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The Effect of Viral Suppression on Cross Sectional Incidence Testing in the Johns Hopkins Hospital Emergency Department

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Abstract

Objective(s)—To determine the effect of viral suppression on cross sectional incidence testing.

Methods—In 2001 and 2003, patients entering the Johns Hopkins Hospital Emergency Department (JHHED) were enrolled into an interview based, identity unlinked serosurvey. All HIV positive samples were tested by the Vironostika-less sensitive (LS) EIA and an avidity assay to determine recent HIV infection. Additionally 16 samples from 8 previously characterized elite suppressors (ES) were tested by cross sectional incidence assays.

Results—HIV prevalence was 12% for the 2001 survey and 11% for the 2003 survey. Of the HIV infected subjects 18% did not know they were infected. Vironostika-LS EIA determined that 6% (11/183) and 7% (17/243) of HIV+ individuals in 2001 and 2003, respectively, were recently infected. Avidity testing confirmed 6 of 11 in 2001, and 5 of 17 in 2003 were newly infected, leaving 17 discrepant samples. All 17 discrepant samples were western blot positive, viral load undetectable and 7/17 had antiretroviral drugs (ARVs) in their serum. Ten individuals were virally suppressed without ARVs, appeared incident by Vironostika-LS EIA but chronic by avidity. These 10 subjects had similar testing profiles to the known 16 ES samples, as 9 of 16 were incident by Vironostika-LS EIA, and 0/16 were incident by avidity.

Conclusions—By removing the viral load negative individuals and by confirming the initial Vironostika-LS EIA results by avidity, the incidence estimate was lowered from 1.73 to 0.94 percent/year in 2001 and from 1.90 to 0.56 percent/year in 2003. Viral suppression affects the performance of the cross sectional incidence tests which rely on antibody titer. In additional 2% (10/426) of all HIV infected individuals who use the JHHED for medical care appear to suppress HIV to undetectable levels without ARVs.

Keywords

Incidence testing; viral suppression; Emergency Department

Introduction

Accurate estimates of HIV-1 incidence are necessary to determine the state of the epidemic, to calibrate and validate epidemic models, and to plan and assess the impact of prevention

interventions. Incidence testing provides a measure of the current level of HIV-1 transmission in a population. Reliable measures of HIV-1 incidence can be obtained through longitudinal cohorts or by serological tests that exploit the difference between newly infected individuals versus chronically infected ones. Samples from recently infected individuals are critical to help define the types of viruses being transmitted, an important component for the development of vaccine strategies to prevent transmission [1].

Longitudinal cohorts are difficult to establish, expensive to maintain and suffer from a 'cohort effect' when determining HIV-1 incidence [2]. To obtain HIV incidence from cross sectional cohorts, methodologies have been developed that take advantage of the biological differences between recently infected and chronically infected individuals [3-11]. In our study, we use two methods based on different aspects of the immune response to HIV infection, the increasing concentration of antibody [4] and increasing binding strength of the antibody antigen complex, known as avidity [7]. A previous study demonstrated that patients receiving highly active anti-retroviral treatment (HAART) have a decrease in anti-HIV antibody titer [12]. In this manuscript we detail how effective viral suppression, either naturally or through medication, can affect the incidence estimation based on antibody titer, and provide an algorithm to obtain better incidence estimates in populations with a mature HIV epidemic receiving anti-retroviral drugs.

Methods

The serosurveys presented in this manuscript occurred during two distinct 7-week periods in the summers of 2001 and 2003 in the Emergency Department (ED) of Johns Hopkins Hospital (JHH), an inner-city university teaching hospital. The ED mainly serves the local socioeconomically disadvantaged minority population. Annual census is approximately 55,000 adult patient visits per year, and 50-60% of patients receive a blood draw during their visit [13,14]. The study was approved by The Johns Hopkins Institutional Review Board (RPN 99-03-12-02).

