Direct and Indirect Estimates of HIV-1 Incidence in a High-Prevalence Population

Farley R. Cleghorn, 1-8 Noreen Jack, 2 Jacquelyn R. Murphy, 3 Jeffrey Edwards, 2 Bisram Mahabir, 4 Rosemary Paul, 4 Thomas O’Brien, 1 Michael Greenberg, 6 Kent Weinhold, 6 Courtenay Bartholomew, 5 Ron Brookmeyer, 7 and William A. Blattner 1-8

While the worldwide AIDS epidemic continues to expand, directly measured incidence data are difficult to obtain. Methods to reliably estimate human immunodeficiency virus type 1 (HIV-1) incidence from more easily available data are particularly relevant in those parts of the world where prevalence is rising in heterosexually exposed populations. The authors set out to estimate HIV-1 incidence in a population of heterosexual sexually transmitted disease clinic attendees in Trinidad who had a known high prevalence of HIV-1 subtype B. Over the period 1987–1995, HIV-1 incidence estimates from serial cross-sectional studies of HIV-1 prevalence, passive follow-up of clinic recidivists, modeling of early markers of HIV-1 infection (p24 antigen screening), and a cohort study of seronegative genital ulcer disease cases were compared. Measuring incidence density in the genital ulcer disease cases directly gave the highest estimate, 6.9% per annum. Screening for the detection of early HIV-1 markers yielded an incidence of 5.0% per annum, while estimating incidence from serial cross-sectional prevalence data and clinic recidivists gave estimates of 3.5% and 4.5% per annum, respectively. These results were found to be internally consistent. Indirect estimates of incidence based on prevalence data can give accurate surrogates of true incidence. Within limitations, even crude measures of incidence are robust enough for health planning and evaluation purposes. For planning vaccine efficacy trials, consistent conservative estimates may be used to evaluate populations before targeting them for cohort studies. Am J Epidemiol 1998; 147:834–9.

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Monitoring the acquired immunodeficiency syndrome (AIDS)/human immunodeficiency virus type 1 (HIV-1) epidemic in a defined population is of enor-

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Abbreviations: AIDS, acquired immunodeficiency virus; CI, confidence interval; GUD, genital ulcer disease; HIV-1, human immunodeficiency virus type 1; STD, sexually transmitted disease.

1 The Viral Epidemiology Branch, Division of Cancer Etiology, National Cancer Institute, National Institutes of Health, Bethesda, MD.
2 The Caribbean Epidemiology Centre, Port of Spain, Trinidad.
3 Research Triangle Institute, Epidemiology and Medical Studies Program, Washington, DC.
4 The Ministry of Health, Government of Trinidad and Tobago, Trinidad.
5 The University of the West Indies, Department of Medicine, Port of Spain, Trinidad.
6 Duke University Center for AIDS Research, Raleigh-Durham, NC.
7 Department of Biostatistics, The Johns Hopkins University, Baltimore, MD.
8 Institute of Human Virology, University of Maryland, Baltimore, MD.

Reprint requests to Dr. Farley R. Cleghorn, Division of Epidemiology and Prevention, Institute of Human Virology, University of Maryland, Suite N445, 725 W. Lombard Street, Baltimore, MD 21201.
explosive epidemics, such as is the case in Trinidad and elsewhere (5–7). Modeling represents yet another approach to estimating incidence, and one recently described method that models the prevalence of early markers of HIV-1 infection has been applied in locales with characteristics similar to those of Trinidad, but it requires further validation (8).

Trinidad and Tobago comprise a Caribbean nation with a population of 1.3 million (1990 midyear estimate). Cases of AIDS were first reported there in 1983 (9), and approximately 2,400 cases have been reported through the end of 1996, for a cumulative AIDS incidence rate of approximately 180/100,000 population (10). As in the rest of the world, the most important mode of spread of HIV-1 infection in Trinidad and Tobago is via sexual activity. We have shown that the epidemic there was initially restricted to homosexual/bisexual males whose major risk factor in 1985 was sexual contact with a North-American male (11). Bisexual activity constituted the bridge for the introduction of the virus to the general heterosexual population, where the presence of efficient cofactors for transmission, including high rates of partner exchange and sexually transmitted disease, facilitated rapid HIV-1 dissemination in the most sexually active age groups (12). This increasing prevalence in a large, susceptible, sexually transmitted disease (STD) clinic population in the face of national prevention efforts has mandated that Trinidad join in the global collaboration in preparation for the evaluation of HIV-1 vaccine efficacy and has led to efforts to quantify HIV-1 incidence there (13).

