Estimates of HIV-1 incidence based on serological methods: a brief methodological review

Estimativas de incidência da infecção pelo HIV-1 baseadas em métodos sorológicos: uma sumária revisão metodológica

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Abstract

The paper reviews the serological methods employed in the estimation of HIV incidence based on cross-sectional studies, as well as the main findings from studies carried out in Brazil that have used such methods. Each method is briefly described, as well as their advantages and limitations. The different methods are also analyzed as a set of complementary but sometimes contradictory strategies under permanent criticism and review, still far from a gold standard. Finally, an additional question — central to the accurate monitoring of the AIDS epidemic using such methods — is discussed: whether the different methods should or should not be adjusted. The debate is open and controversy should be viewed as an unavoidable consequence of a very dynamic research field, informed by the progress in sciences as diverse as epidemiology, biostatistics, mathematical modeling and different branches of basic science, such as immunology, virology, and molecular biology.

HIV-1; HIV Infections; Incidence

Introduction

HIV is one of the most important emergent pathogens in the last century. Around 70 years after its introduction in the human population as a zoonosis from non-human primates, it has become a major public health problem throughout the world. World Health Organization (WHO) estimates point to 33.2 million (30.6-36.1 million) adults and children living with HIV in 2007, roughly 70% of them in Sub-Saharan Africa [1]. Latin America accounts for 1.7 million AIDS cases (1.5-2.1 million; 5%) in this period. In Brazil, 630,000 individuals are estimated to be living with HIV/AIDS. This estimate has been quite stable since the beginning of this century [2].

Although data on HIV seroprevalence are available for all countries in the world, the systematic assessment of incident cases through dedicated reporting systems is still limited to a few countries (e.g. the USA and Germany) that use serological approaches to estimate HIV incidence after repeated population-based studies [3,4]. The estimation of incidence rates at the population level is of paramount importance to assess the dynamics and trends of the HIV epidemic in order to implement adequate preventive measures and to monitor ongoing interventions and the putative impact of treatment at the population level, due to its impact on HIV infectivity and transmission [5]. Moreover, studies aiming to evaluate the immune response and virus

diversity in early infection are of great relevance for vaccine strategies.

The first methodological approaches used to estimate HIV incidence were based on the assessment of seroconversion in cohorts of HIV-seronegative individuals, with the establishment of large cohorts in Western countries, such as the *Multicenter AIDS Cohort Study* (MACS), implemented in different research centers in the US, now celebrating its 25th anniversary [6], the *Vancouver Injection Drug Users Study* (VIDUS) in Vancouver, Canada [7], and the ALIVE study, also comprising injection drug users, and carried out since the late 1980s, in Baltimore, United States [8].

This approach is very expensive and time consuming due to the necessity to follow large cohorts over time. Moreover, behavioral changes can take place during participation in this type of study, as an expected outcome of the adoption of standard of care interventions aiming to prevent HIV acquisition as mandated by ethical requirements of any study with human beings. Such interventions much likely reduce risky behaviors and seroconversion rates over time. Besides such limitations, secondary to the very characteristics of longitudinal studies with human beings, cohort studies also tend to select subgroups of individuals with particular characteristics (such as those more concerned about their health) [9].

In this sense, cohort studies should not be viewed as a gold standard vis-à-vis estimates using biomarkers, but rather as studies subjected to their own specific biases [10]. As such, there is not a gold standard for assessing the validity of new strategies (either biological or epidemiological), and all strategies should be viewed as different attempts (with partial success) to circumvent the limitations and caveats posed by each different method.

In order to overcome such limitations to detect early and recent HIV infections, several methods based on the assessment of biological parameters have been developed over time to detect infection in both pre and post-seroconversion phases.

Methods

This paper summarizes the main methods that have been used to assess HIV seroincidence in cross-sectional studies worldwide. This section aims to be a tutorial for graduate students, clinicians, infectologists, and health managers, as well as researchers working in the fields of epidemiology, immunology, virology, and molecular biology.

