminate the harmful IA, thereby diminishing disease progression. Either way, evidence is lacking, and whether a high turnover of cells is contributing to non-progression is still to be determined.

Thus, it seems reasonable to assume that the level of IA is a determinant for how fast the turnover of T cells is, thereby relating IA to exhaustion (Table 2). Indeed, it has been proposed that IA leads to CD4+ cell depletion because it erodes the naïve T-cell pool [92]. Still, the reason for the strong predictive value of IA in HIV infection is uncertain, but low IA found in controllers suggests that IA forms an important role in lack of progression.

6. Immune Regulation: Pro- and Anti-Inflammatory Cells

The understanding of the immune system is constantly changing as a consequence of rapidly expanding knowledge. Recently, the discovery of T-cell subsets with pro- and anti-inflammatory properties has altered our view on immunology. Regulatory T cells (Tregs) are anti-inflammatory T cells, while Th17 cells have proinflammatory properties. Tregs are crucial in sustaining tolerance to self-antigens [93, 94] and suppressing T-cell activation resulting in down-regulation of immune activation, including reduction in anti-tumor immunity, graft rejection, and graft *versus* host disease ([95], reviewed in [96]). Finally, the role of Tregs in chronic viral infections, including HIV, has gained considerable interest due to their immunosuppressive capabilities. Thus, in theory, Tregs can downregulate the chronic IA seen in HIV infection making Tregs a key element in the understanding of the interaction between the host immune system and HIV (reviewed in [97]). For this reason, Tregs have been suggested as downregulators of the unbeneficial unspecific IA in HIV-infection, expecting high levels of Tregs as being an advantage. However, levels of Tregs in HIV-infected, untreated, progressing patients have been shown to be elevated compared to healthy controls in a number of studies ([98–100] reviewed in [101]). This suggests that high levels of Tregs are actually harmful, possibly because they downregulate beneficial HIV-specific responses. In support of this, the level of Tregs in controllers has been reported to be lower compared to progressors, and closer to healthy controls, although conflicting results have been reported as well [70, 77, 102–106]. Furthermore, it has been shown that the suppressive activity of Tregs in EC is preserved, while it was found to be disrupted in progressors [105]. Finally, Tregs have been suggested to increase with age (reviewed in [107]), possibly contributing to the reported loss of non-progression in some individuals. All together this is supportive of a significant influence of Tregs on non-progression.

However, like most other pieces in the puzzle of understanding the interaction of HIV and the immune system the Treg element has proven to be more complex than expected. Thus, Tregs are closely related to IL-17-producing Th17 cells. Tregs and Th17 cells share a reciprocal maturation pathway and function together in opposing ways to control the inflammatory response upon infection. While Tregs inhibit autoimmunity, Th17 cells have been shown to play a critical role in the induction of autoimmune tissue injury and immune responses [108]. Th17 cells have been shown to be rapidly depleted during acute SIV infection cells, and a disturbed balance of Th17 cells and Tregs has been suggested to be associated with subsequent high IA and disease progression ([109, 110], reviewed in [101, 111]). In controllers, a maintained balance between Tregs and Th17 cells is reported [102] (reviewed in [112]), highlighting the significance of a well-regulated balance between Tregs and Th17 cells. Th17 cells and Tregs have primarily been examined in controllers. However, one study of a group of non-progressors, where most participants met the LTNP criteria, have found elevated levels of Th17 cells compared to progressors [113]. Thus, a high level of Th17 cells may contribute to lack of progression in controllers as well as in LTNP. However, HIV leads to redistribution of CD4+ cells between blood and lymphatic tissue (LT). Thus, it has been demonstrated that HIV binds to resting CD4+ cells and upregulates L-selectin causing the cells to home from the blood into lymph nodes (LNs) at enhanced rates [114, 115]. This has led to the homing theory, offering an explanation for the loss of CD4+ cells due to cells leaving the blood and entering the LT (reviewed in [116]). Indeed, accumulation of Tregs has been found in secondary lymphatic tissue (SLT) compared to peripheral blood in untreated HIV-infected patients [117, 118], indicating that the conclusion drawn from the reported findings of Tregs in peripheral blood is to be considered with caution.

7. Secondary Lymphatic Tissue and Microbial Translocation

CD4+ cell depletion occurs in the blood as well as in the SLT of LN and gut-associated lymphatic tissue (GALT) where the majority of the CD4+ cells reside. During primary HIV infection a vast number of cells are depleted, reaching a loss of more than 50% in LN as chronic infection is established [119, 120]. It has been proposed that HIV damages the structures in the LT, that help sustain the normal CD4+ cell population replacing the functional space with collagen. Thus, the greater the amount of the collagen deposition, the lower the CD4+ cell count and the smaller the number of naive CD4+ cells [121]. Also, LN biopsies from HIV- and SIV-infected individuals show breakdown of the lymph node architecture and evidence of apoptosis [122]. In contrast, a preserved lymph-node architecture was reported in the history of HIV in non-progressors compared to progressors, indicating that progressors host a preserved SLT [123]. GALT is the main defence against infectious microorganisms in the gastrointestinal (GI) tract and consists largely of T cells. Importantly, the main part of Th17 cells reside in the GALT [124]. Th17 cells are important for the integrity of the gut mucosal