MATERIALS AND METHODS

Description of the STD clinic

This centralized service is located in the capital, Port of Spain, and is the single referral center for STDs and HIV-1 in the socialized national health system. Its clientele is drawn from the entire country (14). We have conducted a series of epidemiologic studies in this clinic population, and over the period during which these data were collected, clinic records documented approximately 6,000–8,000 visits per year, of which about one third attended for a new episode of STD (14).

Cross-sectional serologic surveys to monitor trends in HIV-1 prevalence were conducted at the STD clinic from mid-1987 to mid-1988 and from mid-1990 to mid-1991. All persons who attended the clinic for the evaluation of new STD symptoms during the two study periods were offered enrollment, and written informed consent was obtained for participation. Identical recruitment procedures were used for both surveys, and there was no difference in clinic operation or average annual number of attendees between the two time periods. A total of 2,019 persons were enrolled in 1987/1988, while the 1990/1991 study was terminated at 1,606 participants because of the high prevalence of HIV-1. The overall refusal rates were 20 percent in 1987/1988 and 25 percent in 1990/1991 (methodology detailed in reference 12).

Under a separate protocol to ascertain and follow early HIV-1 infection for pathogenesis studies between June 1, 1993, and May 31, 1995, all clinic attendees were offered tests for HIV-1 p24 antigen and HIV-1 antibodies after signed informed consent. In that period, 12,154 person-visits were screened, representing 93.3 percent of all clinic attendance.

Additionally, to measure incidence directly in the highest-risk subgroup of clinic attendees, a cohort study of 200 consecutively ascertained HIV-1-seronegative cases of genital ulcer disease (GUD) was initiated in December 1993. We have previously documented that GUD was the single most important independent predictor of HIV-1 status among males in a case-control study conducted at the clinic in 1993 (12). All cases were counseled at each visit about HIV-1 prevention, the benefits of reduced sexual exposure, and the use of condoms and were followed on a fixed monthly schedule. At each visit, tests for HIV-1 p24 antigen and antibodies were conducted. The seroconversion point was estimated to be the midpoint between the last seronegative and the first seropositive visit, or the first p24 antigenemic visit.

Laboratory methods

HIV-1 antibody detection was performed at the National Institutes of Health by using two enzyme immunosassays, Cambridge Whole Virus (Cambridge, Massachusetts) and Genetic Systems Whole Virus (Redmond, Washington). Confirmation was performed with an immunoblot (Cambridge-Biotech, Rockville, Maryland). Samples were deemed positive if they exhibited reactivity to at least two of gag, pol, and env gene products using standard Centers for Disease Control and Prevention criteria. HIV-1 p24 antigen was detected by using a core profile enzyme-linked immunosorbent assay (Dupont Biotechnology Systems, Boston, Massachusetts) with and without acid dissociation, and positive samples were confirmed by using a neutralization step.

Methods for computing HIV-1 incidence

Method 1. Using serial prevalence data, annualized incidence rates were calculated as the difference in seroprevalence at the two time points divided by the
time period between the two surveys, which in this case was 3.5 years. Recidivists were counted only once in computing prevalence. Ninety-five percent confidence intervals were computed from the variance using standard methods.

**Method 2.** In this passive follow-up method, we linked unique identifiers from the two cross-sectional serosurveys. The person-time contributions from all of those enrolled in both 1987/1988 and 1990/1991 surveys were computed, and the number of HIV-1 seroconversion events in this group was used as the numerator to compute incidence. This could be described as a retrospective cohort approach to estimate incidence density. Ninety-five percent confidence intervals were computed assuming a Poisson distribution.

**Method 3.** In a prospective cohort study of consecutive, seronegative GUD cases, we quantified HIV-1 incidence density directly, i.e., Incidence = number of HIV-1 seroconversion events /observed follow-up time contributed by enrolled subjects (person-time). Confidence intervals were computed assuming piecewise constant hazards over time, i.e., the risk of infection was assumed to be constant over the time interval.

**Method 4.** This recently described method (8) models incidence by relying on the incidence-prevalence-duration relation in short-duration conditions and is based on the equation \( p = I \times D \), where \( p \) is the proportion of HIV-1-negative individuals who are positive for p24 antigen, \( D \) is the duration of p24 antigenemia prior to antibody seroconversion (estimated at 22.5 days), and \( I \) is the incidence rate. Thus, the incidence rate expressed as an annual percentage is \( p \times (365/D) \times 100 \).

