Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis

Mark W. Tenforde,[1,2] Margaret Mokomane,[3] Tshepo Leeme MBBS DTMH,[4] Raju K.K. Patel MBBS DTMH,[5] Nametso Lekwape,[4] Chandapiwa Ramodimoosi,[3] Bonno Dube,[6] Elizabeth A. Williams,[7] Kelebeletse O. Mokobela,[6] Ephraim Tawanana,[8] Tlhagiso Pilatwe,[9] William J. Hurt,[4] Hannah Mitchell,[4] Doreen L. Banda,[4] Hunter Stone,[10] Mooketsi Molefi,[11] Kabelo Mokgacha,[9] Heston Phillips,[12] Paul C. Mullan,[13] Andrew P. Steenhoff,[4,14] Yohana Mashalla,[11] Madisa Mine,[3] Joseph N. Jarvis,[4,11,15,16]

Affiliations

[1] Division of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle, WA USA

[2] Department of Epidemiology, University of Washington School of Public Health, Seattle, WA, USA

[3] Botswana National Health Laboratory, Gaborone, Botswana

[4] Botswana-UPenn Partnership, Gaborone, Botswana

[5] Imperial College London, London, UK

[6] Nyangabwe Referral Hospital, Francistown, Botswana

[7] St. George's University, London, UK

[8] Princess Marina Hospital, Gaborone, Botswana

[9] Botswana Ministry of Health, Gaborone, Botswana

[10] University of Texas Southwestern Medical Center, Dallas, TX USA

[11] University of Botswana, Gaborone, Botswana

[12] UNAIDS, Botswana Country Office

[13] Children's National Health System, Washington, DC USA

[14] Division of Infectious Diseases, Children's Hospital of Philadelphia, Philadelphia, PA USA

[15] Division of Infectious Diseases, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA USA

[16] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Corresponding Author

Mark Tenforde MD MPH

1959 Pacific Street NE

Seattle, WA 98195 USA

Phone: +1 2069406927

Fax: +1 2069445109

E-mail: mark.tenforde@gmail.com

Short summary:

In a Botswana national meningitis survey, we observed a high 2013-2014 incidence of cryptococcal meningitis, particularly in men in their 4th-5th decade, highlighting the need to target populations at high risk for advanced HIV and adopt HIV differentiated care models.

ABSTRACT

Background: Botswana has a well-developed antiretroviral therapy (ART) program which serves as a regional model. With wide ART availability, the burden of advanced HIV and associated opportunistic infections would be expected to decline. We performed a nationwide surveillance study to determine the national incidence of cryptococcal meningitis, and describe characteristics of cases 2000-2014 and temporal trends at two national referral hospitals.

Methods: Cerebrospinal fluid data from all 37 laboratories performing meningitis diagnostics in Botswana were collected 2000-2014 to identify cases of cryptococcal meningitis. Basic demographic and laboratory data were recorded. Complete national data from 2013-2014 were used to calculate national incidence using UNAIDS population estimates. Temporal trends in cases were derived from national referral centers 2004-2014.

Results: 5296 episodes of cryptococcal meningitis were observed in 4702 individuals; 60.6% were male, and median age was 36 years. Overall 2013-2014 incidence was 17.8 cases/100,000 person-years (95%CI 16.6 – 19.2). In the HIV-infected population, incidence was 96.8 cases/100,000 person-years (95%CI 90.0 – 104.0); male predominance was seen across CD4 strata. At national referral hospitals, cases decreased 2007-2009 but stabilized 2010-2014.

Conclusions: Despite excellent ART coverage in Botswana, there is still a substantial burden of advanced HIV, with 2013-2014 incidence of cryptococcal meningitis comparable to pre-ART era rates in South Africa. Our findings suggest a key population of individuals, often men, are developing advanced disease and associated opportunistic infections due to a failure to effectively engage in care, highlighting the need for differentiated care models.

Key words: Cryptococcal meningitis, incidence, HIV, Botswana, sub-Saharan Africa

INTRODUCTION

Botswana was the first country in Africa to establish a national ART program and began providing treatment free-of-charge to its HIV-infected population in early 2002 [1]. National HIV care guidelines in 2012 recommended a CD4 T-cell threshold of <350 cells/μL for ART initiation, up from <200 cells/μL in 2008 [2, 3], and recent population level data suggest that, in rural regions, the program is close to achieving "90-90-90" targets set by the Joint United Nations Programme on HIV/AIDS (UNAIDS) [4]. Despite the success of the national ART program, Botswana, a country of approximately two million people, still has high incidence of new HIV infections in certain populations and an HIV prevalence among the highest in the world, with an estimated adult HIV prevalence of 25% in 2014 [5, 6].