The second part of the paper reviews the available evidence from Brazilian studies using such different methods and comprises a comprehensive review of all published studies available in the main bibliographic databases, such as PubMed, SciELO and Scopus, since the very implementation of such methods in Brazil up to September 2009.

Results

Major methods used in the assessment of HIV incidence in cross-sectional studies

Table 1 outlines the major serological approaches used to detect the pre and post HIV seroconversion phases. These approaches, based on biological parameters assessed in the sera of patients can be used in cross-sectional surveys as they are able to differentiate recent from long-term HIV-1 infections. In this section we will briefly describe their major characteristics and applications.

• **Pre-seroconversion**

During the immunological window, i.e., between infection and seroconversion, it is possible to detect HIV RNA, DNA and p24 antigens in absence of HIV antibodies [11] as follows: p24 antigen and DNA PCR, 26.4 days (95% confidence interval – 95%CI: 12.6-38.7); and RNA PCR, 31.0 days (95%CI: 16.7-45.3). Further analyses with highly sensitive techniques reduced the time of RNA and p24 detection after HIV-1 infection (Table 1) to periods as short as 6 and 12 days, respectively [12].

Early in 1995, p24 antigenemia and HIV antibodies were used as a two-step algorithm to detect the prevalence of p24 in absence of anti-HIV antibodies [13]. The HIV incidence rate was then calculated by using the classical relation between prevalence, incidence, and the duration of the period between the onset of p24 antigen detection and the first HIV antibodies.

The short window period for seroconversion is a limitation for the application of methods detecting HIV RNA or p24 antigen for the estimation of HIV incidence. Indeed, a very large number of HIV seronegative individuals should be tested to have the chance of identifying a few cases of seroconversion in order to get an accurate estimation of HIV incidence at the population level. The application of these methods to estimate HIV incidence is very limited in the literature.

Notwithstanding, the original insight from the American statistician Ron Brookmeyer to use two biomarkers emerging in different moments

Table 1

Major methods used in the estimation of HIV incidence based on cross-sectional surveys.

Timing	Serological assay	Test for	Interval to	Window period (days)
Pre-seroconversion	HIV-RNA	Plasma RNA	Detection	5.6 [12]
	HIV p24	Plasma p24	Detection	12 [12]
Seroconvertion	LS-EIA	HIV Ab titer	>> titer cutoff	130-170
	BED-CEIA	HIV Ab/total IgG	>> HIV Ab proportion	153
	Avidity	HIV Ab Avidity	>> Avidity cutoff	125-142 [34]
	IgG3 isotype	Anti-p24 IgG3 isotype	Undetectable anti-p24 IgG3 isotype	80
	IDE-V3	Ratio ODsample /ODcontrol	>> ratio	180 [38]
	INNO-LIA	Immunoblot	>> intensity of reaction	Not established [41]

Adapted from Parekh & McDougal [16], including additional data from Bush et al. [12], Chawla et al. [34], Barrin et al. [38] and Schüpbach et al. [41].

Ab: antibodies; BED-CEIA [BED (HIV-1 subtype gp41 antigens)]: capture enzyme immunoassay; IDE-V3: imunodominant epitope of the V3 region; INNO-LIA: line immunoassay from innogenetics; LS-EIA: less sensitive enzyme immunoassay; >>: should be read here as a symbol for "increased reaction".

of the HIV infection over time paved the way for all subsequent assessments of HIV incidence based on cross-sectional studies, always profiting from the fact that incidence can be approximately deduced from available data on prevalence and the duration of a given infection (or non-transmissible medical condition). Besides the technical difficulties secondary to the short window period, one must also observe that the very nature of the statistical procedure imposes limitations on the accuracy of the estimates. As shown by Miettinen [14], the actual relation involves the prevalence odds instead of prevalence itself and the estimates have as a basic assumption the stability of the population under analysis, which is far from real in most cases (such issues are discussed in some detail in a former paper) [15].