barrier by stimulating epithelial proliferation and inducing a proinflammatory environment by recruiting neutrophils to fight microorganisms. Upon acute HIV infection follows a significant depletion of CD4+ cells in the GALT [125, 126]. The depletion is linked to a damage of the mucosal barrier that may be due to an imbalance of Th17 cells as the massive depletion of CD4+ cells during acute HIV and SIV infection in particular includes Th17 cells [125, 126]. The damage to the mucosal barrier results in microbial translocation (MT)—a continuing leak of microbial remnants from the GI tract that enters the systemic circulation. These microbial products lead to immune activation [111, 127, 128], thereby contributing to HIV progression. The data on mucosal integrity and the influence of MT on immune activation in non-progressors are limited. One study using a rhesus macaque model has shown that spontaneous restoration of mucosal CD4+ cells upon acute SIV infection is predictive of non-progression [129]. Furthermore, EC and VC present with similar preserved numbers of CD4+ cells in rectal biopsies comparable to HIV-negative individuals, while the number in progressors is reported to be diminished [130, 131]. However, the level of lipopolysaccharide (LPS) used as a marker of MT is comparable in controllers and progressors and elevated compared to HIV-negative individuals [73], indicating that low chronic immune activation in non-progressors might have effect in the long-term despite the appearance of the relatively intact mucosal barrier. Thus, present data indicate that non-progressors are distinct from progressors in several aspects of the integrity of the mucosal barrier and MT, suggesting an important mechanism for the capability of non-progressors to control immune activation and HIV infection. However, to determine the causal relationship between MT and control of HIV infection prospective studies are needed.

8. HIV-Specific Immune Responses

HIV-specific CD8+ and CD4+ cells and neutralizing antibodies are considered an important albeit most often insufficient element in suppressing viral replication (reviewed in [132, 133]). Some of the first studies were made of HIV-infected patients with primary infection. Here it was shown that the level of HIV-specific CD8+ cells paralleled the efficiency of control of primary viremia. Also, patients who mounted strong gp160-specific CD8+ cell responses showed rapid reduction of acute plasma viremia, while viremia in patients with low virus specific CD8+ cell activity was poorly controlled [134]. Another study showed that an absent HIV-specific CD8+ cell response during primary HIV infection was associated with prolonged symptoms, persistent viremia, and low CD4+ T-cell count [135]. Furthermore, it has been shown that in vivo depletion of CD8+ cells eliminates the ability to contain SIV replication [136]. For this reason an HIV-specific CD8+ cell immune response is widely accepted as a contributor to control of viral replication and lack of progression in non-progressors, and this has been evaluated in a number of studies. Thus, it has been shown that non-progressors are able to maintain an established CD8+ cell precursor pool and present with a consistent highly functional HIV-specific response, while this ability is lost in progressors [137, 138]. Also, the capacity of virus-specific CD8+ cells to proliferate in response to stimulation with HIV antigens is reported to be preserved only in non-progressors [139]. This is in agreement with findings from a prospective study of an increase in polyfunctionality in HIV-specific CD8+ cell responses from EC, and a decrease in progressors over time [140], and with findings of a stronger and broader cytokine and chemokine response following HIV-specific stimulation of PBMC from EC compared to progressors [70, 77]. In addition, it has been reported that the inhibitory immunoregulatory receptor CTLA-4 is selectively upregulated in HIV-specific CD4+ cells in progressors compared to non-progressors. CTLA-4 expression was also found to be positive associated with disease progression and negatively associated with the capacity of CD4+ cells to produce IL-2 in response to viral antigen [141]. Furthermore, it has been shown that in non-progressors HIV-specific CD8+ T cells efficiently eliminate primary autologous HIV-infected CD4+ cells [142]. Additionally, it seems of importance if the HIV-specific cells are activated or not, as it has been shown that ECs possess lower levels of activated HIV-specific CD8+ cells and of recently divided HIV-specific CD4+ cells than progressors [70]. Based on these data an ideal HIV vaccine would induce strong HIV-specific immune responses and minimize HIV-specific immune activation. Another goal of vaccine development is induction of antibodies that neutralize a broad range of HIV isolates. Although antibodies can be elicited by HIV infection, those that are broadly neutralizing are undetectable in most individuals (reviewed in [143]). The level and the breadth of neutralizing antibodies are reported to correlate to viral load [144, 145], and the same or lower levels of antibodies are reported in controllers compared to progressors [144, 146]. Furthermore, one study showed that no single anti-HIV antibody specificity was a clear correlate of immunity in controllers [146]. This is consistent with neutralizing antibodies as poor contributors to non-progression. Contrary, antibodies directed against autologous Env variants are reported to be present in non-progressors [147], and efficient elicitation of de novo neutralizing antibodies has been shown in SIV controllers [148].

Taken together these findings unanimously indicate that a virus-specific response by CD8+ cells is a contributing factor to non-progression, while the influence of virus-specific CD4+ cells and neutralizing antibodies is more unclear. Interestingly, similar preserved HIV-specific T-cell responses have been demonstrated in a study of controllers and LTNPs while responses were blunted in progressors [149]. This indicates that HIV-specific responses are crucial in sustaining non-progression, regardless the viral replication, consequently playing a role in non-progression in controllers as well as in LTNP. However, these beneficial HIV-specific responses might be a part of the explanation of why HIV is not being eradicated, even in controllers. Thus, it has recently been shown that high HIV-specific responses are associated with high levels of cell-associated HIV DNA levels in controllers [150].

9. Eradication, Latency, and Reservoirs

Despite effective cART complete eradication of HIV seems unlikely, and complete eradication of HIV is so far only obtained once in the Berlin-patient [30]. In general, low-level HIV replication continues despite cART. This is in part due to the capability of HIV to conceal itself and persist in cellular reservoirs. Furthermore, a major impediment to the eradication of HIV is latently infected resting CD4+ cells that are characterized by proviral DNA integration into the host genome; particulary memory CD4+ cells have proven to be a major cellular reservoir for HIV [151]. The major anatomical site for HIV reservoir is SLT including GALT [152–154]. Thus, viral reservoirs are considered a major obstacle to eradicate HIV and considered to be the reason for rebound viraemia during cART interruptions. Notably, the concept of a functional cure has emerged where lifelong control of viral replication is obtained and disease progression is avoided although provirus is detectable. This might be illustrated by the viral control found EC.