In sub-Saharan Africa as a whole, as in Botswana, although millions now access life-saving antiretroviral therapy (ART), overall HIV prevalence is still not declining and ART is initiated at low CD4 T-cell counts in a majority of HIV-infected individuals [7-9], resulting in a large population at substantial risk of opportunistic disease and with high rates of mortality [10]. Care default and treatment failure further increase the size of this high-risk population, with pooled observational cohort data from African settings suggesting only 65% of patients starting first-line ART remain in care at 36 months and virological failure and acquired ART resistance frequently observed [11-13]. Movement toward an HIV "test and treat" strategy in sub-Saharan Africa, which was adopted in Botswana in June 2016, will necessitate streamlining models of care to quickly expand treatment programs, which has the potential of leaving vulnerable patients further behind.

Cryptococcal meningitis (CM) is a severe fungal infection primarily seen in individuals with defective cell-mediated immunity [14], with the vast majority of cases occurring in the context of advanced HIV infection [15]. CM typically affects individuals with CD4 cell counts <100 cells/μL, and is now frequently described following late ART initiation and ART default, as well as in ART naïve individuals [16-18]. As countries move toward expanded HIV treatment programs, CM serves as an important indicator disease for national programs. An understanding of advanced HIV disease burden and temporal trends in sub-populations will guide program delivery and differentiated models of HIV care targeting groups most at risk for delayed HIV diagnosis, ART initiation, and care default, with the associated morbidity and mortality and high transmission risks.

We collected laboratory records from all facilities in Botswana that perform meningitis diagnostics and UNAIDS country-level HIV prevalence estimates to determine the 2013-2014 national incidence rate of cryptococcal meningitis, describe characteristics of CM cases over a 15-year period, and define temporal trends in cases diagnosed at the two national referral hospitals.

METHODS

Study design

The Botswana National Meningitis Survey was a 15-year retrospective review of routine cerebrospinal fluid (CSF) laboratory records to determine the etiologies of meningitis in Botswana and temporal trends with maturation of the local HIV epidemic. The study was conducted in collaboration with the Botswana Ministry of Health (MoH) and the National Health Laboratory. Institutional review board (IRB) approval was obtained from the University of Botswana, University of Pennsylvania, University of Washington, Health Research Development Council (Botswana Ministry of Health), and at hospitals with independent IRB committees (Letsholathebe Memorial Hospital, Mahalapye Hospital, Nyangabwe Referral Hospital, Princess Marina Hospital, Scottish Livingstone Hospital, and Sekgoma Memorial Hospital).

Participating laboratories and CSF data collection

Thirty-seven laboratories are registered by the Botswana National Health Laboratory to perform cerebrospinal fluid (CSF) testing for the diagnosis of cryptococcal meningitis by India ink stain, fungal culture, and/or cryptococcal antigen (CrAg) testing, including two national referral hospitals, seven district hospitals (six medical, one psychiatric), and 28 primary, private, mining, and military hospital-based, clinic-based, or standalone laboratories. Facilities were visited by the study team for collection of meningitis-related laboratory data. Most visits took place between February 2015 and May 2015. All available CSF records from January 1, 2000 through January 1, 2015 were located at facilities. Records were scanned on a password-protected study laptop, then numbered and entered into a REDCap database. To ensure data accuracy during transcription of paper-based records into the REDCap database, a minimum of two study team members reviewed each entry and discrepancies were adjudicated through consensus.

In addition to paper records, a majority of hospital-based laboratories maintain records on a government-financed electronic medical record (EMR), Integrated Patient Management System (IPMS). At facilities with IPMS, clinical data is entered directly into IPMS with laboratory books used to keep records during internet downtime or entered both in laboratory notebooks and IPMS. IPMS was queried centrally at the MoH to obtain all Botswana CSF-related electronic laboratory records and HIV-related data (CD4 T-cell count nearest to the date of cryptococcal meningitis diagnosis). Paper-based records from the REDCap database were merged with IPMS records using Stata (version 12, College Station, TX), matching by date, testing location, and patient identifiers (name, age, gender), and duplicate entries removed. Three hospitals used additional EMR platforms for limited periods during the study period. Electronic records from these EMR platforms were also queried using CSF-related search terms and this data entered into the REDCap database.

Population denominator data

The UNAIDS Spectrum model version 5.41 was used to determine population numbers [19], national HIV prevalence, new HIV infections, and the distribution of CD4 count. Two module sets were activated for this projection. The demographic set included DEMPROJ – to estimate the population by age and sex, and FAMPLAN – to estimate the impact of family planning services on population size. The HIV set included AIM – to estimate the impact of HIV, GOALS – to estimate the funding needed to achieve the national HIV response targets, and RNM – to estimate the costs of implementing the national HIV program. Model inputs were derived from published country reports, strategic plans, costing studies, programmatic data, and expert opinion [20].