**Method for internal consistency of incidence estimates**

Surveillance conducted at this STD clinic indicates that approximately 20 percent of those who presented for evaluation of a new episode of STD have some form of GUD (14). We have previously documented that GUD was the single most-significant, independent risk factor for HIV-1 in a case-control study conducted at this clinic in 1993, with an unadjusted odds ratio of 6.3 (95 percent confidence interval (CI) 2.7–15.1) for males (12). By using these data, it is possible to generate an estimate of HIV-1 incidence in the overall STD clinic population as follows:

\[
I_N = [p_{\text{GUD}} \times i_{\text{GUD}}] + [(1 - p_{\text{GUD}}) \times (i_{\text{GUD}}/OR_{\text{GUD}})]
\]

where, \( I_N \) = STD clinic population incidence;

\( p_{\text{GUD}} \) = proportion of STD population with GUD;

\( i_{\text{GUD}} \) = HIV-1 incidence in the GUD population; and

\( OR_{\text{GUD}} \) = odds ratio for GUD in HIV-1 infected persons in clinic population.

Confidence intervals for this overall estimate were generated by using the upper and lower bounds for the odds ratio for GUD given above.

**RESULTS**

**Method 1. HIV-1 prevalence by serial cross-sectional surveys (table 1)**

HIV-1 prevalence was 3.0 percent (95 percent CI 2.3–3.9 percent) in 1987/1988 and 13.6 percent (95 percent CI 11.8–15.6 percent) in 1990/1991. This represented a cumulative incidence of 10.6 percent over 3.5 years, annualized to 3.5 percent per year (95 percent CI 2.9–4.1).

**Method 2. Passive follow-up of STD recidivists (table 1)**

A total of 98 (6 percent) of the 1991/1992 survey participants were also enrolled in the 1987/1988 study. They contributed exactly 309 person-years of

![Table 1: Estimates of HIV-1* incidence in STD* clinic attenders in Trinidad, 1987–1997](image)

* HIV-1, human immunodeficiency virus type 1; STD, sexually transmitted diseases; CI, confidence interval; NA, not applicable; GUD, genital ulcer disease.
follow-up and experienced 14 seroconversion events in this person-time. The HIV-1 seroincidence (incidence density) among these STD clinic recidivists was 4.5 per 100 person-years of follow-up (95 percent CI 2.5–7.6).

Method 3. Estimate based on proportion of p24 antigenemic antibody-negative persons (table 2)

Between June 1, 1993, and May 31, 1995, 12,154 persons were screened for HIV-1 p24 antigen and HIV-1 antibodies at the STD clinic. This constituted 93.3 percent of total clinic attendance. HIV-1 prevalence was 6.4 percent overall. There were 158 p24 antigenemic cases (1.3 percent), of whom 111 had a positive Western blot by conventional criteria. Of the remaining 47, 12 did not confirm by the neutralization test, leaving 35 (0.3 percent) cases of primary HIV-1 infection. The estimated incidence as a function of the proportion of individuals who are p24 antigenemic and the mean duration of time to seroconversion (22.5 days; range, 13–42 days) is 5.0 percent per year (95 percent CI 2.7–8.6).

Method 4. Prospective follow-up of high-risk cases of GUD (table 1)

A total of 196 cases of initially HIV-1-seronegative GUD cases were enrolled and followed in this prospective cohort study, with total accrual of 232.5 person-years of follow-up through April 30, 1997. There have been 16 seroconversion events, giving an incidence of 6.9 (95 percent CI 4.0–11.2) per 100 person-years of follow-up.

Internal consistency estimate

With the formula given above, the calculated overall STD clinic population incidence = \{0.2 \times 6.9\} + \{0.8 \times 6.9/6.3\} = 2.3 percent (95 percent CI 1.3–3.7) per annum. The confidence interval for this incidence estimate, which is calculated from the directly measured HIV-1 incidence among cases of GUD, is consistent with the incidence estimates generated from method 1, which is also drawn from the entire clinic population.

DISCUSSION

We have described methods for estimating HIV-1 incidence in an STD clinic in Trinidad and compared the estimates to directly measured incidence density from a cohort study. The resulting picture is one of dynamic HIV-1 incidence in a highly exposed population. While these estimates come from groups with differential risk for HIV-1 within the clinic population, we have presented data to show that they are internally consistent.

Of the methodologies used to determine HIV-1 incidence in potential target populations for HIV-1 efficacy trials, cohort studies provide the most reliable estimates. In a fixed or closed cohort study, subjects are recruited and followed for a given period of time. No new subjects can enter the study after the end of recruitment, although losses may occur as a result of withdrawal, migration, death, or loss to follow-up. The size of the cohort under follow-up decreases with the length of follow-up. Compared with an open cohort, in which enrollment continues throughout a study and new susceptible persons enter the eligible pool, a closed cohort is easier to manage because the recruitment period is short and defined. However, with a closed cohort, HIV-1 incidence may decrease over time as the highest risk subjects are removed from the study as events and the average age of the cohort increase with time. In addition, in cohorts with a low follow-up rate, bias may be introduced since those who return for follow-up have a different risk for infection than those who do not (15). Finally, because the incidence of HIV-1 infection may be low in closed cohorts after extended follow-up, it might be inadvisable to enroll the same subjects in a future vaccine efficacy trial. Both methods use actuarial methods to compute incidence density, which takes into consideration variability in the length of follow-up. Further, the data can be stratified by any number of covariates or calendar time intervals to test for time trends in incidence.