Few studies have examined the capability of non progressors to eradicate or reduce HIV reservoirs and latency. However, low proviral DNA in PBMC in EC compared to patients on cART has been demonstrated [155, 156]. One study even reported differences in the level of proviral loads in EC compared to VC, implying that even low viral replication is of importance [17]. Furthermore, autologous viral replication ex vivo was detected in only 2 out of 14 EC compared to 9 out of 10 VCs, suggesting ECs to have a diminished viral reservoir in peripheral CD4+ cell compartment [17]. Furthermore, impaired viral replication in the early phase of HIV seems to be predictive for VC [157]. The anatomical reservoir in GALT has been examined in a small study revealing lower levels of HIV DNA in rectal tissue in LTNP compared to progressors [158]. However, an SIV macaque model demonstrated that colon mucosa and associated lymph nodes are a major SIV reservoir even in controllers [159].

Conclusively, non-progressors and especially EC seem to have diminished cellular reservoirs, whereas the size of the anatomical viral reservoirs in SLT is uncertain. However, the described traffic of CD4+ cells between plasma and SLT and the reduced microbial translocation indicate that non-progressors harbor a lower viral reservoir in SLT than do progressors. Understanding how ECs control and reduce cellular reservoir might provide the basis to elucidate the needs for a functional cure.

10. Clinical Implications

A common feature in non-progressors is preserved immunology. However, reports of loss of the non-progressor status with declining CD4+ cell counts in LTNP have been observed, and these patients may eventually require cART. Likewise, a loss of viral control in controllers is reported. Interestingly, progression and AIDS events in controllers despite low or undetectable viral loads events are reported as well [2, 8, 22, 29, 39, 48, 73, 160]. As a result of this, cART has been suggested to controllers.

In EC viral replication cannot be measured in commercial assays in EC. However, using ultrasensitive assays HIV RNA can be detected in the majority of these patients [161, 162], and it has been shown that loss of CD4+ cells is more common among ECs with low level viremia [162]. Furthermore, low-level viremia is reported to be associated with measurable T cell dysfunction in EC [163]. Finally, is has been shown that blips are associated with a nonfavourable clinical outcome [26]. Taken together, this indicates that even very low levels of viremia have clinical implications and are involved in disease progression. This is supported by findings of higher levels of immune activation in controllers compared to patients on cART [73, 164]. Moreover, it seems plausible that cART would normalize immunological parameters in controllers. This is confirmed by findings of declining immune activation in EC and increasing CD4+ cell counts in EC as well as EC as due to cART initiation [165, 166]. Thus, cART may be considered in progressing controllers despite undetectable viral replication.

11. Conclusion and Future Directions

Rare groups of HIV-infected patients that do not progress to AIDS or death have been known since the beginning of the HIV epidemic. Some of these non-progressors control viral replication, that is, the controllers, while LTNP, have ongoing viral replication. So far, it is not clear why these patients do not progress, but immunological mechanisms have been suggested. The immunological response to HIV infection can be divided into an immune homeostasis resistant to HIV and an immune response leading to viral control. This paper has focused on immunology in non-progressors. We suggest that two different mechanisms are responsible for preserved CD4+ cell counts in controllers and LTNP.

In summary, data unambiguously show that controllers are immunologically different from progressors in production, destruction, and regulation of cells. Thus, controllers have a preserved CD4+ cell production with a bone marrow function, a lymphopoiesis, a thymic output, and an IL-7/IL7-R balance resembling HIV-negative individuals. Furthermore, controllers have lower destruction of CD4+ cells as evidenced by lower microbial translocation, immune activation, and apoptosis. Likewise, the balance between Tregs and Th17 cells is less disturbed and HIV reservoirs seem to be lower compared to progressors. However, non-progression and preserved CD4+ cells counts in controllers may not be entirely surprising since they are characterized by viral control and thus to be compared with HIV-infected patients on treatment. Thus, preserved immune homeostasis may be a reflection of rather than a reason for the viral control. In contrast, high level of HIV-specific immune response in controllers is probably a contributing factor to non-progression in controllers.

The really intriguing question is how non-progression occurs in LTNP where continuous viral replication is evident and ought to result in destruction of CD4+ cells. Unfortunately, the literature of LTNP is limited, often because they are included in study populations of controllers as viral load is not included in the definitions. An extraordinary

ability to produce cells or a lower rate of destruction is expected in these patients according to the preservation of normal CD4+ cell counts. However, increased rate of production has not been shown so far, and the finding of similar levels of IL-7 between LTNP and progressors suggests that a different CD4+ production is not the explanation for non-progression in LTNP. Likewise, evidence of reduced turnover of cells in LTNP has not been found, and in general LTNPs appear to have an immune system very much like progressors. However, findings of elevated levels of Th17 cells have been reported, suggesting that the immune regulation by pro- and anti-inflammatory cells is different in LTNP compared to progressors. Indeed, it would be interesting to further evaluate immunological parameters, includingTh17 cells, Tregs, and microbial translocation in LTNP, ideally in prospective studies in order to clarify cause and effect. Also, it would be of interest to elucidate immunological parameters in former LTNP who have lost their non-progressor status.

Finally, due to distinct immunologically profiles in LTNP and controllers, we suggest that a clear distinction between patients with and without viral replication is made in future studies in order to improve the possibility to understand the different mechanisms for non-progression in these fascinating patients.