Patients and case definition

Records were obtained of any patient who received a lumbar puncture (LP) for CSF analysis without an age cut-off. In Botswana, routine diagnostic CSF testing for cryptococcal meningitis includes India ink stain and fungal culture. CSF testing for CrAg was performed using latex agglutination tests by several centers and routinely at referral hospital laboratories. The cryptococcal lateral flow assay was not available in Botswana during the study period.

A "case" (or episode) of cryptococcal meningitis was defined as CSF with positive India ink stain, *Cryptococcus* culture, and/or positive CSF CrAg. As CSF analysis may have been repeated on patients receiving multiple LPs for lowering of intracranial pressure or other indications during an admission, all positive CSF samples for a unique patient within a single documented hospitalization (or in the absence of admission dates, within a ≤ 14-day window of any previous CSF sample) were considered part of the same "case."

Data analysis

Data were analyzed using Stata (version 12, College Station, TX). Cases of cryptococcal meningitis in Botswana were enumerated using the case definition. The number of cases, recurrent cases, patient age and gender, and month and season of diagnosis were described using frequencies, percentage or median and interquartile range (IQR), as appropriate. It was not possible to calculate national incidence rates over the full 15-year study period, as records prior to 2013 were partially complete. Complete CSF laboratory data from all laboratories were obtained during 2013 and 2014 calendar years. For this 2013-2014 national data, UNAIDS Spectrum model HIV estimates were used as a population denominator to determine cryptococcal meningitis incidence rate in person-years of observation with 95% confidence intervals (95%CI) derived using a Poisson distribution. Incidence was estimated by gender and ordinal age categories as well as by CD4 T-cell count category stratified by gender in individuals ≥15-years old.

The two national referral hospitals (Princess Marina Hospital and Nyangabwe Referral Hospital) adopted IPMS in 2004 and also maintained paper-based records, yielding a complete dataset for the 11-year period 2004 to 2014. At these two hospitals, annual cases diagnosed over this 11-year period were displayed graphically in relation to the "treatment gap" (UNAIDS-estimated HIV-infected population ≥15 years of age minus population receiving ART) and median CD4 cell count at ART initiation.

RESULTS

15-year cryptococcal meningitis data

We identified 5296 unique cases of cryptococcal meningitis in 4702 individuals from 2000-2014 (**Table 1**). Of these individuals, 90.3% (4248/4702) experienced a single episode, 7.7% (360/4702) two episodes, 1.0% (48/4702) three episodes, and 1.0% (46/4702) four or more episodes of disease. Median age at diagnosis was 36 years (IQR 30-43 years) (**Figure 1**) and more cases were diagnosed in males than females (60.6%

males versus 39.4% females). There was no evidence of seasonality. Case numbers did not differ with respect to month or season at diagnosis.

2013-2014 incidence of cryptococcal meningitis

National incidence of diagnosed cryptococcal meningitis 2013-2014 was 17.8 cases/100,000 person-years (95%CI 16.6 – 19.2 cases/100,000 person-years), with higher incidence observed in males than in females (22.0 cases/100,000 person-years in males [95%CI 20.1 – 24.1 cases/100,000 person-years] versus 13.7 cases/100,000 person-years [95%CI 12.1 – 15.3 cases/100,000 person-years] in females) (**Table 2, Figure 2A-B**). Peak incidence was observed in 40-44 year olds, with an earlier peak among females (30-34 years) and a later peak among males (40-44 years) mirroring national age trends in HIV prevalence.

In the HIV-infected population, overall incidence was 96.8 cases/100,000 person-years (95%CI 90.0 – 104.0 cases/100,000 person-years), or 135.4 cases/100,000 person-years (95%CI 123.4 – 148.3 cases/100,000 person-years) in males and 66.3 cases/100,000 person-years (95%CI 58.9 – 74.4 cases/100,000 person-years) in females. Incidence rate was relatively uniform across adult age categories, peaking in the 4^{th} through 5^{th} decade of life (**Figure 2C**). Although the absolute number of CM cases in children was relatively low, incidence was comparatively high in the two youngest age categories (0-4 years and 5-9 years), declining in the 10-14 year age group, then increasing to adult levels in the adolescent category (15-19 years). Note that the high incidence in the 0-4 year age category may be spuriously high due to an underestimation of the denominator in the

UNAIDS figures and low overall numbers in both the numerator and denominator categories. Incidence increased markedly with declining CD4 T-cell count strata. In those with CD4 T-cell counts <50 cells/μL, a nearly 2% annual incidence was observed, of 1854 cases/100,000 person-years (95%CI 1680.5 – 2042.0 cases/100,000 person-years), with higher incidence in males than females across CD4 count strata (**Figure 3**).