Incidence data are necessary for planning and evaluating national HIV-1 control programs and can also be estimated from serial cross-sectional and modeling studies. Specifically, in the case of HIV-1 infection, the short duration of p24 antigenemia during early infection and the subsequent appearance of antibodies, which are permanent markers of infection, allow the application of statistical modeling computations to

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* HIV-1, human immunodeficiency virus type 1; STD, sexually transmitted diseases; CI, confidence interval.
estimate incidence-duration relation. This method, developed in Baltimore (8), has been applied to the HIV-1 epidemics in India and Thailand to generate incidence estimates (16, 17). These estimates of incidence are subject to a number of caveats, including potential sources of bias. One important caveat is that of applicability to the general population, although this is not relevant to our data since we have attempted to present estimates for the STD clinic population only, focusing on specific higher risk subgroups for directly measured incidence.

However, there are important methodological problems with trying to estimate incidence rates from serial prevalence surveys of HIV-1 antibody (18, 19). One of the main problems is that the population that is surveyed from one time period to the next could be changing because of unknown selection biases. Non-response bias may be relevant in cross-sectional prevalence and other studies. In this respect, refusal rates in our cross-sectional serosurveys were 20 percent in 1987/1988 and 25 percent in 1990/1991. While refusals may have biased the estimate of prevalence in either direction, we have no evidence to suggest that this differed between studies. In addition, there are losses to the population due to HIV-related deaths.

It is noteworthy that a dynamic incidence of infection may occur even in situations where prevalence is relatively stable (20, 21). Indeed, in a steady-state situation where the annual number of new HIV-1 infections is equal to the annual number of new HIV-1 deaths, there would be no change in HIV-1 seroprevalence even though the HIV-1 incidence rate (annual number of new infections) can be quite high. In many developed countries, such as the United States, where the epidemic has matured, HIV-1 seroprevalence rates have remained relatively constant in recent years. It would be incorrect to conclude from that observation that there have been no new infections. Instead, constant HIV-1 prevalence rates are more consistent with the hypothesis that HIV-1 incidence rates are approximately equal to HIV-1 mortality rates. On the other hand, in some developing countries where the epidemic is younger, losses in the population from HIV-1 deaths may be small because of the long incubation period. In such situations, serial prevalence surveys may be useful for estimating HIV-1 incidence rates provided there are no changing selection biases over time in the populations being screened in the prevalence surveys. In the earliest stages of an epidemic, where losses from HIV-1 deaths can be ignored, if incidence rates are growing exponentially, then prevalence rates are as well. It follows under these assumptions that the doubling time of seroprevalence rates is also equal to the doubling time of incidence rates. In this site, the low proportion of recidivists implies that there is a large pool of potential clinic attendees, with long periods between repeat visits. Interestingly, although STD recidivists are usually thought to be at higher risk of HIV-1 infection than one-time attendees, the incidence estimate of 4.5 percent (95 percent CI 2.5–7.6) from this subgroup was found to be similar to that of 3.5 percent (95 percent CI 2.9–4.1) per year for the entire clinic population.

While estimating incidence from prevalence is indeed less costly, a major disadvantage is that no cohort infrastructure is left available for intervention trials. However, this consideration may not be relevant in much of the world, and in any event, such an approach may help to ascertain target populations for cohort-building activities.

STD clinic attendees are members of a subgroup of the general population and have special attributes that preclude generalizability of these findings. Even within this special population, there is differential risk for HIV-1, as evidenced by the higher incidence among those with GUD compared with the entire clinic population. Finally, the internal consistency calculation provided confidence intervals (95 percent CI 1.3–3.7) that overlap those calculated for the entire clinic population (95 percent CI 2.9–4.1), providing additional validation for the methodologies presented.

In conclusion, indirect estimates of incidence may be used for tracking the AIDS epidemic and in planning and evaluating resource distribution. Using these methods to estimate HIV-1 incidence in a developing country setting is both convenient and efficient. For these purposes and in the evaluation of possible sites for HIV-1 vaccine efficacy trials, much time and resources may be saved by adopting indirect measures of incidence. While such approaches do not replace cohort studies to directly measure incidence, they may be used to target suitable populations for cohort studies.

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