• **Post-seroconversion**

In order to improve the serological estimates of HIV incidence, serological methods able to discriminate recent from long-term seroconversion with HIV, denominated as "serological testing algorithm for recent HIV seroconversion" (STARHS) have been developed over time, as reviewed by Parekh & McDougal [16] and Murphy & Parry [17].

While in the pre-seroconversion phase the methods are based on the detection of p24 antigen and/or viral RNA, in the post HIV seroconversion phase the STARHS are based on differences on anti-HIV antibody concentration, proportion, affinity, avidity and immunoglobulin isotype, which are parameters associated to the matura-

tion of the immune response to target antigens (Table 1). Although developed for distinguishing recent from long-term HIV infection, in general the STARHS are not considered sensitive enough to be used at the level of individual cases. Their major applications rely on population-based studies, for estimating the rate of acquisition of new infection, or incidence rate based on cross-sectional serosurveys [17].

Detuned assay

The first serological strategy used to identify recent seroconversion in a cross-sectional population, developed by Janssen et al. [18], was based on a sensitive/less sensitive testing algorithm using commercial ELISA assays (S/LS EIA). This approach, also known as detuned assay, was based on the differences on HIV-antibody concentrations between recent and long-term HIV infection. For this purpose, the firstly developed detuned assay was a modification of the commercial HIV-1 antibody assay 3A11 (Abbott), increasing the serum dilution (1:20.000) and shortening incubation times to render it less-sensitive (3A11-LS) in detecting HIV-1 antibodies. HIV-positive individuals with low titer antibodies to HIV antigens, as seen in early infections, gave results below a pre-determined threshold cutoff in the LS assay, but positive in the S assay, and were considered as recent infections. The window period was defined based on serial samples from known seroconverters, and was found to be 129 days (95%CI: 109-149 days). Due to the nature of this method, an internal calibrator and additional control specimens, provided by

the Centers for Disease Control and Prevention (CDC), were requested to determine the cut-off and to monitor the assay performance [16]. Differences on the window period between HIV-1 subtype infections were however detected in two studies conducted in Thailand, comparing Thai subtype B (B') and CRF01_AE infected individuals. These differences were also detected between the two commercial kits adapted for the S/LS detuned assay. Thus, a window period of 155 days (95%CI: 128-189 days) was found for subtype B using the HIV-1 antibody assay 3A11, but it was of 270 days (95%CI: 187-349 days) for CRF01_AE specimens [19]. Similarly, Vironostika-LS EIA results showed a mean window period of 239 days (95%CI: 208-287 days) for subtype B' and 356 days (95%CI: 318-402 days) for CRF01_AE samples from Thailand [20]. These results demonstrate that both methods have different performances in detecting recent seroconversion between different HIV-1 subtypes. Although this method was applied in several contexts and populations [16,17], limitations in terms of estimates for different HIV subtypes, as well as the availability of commercial kits are limiting issues.

• **BED-CEIA**

A new approach to detect HIV-1 recent infection was developed by Parekh et al. [21] and used a branched peptide containing the gp41 immunodominant sequences from HIV-1 subtypes B, E (CRF01_AE), and D, in a competitive capture EIA, denominated BED-capture enzyme immunoassay (BED-CEIA). This assay is based on the gradual increase on the proportion of anti-HIV-1 specific IgG in the total IgG, as observed along the maturation of the immune response to a given antigen. In order to define the cut-off to distinguish recent from long-term infections, the BED-CEIA normalized optical density (OD-n) was calculated (ODspecimen/ODcalibrator), using a calibrator specimen, to 1.0 OD-n for a seroconversion period of 160 days. Separate analysis of B and E subtype specimens yielded the same optimal OD-n threshold and similar seroconversion periods and the assay was further validated in African specimens [21]. Variations in the window period have been observed for some subtypes (mean of 153 days for subtypes A to E), however if these differences are due to the assay or other confounding factors related to the population structure and other characteristics of the local epidemics, those remain to be determined [16].