Referral hospital temporal trends

At the two national referral hospital laboratories, processing 60% of CSF samples, the number of cases of cryptococcal meningitis cases decreased between 2004 and 2014, with a marked decrease 2007-2009 but only a modest decline in 2010-2014, despite a shift in national ART guidelines recommending ART initiation at a higher CD4 count (<350 cells/μL in 2012 guidelines versus <200 cells/μL in 2008 guidelines) [2, 3]. **Figure 4A** shows cases of cryptococcal meningitis diagnosed at the referral hospitals, with total HIV-infected population in Botswana ≥15 years of age and population on ART over time. Over this period, the median CD4 count at ART initiation increased from 2007-2011 but remained stable 2012-2014 (**Figure 4B**), with similar trends observed in the total population of HIV-infected individuals with CD4 counts <200 cells/μL (**Figure 4C**).

DISCUSSION

These are the first robust national cryptococcal meningitis incidence estimates from a resource-limited country. Collection of complete records from all laboratories in Botswana performing CSF testing allowed us to accurately estimate 2013-2014 national incidence rates, revealing a high incidence of cryptococcal meningitis in Botswana in the context of the highest population antiretroviral therapy coverage in Africa. The 2013-2014 Botswana national cryptococcal meningitis incidence is almost identical to the pre-ART era (2002-2004) laboratory surveillance-based incidence estimates from Gauteng Province, South Africa (17.8 cases/100,000 person-years versus 15.6 cases/100,000 person-years, respectively), a period when estimated adult (15-49 years) HIV prevalence in South Africa was 21.5% [21, 22]. Limited to only HIV-infected populations, 2013-2014 Botswana incidence of 96.8 cases/100,000 person-years is again comparable to South African incidence estimates from the pre-ART era (95 cases/100,000 person-years), and greater than 100-fold higher than the 2009 estimated United States HIV-associated cryptococcal meningitis rate of 7.7 cases/1,000,000 person-years [15].

The rate of decline in cryptococcal meningitis cases diagnosed at referral center laboratories has stagnated in recent years, despite national data showing high rates of HIV testing, ART uptake, and viral suppression [4]. These findings reflect a number of factors, including high ongoing national HIV prevalence, only modest improvements in median CD4 T-cell counts at ART initiation in recent years, from 191 cells/μL (IQR 115 – 239 cells/μL) in 2010 to 258 cells/μL (IQR 147 – 337 μL) in 2013 despite a shift in national guidelines promoting earlier therapy [1-3, 23], and high annual incidence of new HIV infection estimated at 1.5-2.5% in adults [24]. Importantly, these findings suggest that, despite a decade and a half of free and widely available HIV testing and treatment services in Botswana, a population of vulnerable individuals are not being effectively reached, engaged, or retained by current testing and treatment services. This key population is now the main driver of HIV-related morbidity and mortality, and likely to be maintaining the high ongoing incidence of HIV acquisition in this setting.

With stabilizing or rising adult HIV prevalence in Botswana and the African region, eliminating the remaining treatment gap and advanced HIV-disease will require innovative solutions to effectively reach key populations who are being missed by current testing and treatment models, diagnose and treat early asymptomatic infection, and promote lifelong engagement with treatment services. Updated 2016 guidelines in Botswana, in line with 2015 World Health Organization guidelines, now recommend the "test and treat" strategy of ART at any CD4 count for people living with HIV [25, 26]. This represents a critical step toward reducing advanced HIV disease and opportunistic infections like cryptococcal meningitis and tuberculosis. However, the latest Botswana AIDS Indicator Survey data estimated 30% of the population aged 10-64 years had never undergone HIV testing in 2013 and ART coverage [24], at about 250,000 HIV-infected individuals by 2015 [23], needs to expand by 100,000, which will put a strain on existing services [27]. Similar scale-up will likely be even more disruptive throughout the rest of Africa, where the number of people receiving ART needs to double to cover all HIV-infected individuals [28]. To avoid leaving vulnerable individuals behind, differentiated care models should be considered to streamline care for populations with well-controlled disease and focus more intensive resources on those with higher need who are now driving the epidemic.