Conflict of Interests

The authors have no conflicts to disclose.

Acknowledgments

The authors thank the Novo Nordisk Foundation and University of Copenhagen for financial support.

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Hindawi Publishing Corporation
AIDS Research and Treatment
Volume 2012, Article ID 390406, 7 pages
doi:10.1155/2012/390406

Research Article

Causes of Death in HIV Patients and the Evolution of an AIDS Hospice: 1988–2008

Ann Stewart,[1,2] Soo Chan Carusone,[1,3] Kent To,[4] Nicole Schaefer-McDaniel,[1] Mark Halman,[5,6,7] and Richard Grimes[8]

[1] *Casey House Hospice, Toronto, ON, Canada M4Y 2K8*
[2] *Department of Family & Community Medicine, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 1A8*
[3] *Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada L8S 4K1*
[4] *Black Creek Community Health Centre, Toronto, ON, Canada M3N 3A1*
[5] *Department of Psychiatry, St Michael's Hospital, Toronto, ON, Canada M5B 1W8*
[6] *Centre for Research on Inner City Health, The Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, ON, Canada M5B 1W8*
[7] *Department of Psychiatry, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 1A8*
[8] *Division of General Internal Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA*

Correspondence should be addressed to Ann Stewart, astewart@caseyhouse.on.ca

Received 1 December 2011; Accepted 7 March 2012

Academic Editor: Anders Sonnerborg

This paper reports on the transformation that has occurred in the care of people living with HIV/AIDS in a Toronto Hospice. Casey House opened in the pre-HAART era to care exclusively for people with HIV/AIDS, an incurable disease. At the time, all patients were admitted for palliative care and all deaths were due to AIDS-defining conditions. AIDS-defining malignancies accounted for 22 percent of deaths, mainly, Kaposi sarcoma and lymphoma. In the post-HAART era, AIDS-defining malignancies dropped dramatically and non-AIDS-defining malignancies became a significant cause of death, including liver cancer, lung cancer and gastric cancers. In the post-HAART era, people living with HIV/AIDS served at Casey House have changed considerably, with increasing numbers of patients facing homelessness and mental health issues, including substance use. Casey House offers a picture of the evolving epidemic and provides insight into changes and improvements made in the care of these patients.

1. Introduction

Remarkable changes have taken place since AIDS was first described in 1981. The face and complexity of both living and dying with HIV have evolved significantly over the last three decades. Today, incidence is increasing in new subpopulations, new treatments are becoming available and individuals are living longer on treatment. There are also new and unanticipated complications of this virus which result in changing causes of death. In order to effectively research, plan, and provide the best care possible for people living with HIV/AIDS, it is essential that we understand both trends and the current state of the epidemic today, including differing causes of mortality.

In a classic paper examining deaths of individuals who were diagnosed with AIDS prior to 1986, the one-year mortality rate was 51.2% and the 5-year rate was 84.8%. The principal causes of death were *P. carinii* pneumonia (now referred to as *P. jirovecii* pneumonia) and Kaposi sarcoma in 82% of the cases [1]. By the mid-1990s antiretroviral combination therapy became available. These drugs suppressed HIV replication and, as a result, deaths due to HIV infection were greatly reduced. The number of deaths in HIV-infected persons in the United States dropped from approximately 50,000 in 1995 to 18,000 in 2008 [2]. AIDS deaths captured by the Public Health Agency of Canada, which counts only voluntary reporting of death in previously reported HIV/AIDS cases, significantly underestimates the number of AIDS deaths in Canada but shows a similar decline with 1501 deaths in 1995 and 53 in 2008 [3]. Effective medications also led to the near disappearance of many

familiar manifestations of HIV. In 1994, over 95% of the deaths in HIV-infected persons in San Francisco were HIV related [4]. Tracking changes in death as a result of antiretroviral therapies, the San Francisco study [4] found, between 1994 and 1998, deaths from wasting in HIV infected persons declined from 252 to 44; deaths from cytomegalovirus infection from 274 to 34; from Kaposi sarcoma from 246 to 32; and from *P. carinii* from 187 to 29 [4]. Declines in these conditions, and other HIV-related conditions, have continued. A recent study of 1597 deaths that occurred in 13 cohorts between 1996 and 2006 found that over half of deaths in people with HIV/AIDS were not attributed to diagnoses that have been traditionally related to HIV infection. Of these, 23% were non-AIDS-defining malignancies; 16% were non-AIDS-defining infections; 15% were related to drugs and/or violence; 14% to liver disease [5].

The changes in prognosis and associated diagnoses have resulted in a new burden of care on organizations that serve HIV-infected persons. One type of healthcare organization dramatically affected is the hospice that initially served those dying with HIV. In the early years of the epidemic, individuals arrived at these institutions with a devastated immune system that resulted in multiple HIV-related opportunistic infections and death within months. With the advent of combination antiretroviral therapy and an increased understanding of the clinical and medical management of HIV-related conditions, HIV/AIDS patients are now frequently discharged to return home to their community. However, this population requires a range of healthcare services to manage the extensive medication regimes and complex medical and psychiatric comorbidities, many of which are non-AIDS defining [6–9]. With the decline in the number of deaths in HIV-infected persons, many AIDS hospices have closed, evolved, or expanded to provide rehabilitation care. Thus, the changes in hospice care over the last 30 years provide a bellwether, giving an insight into the transformation that has occurred in HIV care.