This assay has been largely used in multiple cross-sectional studies to identify recent infections and to estimate HIV incidence in differ-

ent countries and epidemiological scenarios [22,23,24,25,26]. Indeed, this method was applied in a national study to estimate HIV incidence in the US [3].

Taking together, these studies provide trends of incidence and identify associated risk factors, which may contribute to establish preventive measures to control HIV-1 infection. The BED-CEIA is commercially available (Calypte Biomedical, US) and the CDC provides periodic proficiency panels to assess the performance of this technique in the US and international laboratories for both sera and dried blood spots (DBS). Based on some evidence that the BED-CEIA approach misclassifies as recent a substantial minority of long-term infections and consequently leads to overestimation of the incidence rates, the UNAIDS (The United Nations Joint Programme on HIV/AIDS) Reference Group [27] recommended that the BED-assay should not be used for routine surveillance applications, nor for absolute incidence estimates, nor monitoring trends. Among other studies, an unexpectedly high incidence of 6.1%/year 95%CI: 4.2-8.0 was observed in Masaka and 6.0%/year (95%CI: 4.3-7.7) in Kakira, Uganda, while prospective incidence data in Masaka from the same population were found to be 1.7%/year before and 1.4%/year after the study [28]. In order to improve the accuracy of this method, different types of adjustments have been suggested and will be discussed further in this article [29,30].

• **Avidity index method**

Increases in the antibody avidity are observed along the maturation of the immune response to a given antigen. Differences in the IgG avidity to HIV-1 antigens have been described as a useful tool to discriminate recent from long-term HIV-1 infections [31,32,33]. In order to measure the differences on IgG avidity, a commercial method used for HIV diagnostic (AxSYM HIV1/2gO test; Abbott) was modified and the serum samples were tested in parallel diluted in phosphate-buffered saline and in 1 M guanidine. A low avidity (less than 50%) was found in recently infected patients (less than 6 months), compared to long-term infected patients (more than 12 months), who had a higher avidity (80 to 100%) [31]. The avidity index [AI], determined as the ratio of the sample/cut-off value for the guanidine aliquot to that of the phosphate-buffered saline aliquot, was calculated based on serum samples with well documented dates of HIV infection, showed that the mean AIs were 0.68±0.16, within 6 months of infection, and 0.98±0.10 after 6 months. An AI of < 0.90 correctly identified 88.2% of recent infec-

tions, but misclassified as recent infections 13.2% of serum samples collected afterward [33]. Avidity indices of ≤ 0.75 and ≤ 0.80 consistently identified seroconversion within 125 (95%CI: 85-164) and 142 (95%CI, 101-183) days, respectively [34]. AIDS-defining conditions were also found to interfere with the accuracy of this method, as previously described for the detuned and BED-CEIA assays. However, low CD4 counts and viral load, as well as antiretroviral treatment, had no effect in the AI [32]. Comparative studies of the avidity assay with detuned or BED-CEIA showed similar performances for the three assays to identify recent infections in a region where subtype B predominates [35]. More recently, evaluating known seroconversion rates of HIV-seropositive samples from Uganda, where subtypes D and A predominate, the mean AI found for patients with recent infection was 0.55±0.21, while AI of 0.93±0.14 was detected for those from long-term infections [36]. In this study, a sensitivity of 85.2% and a specificity of 85.4% for a cut-off of 0.80 were observed, with no association between HIV subtypes and misclassification, suggesting that this method can be used in field studies in both B and non-B endemic regions.