Our study provides important insights into the characteristics of individuals presenting with advanced HIV and cryptococcal meningitis in Botswana. We found peak incidence of disease in adults in the 4th and 5th decade of life, suggesting a need to better engage young, working-age adults through work- or community-based care models that minimize lost opportunity costs. Strikingly, we observed a greater than two-fold higher incidence of disease in HIV-infected males than HIV-infected females. Although this did not completely correct when the analysis was stratified by CD4 count, perhaps in part reflecting a true gender-related biological predisposition to cryptococcal disease for which the pathogenesis is incompletely understood [29, 30], it highlights the high numbers of men presenting late to care or failing to engage with testing and treatment services. Young women may also have greater likelihood of HIV diagnosis and routine care with pre-natal, post-natal, and contraceptive services.

This study had several important limitations. First, these estimates likely represent the lowest range of true cryptococcal meningitis incidence rates in Botswana due to the limitations of deriving incidence estimates from laboratory-based surveillance, including missing individuals who died before seeking medical care or who sought care but went undiagnosed due to misdiagnosis, stock out of lumbar puncture equipment or laboratory reagents, or lumbar puncture refusal [31]. Although we applied a rigorous approach to data collection and estimated incidence only for 2013-2014 when we believed laboratory data to be complete, missed case ascertainment may also have led to spuriously low incidence estimates. A small number of individuals may have sought care from neighboring countries, but this is not likely to have significantly impacted our findings.

Over 85% of cases in the IPMS dataset had a Botswana national identification number and the vast majority of the remainder could be identified as citizens using available identifying data. Uncertainty in UNAIDS Spectrum model denominator estimates could lead to either overestimation or underestimation, although figures are based on the most robust and contemporary data available and are validated against reliable national census and HIV population prevalence survey results [24]. Secondly, as a laboratory-based surveillance study we did not have national data on ART treatment history and were unable to stratify incidence based on ART status. However, single-center data from Princess Marina Hospital indicates that 51% of cryptococcal meningitis patients are now presenting on ART [32], in keeping with other regional data [16, 17]. Thirdly, we were unable to ascertain outcomes, although 2012-2014 data from Princess Marina Hospital showed a 10-week cryptococcal meningitis mortality of 50% and 1-year mortality of 65% [33]. The competing risk of high mortality could explain, in part, the relatively low observed relapse rate. Finally, we were unable to evaluate temporal trends in cryptococcal meningitis outside of the two referral hospitals due to incomplete records, which might differ from urban settings where referral centers are located. However, as almost two-thirds of cases were diagnosed at referral centers, this provides meaningful information regarding national trends.

In summary, we provide evidence for a high ongoing burden of advanced HIV disease in Botswana, a country with a mature ART program and the highest population ART coverage in Africa. Our findings highlight a need not only to adopt disease-specific measures to reduce cryptococcal meningitis, such as CrAg screening in patients with advanced disease [34-36], but the broader need to engage and link key populations to care who are being missed with current strategies. Adoption of HIV test and treat could substantially reduce advanced HIV disease but must be coupled with paradigm shifts in testing, care linkage, and ART delivery such as differentiated care models and decentralized testing and HIV care delivery.

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Figures

Figure 1. Age distribution of cryptococcal meningitis cases in Botswana, 2000-2014.

Figure 2. Incidence of cryptococcal meningitis in Botswana by age category, 2013-2014. 2A) Overall incidence and interquartile range; 2B) Incidence by gender (males solid bars and females striped bars); 2C) Incidence in HIV-infected population.

Figure 3. Incidence of cryptococcal meningitis in Botswana in HIV-infected population by CD4 strata, 2013-2014. 3A) Overall incidence; 3B) Incidence by gender (males black lines, females grey lines).

Figure 4. Trends in diagnosed cases of cryptococcal meningitis at the 2 national referral hospitals in Botswana, 2004-2014. 4A) Cases diagnosed at referral hospitals (thick black line) and treatment gap in adults (≥15 years) [shaded grey area]; 4B) UNAIDS estimate of median CD4 count at antiretroviral therapy initiation in Botswana; 4C) UNAID estimate of total number of HIV-infected individuals with CD4 cell counts <200 cells/µL.

Tables

Table 1. Description of cryptococcal meningitis cases in Botswana, 2000-2014.

Total Cryptococcal Meningitis Episodes			5296
Total Cryptococcal Meningitis Patients			4702
	Single episode	4248	90.3%
	Two episodes	360	7.7%
	Three episodes	48	1.0%
	Four or more episodes	46	1.0%

Variable	**Value**	**Number with data**	**Result (N, %)***	
Age****	(Median, IQR)	*4056*	36 years	30 – 43 years
Sex****	Male	*4407*	2670	60.6%
	Female		1737	39.4%
Site†	Tertiary	*5296*	3161	59.7%
	Primary/Secondary		2135	40.3%
Month†	January	*5296*	430	8.1%
	February		442	8.4%
	March		481	9.1%
	April		398	7.5%
	May		432	8.2%
	June		418	7.9%

	July		449	8.4%
	August		490	9.3%
	September		435	8.2%
	October		423	8.0%
	November		456	8.6%
	December		442	8.4%
Season†	Summer (Nov-Jan)	*5296*	1328	25.1%
	Autumn (Feb-Apr)		1321	24.9%
	Winter (May-Jul)		1299	24.5%
	Spring (Aug-Oct)		1348	25.5%

*Unless otherwise indicated

**De-duplicated to represent individual patients rather than cryptococcal meningitis episodes.

†Data from all cryptococcal meningitis episodes including relapses.