As a means of tracing this historical change, we performed a retrospective study to compare the causes of death of those who died in a Toronto hospice, known as Casey House, between 1988 and 2006–2008. These two time-periods were chosen to represent the first year of operation of the hospice, before the development of antiretroviral therapies, and a recent portrayal of patients in a time where highly active antiretroviral therapies (HAART) are widely available in developed countries. A longer time-frame in the post-HAART period was necessary to capture similar numbers of deaths. In another report we have described the demographics and medical, and psychiatric comorbidity of the entire population of patients admitted to the facility in 2008 [10]. We include some of that data here to provide broader context of the morbidity as well as mortality currently experienced by the patients needing care.

2. Methods

2.1. Setting. Casey House was originally established in 1988 as Canada's first hospice for individuals with HIV infection. Today, Casey House is a 13-bed hospital which provides both in-patient and home care services to people living with HIV/AIDS. There are approximately 100 in-patient admissions a year. Individuals with HIV may be admitted for sub-acute rehabilitative care, medical and psychiatric symptom control (including pain management), post-hospitalization support, end-of-life palliative care, or respite care. Care is provided by an inter-professional team including primary and consultant specialist physicians, nurses, social workers, and rehabilitation therapists.

2.2. Data Collection and Analysis. A retrospective review of patient charts and death certificates was undertaken at Casey House, Toronto, Canada. The first set of the analyses focused on patients who had died at Casey House. We defined the first 10 months of Casey House operation as the "pre-HAART" period, thus including records of all patients who had died between the first day Casey House opened its doors, March 1, 1988, and December 31, 1988. In this era, Casey House operated solely as a hospice and patients were admitted only for end-of-life care. We compared characteristics of these pre-HAART patients who died to all Casey House patients who died during a 36-month time period from January 1, 2006 to December 31, 2008. The years 2006–2008 were chosen to represent the current epidemic in an environment where highly-active antiretroviral therapies are widely available. We describe differences in causes of death in the two time-periods. Uneven time-periods were necessary to obtain comparable numbers of deaths in each time.

In addition to focusing on patients who died during their tenure at Casey House, we describe the characteristics of all patients admitted to Casey House during the entire year of 2008 (January 1–December 31) to demonstrate how the facility had evolved as the HIV epidemic, and consequently survival, changed [10].

Clinical and sociodemographic characteristics were collected from patient charts and death certificates. Due to developments in medical knowledge and changes in the scope of practice, not all variables were available or collected for all deaths. Causes of death and types of cancer death were identified and classified using the International Classification of Diseases, the 10th edition. Causes of death were identified as AIDS-defining conditions according to the Centers for Disease Control and Prevention (CDC) [11]. Malignancies were identified as AIDS-defining malignancies (including all malignancies listed by the CDC as an AIDS-defining condition: Kaposi sarcoma, Burkitt lymphoma (or equivalent term), immunoblastic lymphoma, lymphoma (primary of brain), and invasive cervical cancer) or non-AIDS-defining malignancies. Simple descriptive and chi-square statistics were used to analyze the data.

This research project was approved by the Research Ethics Board at St. Michael's Hospital, Toronto, Canada.

3. Results

3.1. Patient Characteristics

Pre-HAART Era. Fifty-nine patients died at Casey House in the 10 months between March 1 and December 31, 1988. All

Table 1: Demographics of patients who died in the pre-HAART and post-HAART periods.

	Pre-HAART (1988) ($n = 59$)	Post-HAART (2006–2008) ($n = 48$)	Statistical difference
Average age at death ($n = 59$; $n = 48$)	39.0 (SD = 9.3; range 23–65)	48.1 (SD = 8.6; range 30–69)	$t(105) = 5.2$, $P < .001$
Gender ($n = 59$; $n = 48$):			$x^2(1) = 6.4$,
Male	59 (100%)	43 (89.6%)	$P = .001$
Female	0	5 (10.4%)	
MSM ($n = 59$; $n = 42$)	58 (98.3%)	34 (80.9%)	$x^2(1) = 9.1$, $P < .01$
Average number of years since HIV/AIDS diagnosis until death ($n = 59$; $n = 48$)	1.5 (SD = .9; range 0–4)	13.5 (SD = 6.5; range 1–25)	$t(47) = 12.6$, $P < .001$
Average number of days in hospital prior to death ($n = 59$; $n = 48$)	31.6 (SD = 29.8; range 1–159)	44.8 (SD = 57.9; range 0–228)	$t(67) = 1.4$, $P = .16$
Has mental illness ($n = 43$; $n = 46$)	16 (37.2%)	31 (67.4%)	$x^2(1) = 9.4$, $P = .002$
Homeless ($n = 38$; $n = 47$)	2 (5.3%)	7 (14.9%)	$x^2(1) = 2.1$, $P = .15$
Current smoker ($n = 56$; $n = 46$)	28 (50.0%)	25 (54.3%)	$x^2(1) = .19$, $P = .66$
Recreational drug use ($n = 56$; $n = 47$)	12 (21.4%)	30 (63.8%)	$x^2(1) = 19.0$, $P < .001$
Current IV drug use ($n = 55$; $n = 47$)	4 (7.3%)	11 (23.4%)	$x^2(1) = 5.7$, $P = .017$

patients were male and all but one individual (98.3%) self-identified as men having sex with men (MSM). Individuals lived an average of 1.5 years (SD = 0.9) with an HIV/AIDS diagnosis before dying. The mean age at death was 39.0 years (SD = 9.3).

Post-HAART Era. Forty-eight patients died at Casey House in the 36 months between January 1, 2006 and December 31, 2008. Forty-three (89.6%) patients who died were male. Thirty-four (80.9%) persons identified MSM. The average age at death was 48.1 years (SD = 8.6) after living an average of 13.5 years (SD = 6.5) since HIV/AIDS diagnosis.