Using a well defined identity unlinked technique [13], all patient identifiers were removed and excess sera and information from interview or chart review were coded with a patient study number. The coded serum was analyzed at the conclusion of the 7-week sample collection phase of the study. All samples were tested for HIV-1 by Enzyme Linked Immunosorbent Assay (ELISA) (Vironostika HIV-1 microelisa, Organon-Teknika, Charleston, SC) and confirmed by western blot (Calypte, Berkley, CA). Viral load testing was accomplished by Roche Amplicor v1.5 (Roche, Indianapolis, IN). Unrecognized infection was defined as confirmed HIV seropositivity in a subject who gave no acknowledgement of HIV infection or who did not have a documented history of HIV in their chart or electronic medical records.

Incidence testing was performed using Vironostika-Less sensitive (LS) EIA with a standardized optical density (SOD) cut off of 1.0 of the 1:20,000 diluted sera for a window period of 170 days [4]. The avidity assay was performed on all available HIV+ samples, as previously described [7], with the following modifications: Genetic Systems HIV-1/HIV-2 Peptide EIA (Bio-Rad Laboratories, Redmond, WA) was used in place of the Abbott AxSym assay, samples were diluted 1:10, incubated at 4°C for 30 minutes for the initial antibody binding step and then incubated with 0.1M Diethylamine for 30 minutes at 37°C for the chaotropic disassociation step. An avidity index (AI) cutoff of 35% was used for a window period of 170 days (Dr. Michele Owen personal communication). Incidence estimates were calculated using the calculation as previously described (4). Detection of ARVs was performed by The Clinical Pharmacology Laboratory at the University of North Carolina at Chapel Hill in Chapel Hill, NC [15] (<http://cfar.med.unc.edu>). Statistical analysis was performed using

STATA (StataCorp, College Station TX), significance of differences was determined using a student's t-test, and where appropriate, a Fisher's exact test.

A control population of eight chronically infected HIV positive individuals previously identified as elite suppressors (ES), were also tested by Vironostika-LS, and the avidity assay. The ES subjects repeatedly had viral loads below 50 copies/ml without receiving antiviral therapy. The sera tested are from ES individuals 2 to 4 and 6 to 10 are described on Table 1 in a previous publication [16].

Results

Sufficient amounts of excess sera, retrieved from 1549 patients in 2001 and 2544 in 2003, were used for identity-unlinked HIV testing. Basic epidemiologic information did not change significantly between 2001 and 2003 with 48% male, 73% African American and a median age of 46. Interview or chart review information data was available on 31% (57/183) of subjects from the 2001 survey and 58% (141/242) of subjects from the 2003 survey. Patients with unrecognized HIV-1 infection represented 19% (13/70) in 2001 and 18% (25/141) in 2003, for HIV-1 positive patients with interview data. Of those HIV+ subjects interviewed, 42% (24/57) in 2001 and 40% (57/141) in 2003 were on HAART. The median HIV-1 viral load for both the 2001 and 2003 populations surveyed was 1.2×10^4 copies/ml. For ARV treated subjects 45% (36/80) had viral loads below 400 copies/ml for both surveys. The median viral load for individuals with known infection but not on HAART was 4.6×10^4 copies/ml.

Vironostika-LS EIA identified 6% (11/183) of samples from HIV+ subjects in 2001 and 7% (17/242) from HIV+ subjects in 2003 as recently infected with SOD < 1.0. Incidence based on the Vironostika-LS EIA was 1.73 percent/year in 2001 and 1.90 percent/year in 2003. The avidity classified only 3% (6/183) of samples from 2001 and 2% (5/242) of samples from 2003 as recently infected. The incidence calculated based on avidity assay was 0.94 percent/year in 2001 and 0.56 percent/year in 2003. The discordant samples that were recent by Vironostika-LS EIA but chronic by the avidity assay all had viral loads below 400 copies/ml. Approximately 40% (2/5 from 2001 and 5/12 from, 2003) of samples that were classified as recent infections by Vironostika-LS EIA, but chronic infections by the avidity assay had undetectable viral loads and had ARVs present. The median viral load samples determined incident by both Vironostika-LS EIA and avidity was 2.7×10^5 copies/ml, significantly higher than the known positive untreated population ($p < 0.05$).