IQR = interquartile range

Table 2. National incidence of cryptococcal meningitis in Botswana, 2013-2014.

Strata	Category	Case Number	Person-years	Incidence (per 100,000 PYO)	95% Confidence Interval (per 100,000 PYO)
Overall Incidence					
All	-	755	4,231,095	17.8	16.6 – 19.2
Sex	Male	466	2,115,031	22.0	20.1 – 24.1
	Female	289	2,116,063	13.7	12.1 – 15.3
Age					
(years)	0-4	10	475,589	2.1	1.0 – 3.9
	5-9	4	467,021	0.9	0.2 – 2.2
	10-14	5	458,710	1.1	0.4 – 2.5
	15-19	20	457,334	4.4	2.7 – 6.8
	20-24	37	450,494	8.2	5.8 – 11.3
	25-29	79	424,031	18.6	14.8 – 23.2
	30-34	156	371,214	42.0	35.7 – 49.2
	35-39	138	295,495	46.7	39.2 – 55.2
	40-44	141	212,707	66.3	55.8 – 78.2
	45-49	76	158,636	47.9	37.8 – 60.0
	50-54	40	125,781	31.8	22.7 – 43.3
	55-59	27	107,792	25.1	16.5 – 36.4
	60-64	10	83,350	12.0	5.8 – 22.1
	65-69	4	55,035	7.3	2.0 – 18.6

	70-74	3	42,068	7.1	1.5 – 20.8
	75+	3	45,839	6.5	1.4 – 19.1
HIV infected (Any CD4 Count)					
All	-	755	779,997	96.8	90.0 – 104.0
Sex	Male	466	344,120	135.4	123.4 – 148.3
	Female	289	435,877	66.3	58.9 – 74.4
CD4 cell count <200 cells/µL*					
All	-	690	241,445	285.8	264.9 – 307.9
Sex	Male	432	119,120	362.7	329.3 – 398.5
	Female	259	122,325	211.7	186.7 – 239.2
CD4 cell count <100 cells/µL*					
All	-	590	74,845	788.3	726.0 – 854.6
Sex	Male	370	39,304	941.4	847.9 – 1042.4
	Female	221	35,541	621.8	542.5 – 709.4
CD4 cell count <50 cells/µL*					
All	-	415	22,376	1854.7	1680.5 – 2042.0
Sex	Male	260	11,846	2194.8	1936.1 – 2478.5
	Female	156	10,530	1481.5	1258.1 – 1733.1

*Restricted to individuals aged 15 years old or greater.