Characteristics of patients who died in the pre- and post-HAART period are summarized in Table 1. In the pre-HAART era 37.2% of patients had mental health issues, this almost doubled in the post-HAART era to 67.4%. In 1988, only 5.3% were homeless, however the number of homeless and socioeconomically marginalized patients increased significantly in the post-HAART era. In terms of substance use, the number of smokers remained relatively constant at approximately 50%. In the pre-HAART era, 21.4% endorsed recreational drug use and 7.3% reported injecting drugs. The self-reported incidence of both behaviours nearly tripled in the post-HAART era to 63.8% and 23.4%, respectively.

3.2. Cause of Death

Pre-HAART Era. The primary cause of death was identified as an AIDS-defining condition in all 59 deaths in 1988. Twenty-two percent ($n = 13$) were due to AIDS-defining malignancies. Forty-two percent ($n = 25$) of deaths were due to AIDS-defining opportunistic infections, most commonly disseminated *Mycobacterium avium* complex disease (MAC) ($n = 5$), toxoplasmosis ($n = 5$), and *Pneumocystis* pneumonia (PCP) ($n = 3$). Thirty-six percent ($n = 21$) of deaths were identified as other AIDS-defining conditions. In 19 of these cases the cause of death was listed only as "AIDS."

Post-HAART Era. Forty-eight percent of deaths in the post-HAART era were attributed to AIDS-defining conditions. Thirty-one percent ($n = 15$) of individuals died of AIDS-defining opportunistic infections; 6% ($n = 3$) from AIDS-defining malignancies; 10% ($n = 5$) from other AIDS-defining conditions. The remaining 25 deaths (52%) were attributed to: non-AIDS-defining malignancies ($n = 13$; 27%); liver disease ($n = 3$; 6%); other causes ($n = 9$; 19%) including multi-organ failure ($n = 3$) and respiratory failure ($n = 2$). Fourteen patients had hepatitis B or C listed as a contributing cause of death.

Figure 1 presents a summary and comparison of the primary causes of death in the two time periods.

3.3. Malignancies. Malignancies accounted for 22% ($n = 13$) of deaths in the pre-HAART era. All of these were AIDS-defining malignancies: 11 were attributed to Kaposi sarcoma and 2 to lymphoma. An additional 4 deaths had Kaposi sarcoma listed as a contributing factor. In the post-HAART era, malignancies accounted for the primary cause of death in 33% ($n = 16$) of deaths: 6% ($n = 3$) were due to AIDS-defining malignancies and 27% ($n = 13$) were due to non-AIDS-defining malignancies. Kaposi sarcoma was not listed as the primary cause of death for any patients in the post-HAART period but was a contributing factor in one death. Figure 2 illustrates the frequency of different types of

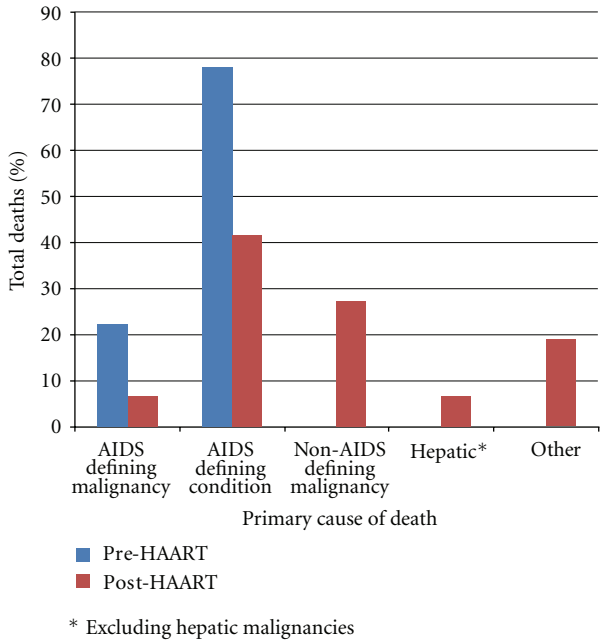

Figure 1: Primary cause of death in individuals who died at Casey House in 1988 (pre-HAART) in comparison to 2006–2008 (post-HAART).

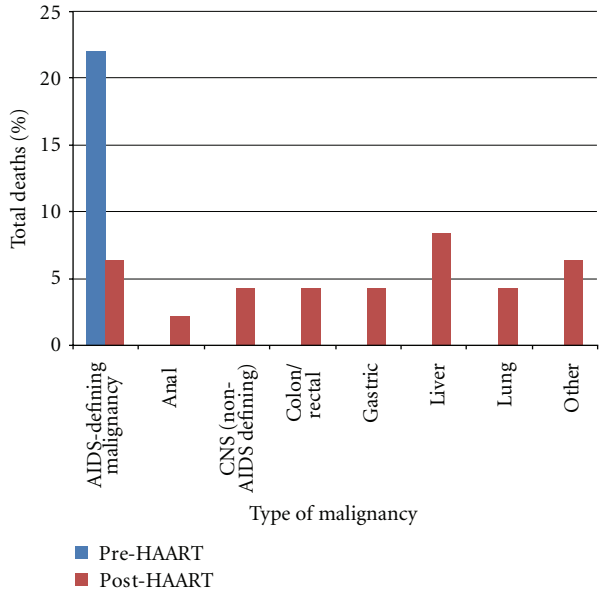

Figure 2: Types of malignancy in individuals who died at Casey House in 1988 (pre-HAART) compared to 2006–2008 (post-HAART).

malignancies present in individuals at the time of death for the two time-points. This figure includes three individuals in the post-HAART period that had a malignancy listed as a contributing cause of death but not as the primary cause of death.