To determine if the JHHED samples from HIV antibody positive subjects with low antibody titer, strong avidity, and undetectable viral load without the presence of ARVs, could be from elite suppressors (ES) we tested 16 samples from eight known elite suppressors previously identified at JHU by Vironostika-LS EIA, and the avidity assay. All samples were from chronically infected individuals with a median of 12 years after initial diagnosis of HIV infection. A majority (9/16) of ES samples tested as incident by Vironostika-LS EIA, three subjects tested as incident for both time points tested, two subjects tested as chronically infected for both time points and three samples had mixed results. No samples from ES subjects tested incident by the avidity assay (Figure 1.).

Discussion

The HIV prevalence in patients presenting to the Johns Hopkins ED has remained stable at approximately 10% since 1996. It is a mature epidemic where 48% of the HIV positive individuals surveyed in 2001 and 2003 were at least 45 years old. The proportion of infected individuals who know their serostatus has increased over time, from 27% in 1988 [13], 61% in 1992 [12], to 81% in 2001 and 82% in 2003. Of the HIV infected patients presenting to the

JHH ED, 42% were on ARVs, with viral suppression in 46% of these individuals. Of the 28 putative samples from recently infected individuals, 61% (17/28) had undetectable viral loads. Of the virally suppressed individuals who appeared incident by Vironostika-LS EIA, 40% (7/17) were a result of ARV treatment. The other 60% (10/17) appeared to be elite suppressors, as they had undetectable viral loads, had a low antibody titer (SOD <1.0 by Vironostika-LS EIA) that bound efficiently to the HIV antigen (avidity index > 35%) and had no ARVs in their blood. These results imply that 2.3% (10/425) of HIV positive individuals that presented to the JHHED during the early part of this decade were elite suppressors. The consequence of viral suppression on a population level dramatically increased the incidence estimate determined by Vironostika-LS EIA for this population (0.94 to 1.73 percent/year in 2001 and 0.56 to 1.90 percent/year in 2003). As the proportion of virally suppressed HIV positive individuals becomes greater, through the survival of individuals who suppress their own infection naturally or those who do so through the treatment of ARVs, the reliability of cross sectional incidence testing based only on antibody titer will need to be addressed.

This study demonstrates the need for adequate epidemiological data on samples being tested by antibody titer based assays to accurately determine incidence in a population with a mature epidemic and access to ARVs. By removing the viral load-negative individuals that have been confirmed to be chronically infected by avidity testing from the incidence calculation, the incidence estimate was lowered more than 50% in this population. Previously the effect of HIV-1 subtype was demonstrated to affect incidence calculations generated by the Vironostika-LS EIA protocol [17]. Our study demonstrates that populations with HIV infected and virally suppressed individuals can exaggerate the incidence estimate based on antibody titer methodology.

Based on the results, we derived an algorithm for determining samples from recently infected individuals (Figure 2.) Samples are initially tested for the presence of HIV antibodies using standard testing procedures. Antibody negative samples could be tested for the presence of HIV virus using an RNA test to find incident samples [18]. The window period for this phenomenon (HIV RNA positive/Antibody negative) is about 3 weeks, therefore identifying 10 such individuals requires the screening of more than 16,000 persons for a population with an annual incidence of 1%. Using the derived algorithm, HIV antibody positive samples would be tested by Vironostika-LS EIA. New avidity based assays exist that may serve as the initial screen to determine if the HIV+ sample is from a recently infected individual, as these assays seem to have a higher specificity for detecting recently infected samples than antibody titer based assays [19]. Putative incident samples would have their viral load tested. Samples with undetectable viral loads would then be tested for the presence of ARVs, which if present would indicate the specimen is from a subject with viral suppression induced by medication. If no ARVs are found, these samples could be from elite suppressors. Samples from individuals which appear incident by Vironostika-LS EIA and are viral load positive could be from recently infected individuals with a subset being in an advanced stage of immune suppression (approximately 5% when using a SOD of 1.0 in a subtype B infected population) [20]. Differentiating immunosuppressed individuals from recently infected could be made by using the avidity assay since patients with AIDS still have a mature binding avidity, though the actual antibody titer may be low.