PYO = person years of observation

Figure 1

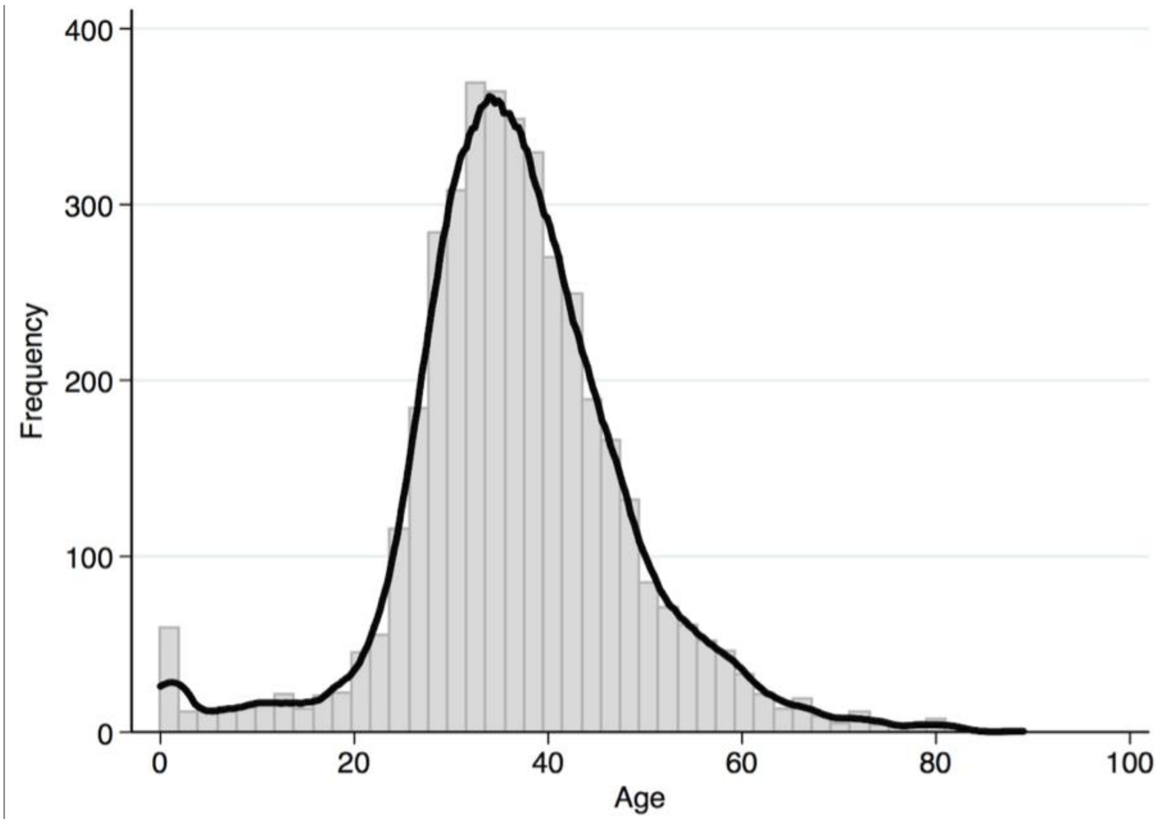


Figure 2

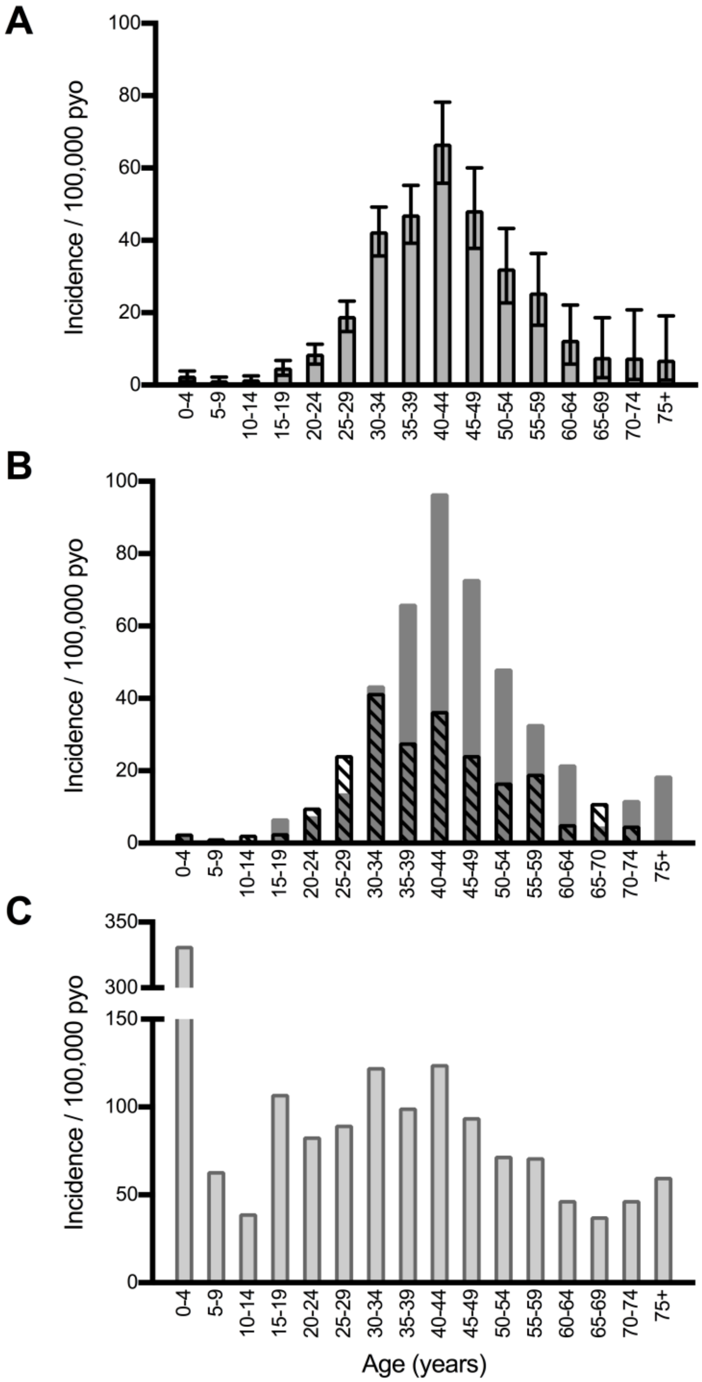

A
B
C
Incidence / 100,000 pyo
0
20
40
60
80
100
50
150
300
350
0-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
65-70
70-74
75+
Age (years)