In the post-HAART era, many of the non-AIDS-defining malignancies occurred in patients who were on HAART therapy, with reasonably well-controlled disease. Admission CD4 count was available on ten patients who died from non-AIDS-defining malignancies, of whom 70% had a CD4 count greater than 200 cells/mm^3. CD4 counts ranged from 25–800 cells/mm^3. Of the eight patients who had admitting viral loads, six were undetectable, and the other two had 150 and 400,000 copies/mL.

3.4. HIV/AIDS Patients Admitted in 2008. Between January 1 and December 31, 2008, 87 individuals were admitted to Casey House. Of the 87 individuals who were admitted, 17 (n = 19.5%) died during their stay at Casey House: seventy-percent of whom had a CD4 count below 200 cells/mm^3. Of the individuals who died with decreased immune system function, 25% were homeless and 25% lived in supportive housing. All individuals who died with a CD4 count above 200 cells/mm^3 reported renting their own housing (excluding supportive housing). Table 2 includes descriptive data for all patients admitted in 2008 and compares individuals who died during their stay to those who were discharged. There were no statistically significant differences (at the $P <$ .05 level) between patients who died and those who were discharged, although there were slightly more individuals with a CD4 count below the median in those who died (P = .07). Eighty percent of patients admitted were male. The average age at admission was 48.9 years (SD = 10.5) and the median CD4 count was 150 cells/mm^3. The average number of medical comorbidities experienced by patients at the time of admission was 5.9 (SD = 2.3). The most common being: AIDS-defining opportunistic infections (n = 27; 65.9%); respiratory conditions, such as chronic obstructive pulmonary disease, (n = 16; 39.0%), and non-AIDS-defining malignancies (n = 10; 24.4%).

4. Discussion

Looking at the evolution in rate and cause of death in a hospice, over a twenty-year period, gives important insight into the development of the HIV epidemic. In 1988, HIV infected persons admitted to this facility were suffering from a ravaged immune system that resulted in deaths caused by a spectrum of diagnoses defined as AIDS-related conditions. All admissions were for palliative care. At the time, AIDS was felt to be a hopeless diagnosis. Casey House embraced the caring and compassionate hospice care model—often demedicalizing the treatment of the patients, and as a result specific investigations were not made into the particular cause of death and many certificates simply state this patient died of "AIDS." In contrast, by 2008 less than 20% of patients admitted to Casey House died during their stay. And thus, 80% were discharged, often following an in-patient stay which included investigations and treatment for HIV as well as management of medical comorbidities and mental health issues. This highlights a significant change in the type of care needed by patients and also the lived experience of HIV-positive individuals.

Over the 20-year period examined in this study, there were significant changes in both the primary cause of death and the characteristics of those who died. In 1988, all deaths

Table 2: Patient characteristics for all admissions in 2008.

	All patients($n = 87$)	Patients who died ($n = 17$)	Patients who survived ($n = 70$)	Statistical difference
Average age ($n = 17$; $n = 70$)	48.9 (SD = 10.5)	49.4 (SD = 10.1)	48.8 (SD = 10.7)	$t(85) = .22$, $P = .83$
Gender ($n = 17$; $n = 70$)				
Male	70 (80.5%)	14 (82.3%)	56 (80.0%)	
Female	17 (19.5%)	3 (17.6%)	14 (20.0%)	$x^2(1) = .05$, $P = .83$
Median CD4 at admission ($n = 16$; $n = 67$)	150.0	68.8% below median	43.3% below median	$x^2(1) = 3.4$, $P = .07$
CD4 admission categories ($n = 16$; $n = 67$)				
<200	48 (57.8%)	12 (75.0%)	36 (53.7%)	$x^2(2) = 2.9$, $P = .24$
200–500	24 (28.9%)	2 (12.5%)	22 (32.8%)	
>500	11 (13.2%)	2 (12.5%)	9 (13.4%)	
Average number of comorbidities ($n = 17$; $n = 70$)	5.9 (SD = 2.3)	5.6 (SD = 2.2)	5.9 (SD = 2.3)	$t(85) = .53$, $P = .60$
AIDS-defining malignancy ($n = 17$; $n = 70$)	5 (5.7%)	2 (11.8%)	3 (4.3%)	$x^2(1) = 1.4$, $P = .24$
Non-AIDS defining malignancy ($n = 17$; $n = 70$)	14 (16.1%)	5 (29.4%)	9 (12.9%)	$x^2(1) = 2.8$, $P = .10$
Psychiatric disorder ($n = 17$; $n = 70$)	80 (92.0%)	14 (82.4%)	66 (94.3%)	$x^2(1) = 2.6$, $P = .11$
Cognitively impaired	41 (47.1%)	9 (52.9%)	32 (45.7%)	$x^2(1) = .29$, $P = .59$
Substance use disorder	36 (41.4%)	5 (29.4%)	31 (44.3%)	$x^2(1) = 1.2$, $P = .26$

were attributed to an AIDS-defining condition, while less than half were in the 2006–2008 time period. Malignancies were a common cause of death in both time periods, although the types of malignancy changed considerably. Although the population remained largely male and mostly among men who have sex with men, in the post-HAART period 10% of deaths were in women. Individuals who died in the post-HAART period also reported greater IV drug and recreational drug use and more individuals were identified as homeless and with a mental illness.