• **Immunoglobulin isotype changing**

Based on the analysis of serial HIV-1 seropositive samples with known seroconversion dates by isotype-specific western blot it was possible to identify differences on antigen reactivity according to the IgG isotype [37]. While IgG1 was directed toward a range of viral proteins, IgG3 was basically directed to p17 and p24 antigens and, as measured by densitometry, the intensity of the IgG3 anti-p24 response was highest early after infection (days 58 and 86 post-infection), declining thereafter. This observation led to the isolation of p24 protein and further standardization of an in house IgG3-specific anti-p24 enzyme-linked immunoabsorbent assay able to discriminate recent (1-4 months) from long-term HIV-1 infection. As of our knowledge, no application of this method to estimate HIV-1 incidence in the field has been published so far.

• **gp41 IDE – gp120V3 assay**

In order to overcome issues related to the availability and utilization of commercial kits, an in-house indirect enzyme-linked immunosorbent assay was developed to quantify antibodies toward four HIV-1 antigens: consensus peptides of the immunodominant epitope of gp41 (IDE), consensus V3 peptides, recombinant integrase, and recombinant p24 [38]. Among them, the IDE and V3 peptides were selected due to their capacity for discriminating recent (< 6 months) from long-term infections. The results are expressed as a ratio of the optical density (OD) sample/OD negative control sera, and the median ratios for antibodies to IDE and V3 were shown to be more than 20-fold higher for sera from chronically infected patients than for sera from recently infected patients [38]. The IDE antigen corresponded to two consensus oligopeptides of 30 amino acids, one HIV-1 group M and one from subtype D, while a mixture of five V3 peptides representing consensus sequences of clades A, B, C, D, and CRF01-AE. Combined quantification of antibody binding to these antigens led to the identification of recent infection with high sensitivity (88.3%) and specificity (97.6%), when analyzing 971 serum samples from HIV-1 positive untreated patients in France [38]. This assay was also validated for dried blood spots, which improves its utility for population studies. Indeed, it has been used to estimate the incidence rate of HIV-1 infection in France [39]. However, in a study conducted in Ivory Coast [40], although the IDE-V3 technique had a specificity of 96.3% for HIV recent infections, a sensitivity of around 40% was observed with this method, which would suggest that differences on HIV-1 subtype infections could compromise the accuracy of this method.

• **HIVI/II confirmatory line immunoassay**

More recently, the ability of an HIV confirmatory line immunoassay (INNO-LIA HIVI/II) was tested to distinguish recent from long-term HIV infection [41]. The authors evaluated the performance of various algorithms for detecting recent infections, based on reactivity, and classified them into one of six possible intensity scores of reaction to five HIV antigens measured in the western blot. They analyzed 356 serum samples, with well documented recent and long-term infections to define the sensitivity and specificity of the proposed algorithms as compared to the HIV-1 BED-CEIA. In general, the specificity of these INNO-LIA algorithms was above 90%, and higher than BED-CEIA in the study (80.1%), but the sensitivity, even after several adjustments, was not satisfactory. According to the authors, recency information could be extracted from INNO-LIA based confirmatory assay at no extra cost. However, in general this confirmatory procedure is not available in developing countries due to its high cost. No additional papers using this approach have been made available so far, making its application to the estimation of HIV incidence still tentative.