Figure 3

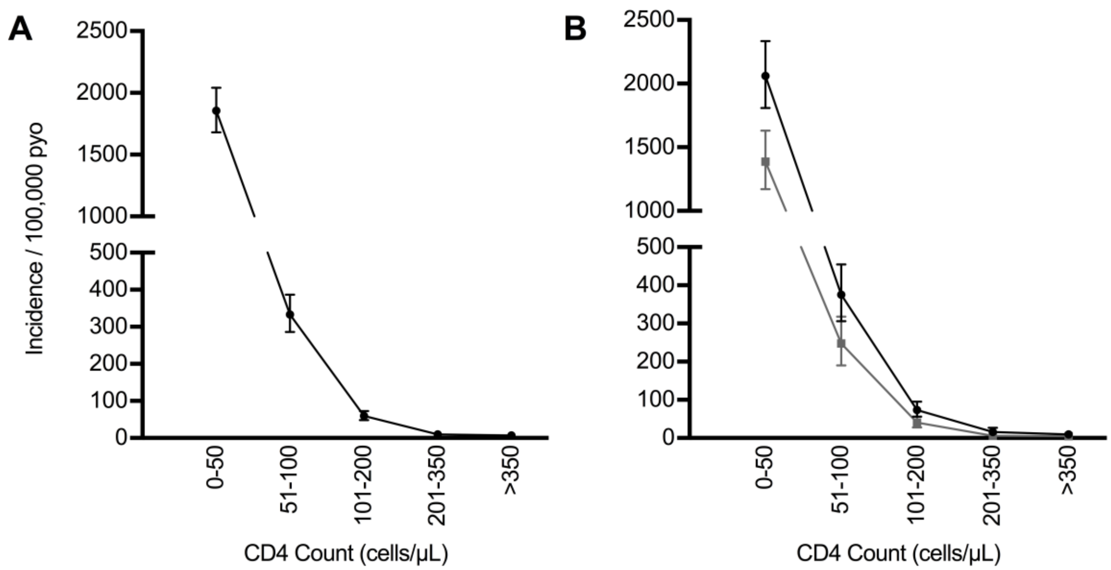
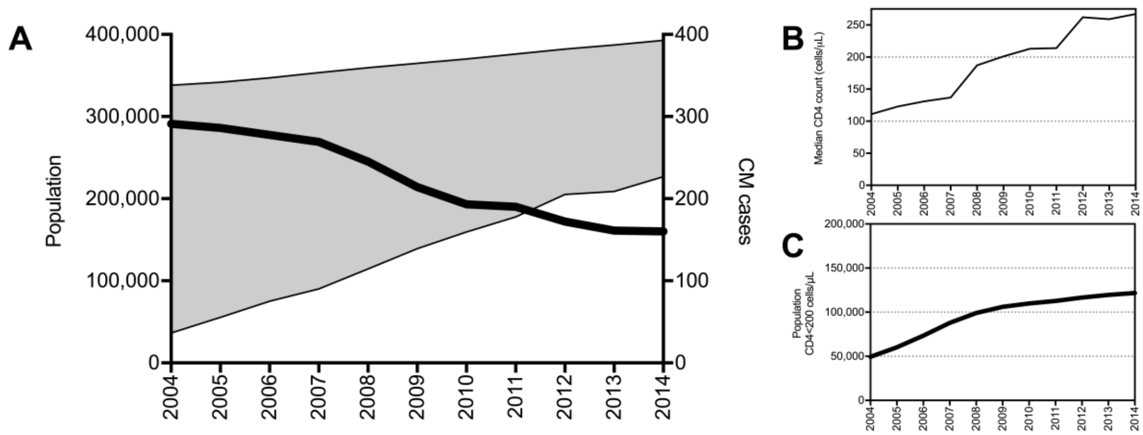

A
B
Incidence / 100,000 pyo
2500
2000
1500
1000
500
400
300
200
100
0
0-50
51-100
101-200
201-350
>350
CD4 Count (cells/µL)

Figure 4


A
Population
CM cases
400,000
300,000
200,000
100,000
0
400
300
200
100
0
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
B
Median CD4 count (cells/µL)
250
200
150
100
50
0
C
Population CD4<200 cells/µL
200,000
150,000
100,000
50,000
0