Surveillance data and other research reports comparing the pre-HAART and post-HAART era show similar changes in the demographics of the HIV epidemic and causes of death. Although HAART has significantly decreased mortality in HIV-positive individuals, mortality is still significantly higher than in the general population and there are subgroups that are at substantially greater risk such as injection drug users [5]. Studies linking HIV/AIDS databases and cancer registries over approximately the same time period have shown a dramatic decrease in AIDS-defining malignancies and an increase in non-AIDS-defining malignancies [12, 13]. As experienced at Casey House, the most common cancers of the pre-HAART era were Kaposi sarcoma and non-Hodgkin lymphoma. Both are typically linked with low CD4 count and coinfection with viral agents. Although such AIDS-defining malignancies remain prevalent in the HIV/AIDS population in the post-HAART era, numbers of non-AIDS-defining malignancies have increased as much as 20% in the US [14]. Between 2001 and 2005, the most common types of non-AIDS-defining cancers were lung cancer, anal cancer, liver cancer, and Hodgkin lymphoma [13]. This is reflected at Casey House. The Swiss cohort study notes that certain non-AIDS-defining malignancies associated with smoking such as lip, mouth, pharynx, and lung cancers increased in the post-HAART era. In addition, cancers of the liver associated with Hepatitis B and/or Hepatitis C coinfection, as well as human papilloma virus-related cancers, such as anal cancer, were also increased. It was speculated that living longer with disease, combined with partial immune reconstitution, allowed long-latency cancers to progress and manifest [12].

The studies make the point that people living with HIV/AIDS are at greater risk of cancer than the general population, and there is a great deal of speculation as to why this occurs. Increased cancer risk is believed to be a complex interaction, with multiple factors, including impaired immunity, co-infection with biologic agents carcinogenic to humans (such as HPV, EBV, and hepatitis), aging, HAART, and traditional risk factors for cancer, such as smoking and sun exposure [15]. There is some evidence that prolonged immunosuppression—as assessed by CD4 nadir—is independently linked to increased incidence of non-AIDS-defining malignancies [16]. In addition, the presence of HIV is linked to cytokine dysregulation and this may also play a role in increasing cancer risks [15].

Certainly there are complex factors at play in the patients seen at Casey House, who may have as many as 6 medical comorbidities, including coinfection with hepatitis, as well as risk factors such as unstable housing, injection drug-use, and smoking (approximately 50% of our patients smoke in comparison to 21% in individuals over 12 in Canada [17]). Individuals are now living for more than a decade with HIV and although some have maintained CD4 counts above 200,

with suppressed virus, many individuals are not on HAART therapy or do not adhere to their medication regimes. It is interesting to note that patients with reconstituted immune systems remain at risk from cancer [18]. And, as reflected in the broader epidemic, our patients include more marginalized individuals who have significant social determinants of health risk factors such as homelessness and mental illness and this association also places them at risk [6, 7, 19]. In response, we need to adopt effective health promotion models for HIV-infected individuals and the health care response must be comprehensive and collaborative. Research and service initiatives should expand their focus to address medical preventive care including cancer screening and treatment initiatives such as smoking cessation, as well as social and psychosocial needs. These services and supports must be available in various settings to address the spectrum of needs, from case management and chronic symptom management to acute medical interventions and end-of-life care.

We acknowledge that this retrospective study has several important limitations. Data extracted from charts are susceptible to variations in reporting and diagnostic criteria and missing data are common. The changes in reporting practices are amplified in this study where data were collected from time-points 20 years apart in a disease that has been described for only 30 years. In 1988, many primary causes of death were written as "AIDS." It is most likely that these individuals and deaths would have been evaluated and recorded differently today. This may underestimate the number of individuals who died of AIDS-defining malignancies and possibly other non-AIDS-related causes of death. In this study, death certificates and primary cause of death were identified for all individuals but data were incomplete for many clinical and demographic variables. Our ability to clinically describe these patients is also limited by the state-of-knowledge and treatments available in 1988. Many tests routinely issued to HIV-positive individuals today, such as CD4 counts and viral loads, either did not exist or were not widely used in 1988. Hepatitis C was not yet identified [20]. These findings are strengthened however by the complete inclusion of deaths that occurred at a single institution during the two periods of study. The description of all patients admitted in 2008 provides further context to the changes in the epidemic and the health care services provided. However, because Casey House is not an acute care hospital our study will not accurately represent the occurrence of certain types of death such as cardiac arrest, suicide, and trauma. It is also possible that individuals were differentially referred to Casey House in the two time periods as clinical knowledge and stigma of the disease evolved.

5. Conclusion

In summary, our findings are consistent with other studies in identifying changing trends in both the demographics and causes of death in individuals dying from HIV/AIDS. This study focuses on the patients and care provided at an AIDS hospice that evolved with the epidemic into a subacute hospital, giving some perspective to the ensuing changes that have occurred in organizations and the care supporting individuals with HIV/AIDS. As our knowledge and treatments for HIV improve, individuals are living longer but often with substantial psychosocial risk factors and significant medical and psychiatric comorbidities. Fewer HIV-positive individuals are dying from AIDS-defining conditions and increasing numbers of individuals are dying from non-AIDS-defining malignancies and other non-AIDS-defining conditions such as liver disease. The palliative care model of symptom management remains important for these patients. In order to plan and provide the care that is needed, it is crucial that we address both the medical and psychiatric complications and the social and psychosocial needs and risks of living with a chronic disease that has such a devastating and stigmatizing history. And, as long as HIV has no cure, we must also continue to acknowledge and provide care for individuals with HIV that embraces the palliative care model.

Acknowledgments

The authors would like to acknowledge the support of David Lightfoot, Information Specialist, for assistance in literature searches, Sarah Stranks for data abstraction, and Maureen Mahan for reviewing this paper. This publication was made possible with help from the Baylor-UTHouston Center for AIDS Research (CFAR), an NIH funded Program (AI036211).

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