Application of serological methods to estimate HIV-1 incidence in Brazil

The above discussed serological assays delineated to detect recent HIV-1 infections have been implemented in Brazil since the end of the 1990s as new tools to estimate the incidence rates of HIV-1 in different scenarios. However, no national data about the incidence of HIV-1 infection have so far been made available. The studies were mainly conducted in the Southeastern region of the country and employed the serologic testing algorithm for detecting recent HIV seroconversion (STARHS), previously denominated sensitive/less sensitive assay. Using this approach, an estimated HIV seroincidence of 1.9 (95%CI: 0.9%-3.9%) and 2.8 (95%CI: 1.4%-5.3%) per 100 person-years among high-risk heterosexual women and men seen at an HIV testing site in Rio de Janeiro in 1998 [42]. A few years later [43], an update of the former analysis found similar results, with an estimated HIV incidence among heterosexual men (1.62%; 95%CI: 0.61-3.52) and women (1.5%; 95%CI: 0.45-3.69), screened in 2002. However, a higher incidence rate, of almost seven fold, was observed for homosexual men, with a seroincidence of 10.89% (95%CI: 4.28-24.81) [43]. A non-significant increase of recent HIV-1 infections (0% *vs.* 0.8%) was observed among injection drug users (IDU) and ex-IDU [44,45] surveyed in 1994-96 and 1999-2001, in Rio de Janeiro. Similar studies were conducted in Sao Paulo, estimating the incidence of HIV-1 infection in inmates [46], cocaine users [47], and among people seeking HIV diagnosis in voluntary centers for counseling and testing from Santos (São Paulo State) [48] and Sao Paulo city [49,50]. In the former study, sera collected between 1996 and 1999 in Santos yielded an overall estimated HIV incidence of 2% (95%CI: 1.1-3.5), with 1.2% (95%CI: 0.5-2.6) in women and 2.7% (95%CI: 1.3-5.0) in men, quite similar to the results obtained in a voluntary center for counseling and testing in Rio de Janeiro for the same period [42]. The incidence of HIV-1 infection based on the STARHS has already been assessed in pregnant women from São Paulo [51], Rio de Janeiro and Porto Alegre (Rio Grande do Sul State). In the former, a cross-sectional study including samples collected from 1999 to 2002 in São Paulo had an incidence of HIV-1 infection of 0.2 per 100 pregnant women per year (95%CI: 0.041-0.608). Similar results were obtained by our group for pregnant women from Rio de Janeiro (0.41%; IC95%: 0.11-0.72), diagnosed for HIV infection at delivery in the same time period, whereas HIV incidence of 1.93% (IC95%: 0.88-2.97) was observed in southern Brazil (Morgado MG, unpublished data).

Incidence of HIV-1 infection was also estimated among blood donors from São Paulo [52], Brazil (2.7 per 10,000 person-years), in a study comparing first time community and replacement blood donors from 1995 and 2001. Moreover, around 21% of potential recent infections were detected using BED-CEIA among HIV-1 seropositive discharged blood units from Brazilian blood donors [53].

More recently, BED-CEIA has been employed in Brazil for estimating HIV incidence and to assess the molecular characterization of newly identified HIV-1 infections in people seeking HIV diagnosis in voluntary centers for counseling and testing in Rio de Janeiro [54], and Curitiba (Paraná State) [55], and in a Public Health Laboratory serving communities with high prevalence of HIV infection in Santa Catarina State (Camboriú and Itajaí) [56]. Indeed, a preponderance of subtype C was detected among males with recent infection in Curitiba, and the overall incidence in this study was of 2.86 persons/years (95%CI: 1.04-4.68). Similar results were obtained for the two Santa Catarina cities, that presented an overall incidence estimate of 2.6 persons/year (95%CI: ±0.8) [56], in a virological scenario where subtype C predominates. Considering the WHO criticisms [30] about the sensitivity and specificity of the BED-CEIA to estimate HIV incidence in epidemiological scenarios including non-B HIV-1 subtypes, in a preliminary study we proposed a criterion based on the matching results of two assays (BED-CEIA and Avidity index) aiming to improve the accuracy of these serological approaches to estimate HIV incidence rates [57].

One major limitation of most Brazilian studies is secondary to the small sample size (and then large confidence intervals and less accurate estimates), as well as some degree of heterogeneity in some studies, such as the ones carried out by our research group on both active and former injection drug users (as stated in the original protocol, from WHO, but not necessarily defining a homogeneous sample) [44,45].