Incidence testing assays need to be refined to accurately determine incidence estimates within the population tested. Biomedical research often relies on commercial methods that are subject to market fluctuations. When these methods are removed from the market or are replaced with different components, replacement tests must be found and must undergo rigorous reevaluation. Unfortunately, the Vironostika-LS EIA is no longer a viable option for determining incidence estimates since the Organon-Teknika is no longer producing the Vironostika HIV-1 microelisa. Additionally, the Bio-Rad HIV-1/HIV-2 Peptide EIA has been

replaced with the HIV-1/HIV-2 Peptide EIA + O which suggests that the avidity assay used in this study needs to be validated again.

Changes in commercial assays complicate the ability to accurately estimate HIV incidence because the tools used to determine these estimates need to be recalibrated using serial samples from known HIV seroconverters, and these samples are precious and rare. Vironostika-LS EIA was supported by the CDC who had established quality control procedures for the laboratories that run the assay. Furthermore, Vironostika-LS EIA had been calibrated for use on populations infected with different subtypes [17]. The study presented here proposes an algorithm that could be used when implementing incidence testing in a population with a mature HIV epidemic receiving antiviral treatment. At this time, the only manner to accurately determine HIV-1 incidence around the world is to continue to validate and improve current incidence testing assays.

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References

1. Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, et al. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. *Science* 2004;303:2019–2022. [PubMed: 15044802]
2. Nelson KE, Galai N, Safaeian M, Strathdee SA, Celentano DD, Vlahov D. Temporal trends in the incidence of human immunodeficiency virus infection and risk behavior among injection drug users in Baltimore, Maryland 1988–1998. *American Journal of Epidemiology* 2002;156:641–653. [PubMed: 12244033]
3. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* 1998;280:42–48. [PubMed: 9660362]
4. Rawal BD, Degula A, Lebedeva L, Janssen RS, Hecht FM, Sheppard HW, Busch MP. Development of a new less-sensitive enzyme immunoassay for detection of early HIV-1 infection. *J Acquir Immune Defic Syndr* 2003;33:349–55. [PubMed: 12843746]
5. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Res Hum Retro Viruses* 2002;18:295–367.
6. Schüpbach J, Gebhardt MD, Tomasik Z, Niederhauser C, Yerly S, Bürgisser P, Matter L, Gorgievski M, Dubs R, Schultze D, Steffen I, Andreutti C, Martinetti G, Güntert B, Staub R, Daneel S, Vernazza P. Assessment of recent HIV-1 infection by a line immunoassay for HIV-1/2 confirmation. *PLoS Med* Dec;2007 4(12):e343. [PubMed: 18052604]
7. Suligoi B, Galli C, Massi M, Di Sora F, Sciandra M, Pezzotti P, Recchia O, Montella F, Sinicco A, Rezza G. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. *J. Clin. Microbiol* 2002;40:4015–20. [PubMed: 12409368]
8. Soroka SD, Granade TC, Candal D, Parekh BS. Modification of rapid human immunodeficiency virus (HIV) antibody assay protocols for detecting recent HIV seroconversion. *Clin Diagn Lab Immunol* 2005;12:918–21. [PubMed: 16085908]

9. Wilson KM, Johnson EI, Croom HA, Richards KM, Doughty L, Cunningham PH, Kemp BE, Branson BM, Dax EM. Incidence immunoassay for distinguishing recent from established HIV-1 infection in therapy-naïve populations. *AIDS* 2004;18:2253–9. [PubMed: 15577537]
10. Barin F, Meyer L, Lancar R, Deveau C, Gharib M, Laporte A, Desenclos JC, Costagliola D. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. *J Clin Microbiol* 2005;43:4441–7. [PubMed: 16145089]
11. Li H, Ketema F, Sill AM, Kreisel KM, Cleghorn FR, Constantine NT. A simple and inexpensive particle agglutination test to distinguish recent from established HIV-1 infection. *Int J Infect Dis* 2007;11:459–65. [PubMed: 17369067]
12. Morris MK, Katzenstein DA, Israelski D, Zolopa A, Hendry RM, Hanson CV. Characterization of the HIV-1 specific humoral immune response during highly active antiretroviral therapy (HAART). *J Acquir Immune Defic Syndr* Dec 15;2001 28(5):405–15. [PubMed: 11744827]
13. Kelen GD, DiGiovanna T, Bisson L, Kalainov D, Sivertson KT, Quinn TC. Human immunodeficiency virus infection in emergency department patients. Epidemiology, clinical presentations, and risk to health care workers: the Johns Hopkins experience. *JAMA* 1989;262:516–22. [PubMed: 2739054]
14. Kelen GD, Hexter DA, Hansen KN, Humes R, Vigilance PND, Baskerville M, Quinn TC. Feasibility of an emergency department —based risk-targeted voluntary HIV screening program. *Annals of Emergency Medicine* 1996;27:687–692. [PubMed: 8644953]
15. Commercial testing for the presence of ARVs in sera is available at: The Clinical Pharmacology Laboratory at the University of North Carolina at Chapel Hill in Chapel Hill, NC <http://cfar.med.unc.edu>. The Laboratory for Antiviral Research at The University of Buffalo in Buffalo, NY <http://tdm.buffalo.edu/>., The National Jewish Medical and Research Center in Denver, Colorado <http://www.njc.org/idpl.html#RTFTtoC3>., Consolidated Laboratory Services in Van Nuys, California (818-994-9714); and the Children's National Medical Center in Washington, DC <http://www.cnmc.org>.
16. Bailey JR, Lassen KG, Yang H-C, Quinn TC, Ray SC, Blackson JN, Siliciano RF. Neutralizing antibodies do not mediate suppression of human immunodeficiency virus type 1 in elite suppressors or selection of plasma virus variants in patients on highly active antiretroviral therapy. *J. Virol* 2006;80:4758–70. [PubMed: 16641269]
17. Young CL, Hu DJ, Byers R, Vanichseni S, Young NL, Nelson R, et al. Evaluation of a sensitive/less sensitive testing algorithm using the bioMérieux Vironostika-LS assay for detecting recent HIV-1 subtype B' or E infection in Thailand. *AIDS Res Hum Retroviruses* 2003;19:481–6. [PubMed: 12882657]
18. Pilcher CD, Fiscus SA, Nguyen TQ, Foust E, Wolf L, Williams D, Ashby R, O'Dowd JO, McPherson JT, Stalzer B, Hightow L, Miller WC, Eron JJ Jr, Cohen MS, Leone PA. Detection of acute infections during HIV testing in North Carolina. *N Engl J Med* May 5;2005 352(18):1873–83. [PubMed: 15872202]
19. Martró E, Suligoi B, González V, Bossi V, Esteve A, Mei J, Ausina V. Comparison of the avidity index method and the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion, two methods using a single serum sample for identification of recent HIV infections. *J Clin Microbiol* 2005;43:6197–9. [PubMed: 16333129]
20. Kothe D, Byers RH, Caudill SP, Satten GA, Janssen RS, Hannon WH, Mei JV. Performance characteristics of a new less sensitive HIV-1 enzyme immunoassay for use in estimating HIV seroincidence. *J Acquir Immune Defic Syndr* Aug 15;2003 33(5):625–34. [PubMed: 12902808]

Fig. 1. Results of Vironostika-LS, and avidity testing on samples from chronically infected Elite Suppressors

Results of 16 samples from 8 individuals who control their virus (viral loads below 50 copies/ml without receiving antiviral therapy) 8 tested by **Vironostika-LS** (Panel A), and avidity (Panel B). Samples tested were a median of 10 years after infection and one more than one year apart for each individual tested.

Fig. 2. Algorithm for determining samples from recently infected individuals

Testing procedures are inside a diamond. Positive outcomes are noted as a solid arrow, and negative outcomes with dashed arrows.