Final remarks

Incidence estimates by serological assays using cross-sectional surveys are very attractive as such studies are much cheaper than longitudinal studies, much simpler to be implemented and less subject to some of the usual bias affecting longitudinal studies. Such biases comprise the decrease of both risk behaviors and seroincidence over time as a consequence of the full implementation of state-of-the-art interventions aiming to prevent new infections (as mandated

by ethical requirements in any study with human beings) and the loss for follow-up (usually a differential loss), affecting the most vulnerable and/or those most likely to be infected vis-à-vis those who tend to remain in the cohort over time, who are more likely to be concerned with their health and to adopt safer behaviors, as discussed by Harrison et al. [9].

However, several drawbacks may compromise the accuracy of such biomarkers as tools for estimating HIV incidence in cross-sectional studies. In general, false positive and or false negative results have been described for all the available methods, biasing, in opposite directions, the estimates of incidence of the samples under study, with obvious consequences for its extrapolation for the reference population. Moreover, differences on HIV-1 subtype infections, low CD4 counts, long term undetectable viral loads, antiretroviral therapy, total IgG concentration, among others, are also associated to the misclassification of recent infections. Due to concerns about studies based on the use of BED-CEIA, conducted in scenarios with high HIV prevalence and/or viral diversity [27], different adjustments have been proposed aiming to improve its accuracy for HIV estimates. One such adjustment proposes "*the exclusion of certain specimens on clinical grounds, by relying on trend differences rather than absolute incidence estimates, by secondary confirmatory testing, or by analytic adjustments for misclassification*" [29] (p. 945) and a second strategy adjusts some parameters, with "*further estimates of epsilon and of the window period*", in order to avoid misclassification of subtype C infected individuals [30] (p. 511).

However, based on the recent literature, there is no consensus of whether or not the proposed adjustments may improve the accuracy of HIV incidence estimates. The pioneer of such strategies from the perspective of statistical methods, Ron Brookmeyer, has recently published a paper where he discusses at length his position against the available adjustments [10]. One must observe, however, that his position was criticized by three research groups (Hargrove [58], Welte et al. [59] and McDougal [60]), with a further treplica by Brookmeyer, who maintained his point of view despite the criticisms [61].

McWalter & Welte [62] further developed the position outlined in their letter in response to Brookmeyer's paper, and took a firm position in favor of adjustment. They compared the accuracy of their adjustment strategy with the adjustment strategies proposed by the authors of the other two letters in response to Brookemeyer's paper (i.e. Hargrove [58] and McDougal [60], and their respective research teams). The authors conclude

that their own strategy is the one which can work under the less restrictive assumptions. One must observe that in spite of their position, the authors admit themselves in their final remarks: "*all the methods make the assumption that survival for assay non-progressors and assay progressors is the same. As we have shown, relaxing this assumption means that the long-term specificity becomes epidemic state dependent*" [62].

From a complementary perspective, a recent paper by Hallet et al. [63], simulating scenarios for the AIDS epidemic in six different African countries, highlighted another caveat putatively associated with the dynamics of each specific epidemic. For Hallet et al. [63] the key question to be urgently evaluated is secondary to whether misclassification based on the use of BED-CEIA may or may not vary over time elapsed since primary infection. The authors conclude that in the case of a non-stationary misclassification bias (i.e. a bias that may vary over time, in this specific case, hypothetically increasing over time), estimates would be influenced by the different trajectories of specific epidemics in each given place, as well as by the underlying demographic composition of each affected population. If such hypothesis proves to be a valid one (i.e. in the case that bias actually increases over time since primary infection), post-assay adjustments will not improve the accuracy of the estimates using BED.

The association of more than one biomarker to improve the estimates of HIV incidence has also been recently proposed. In this sense, a good agreement (90%) between BED-CEIA and Vironostika S/LS methods was obtained for the classification of recent HIV-1 infection [64], when compared to repeat testing in a population of men having sex with men from San Francisco, US. Lower agreement [65], however, was observed when Vironostika S/LS and Avidity assays were compared to identify recent HIV-1 infections among patients entering the Johns Hopkins Hospital Emergency Department in 2001 and 2003. All discrepant cases had undetectable viral load, that were identified as recent by the Vironostika S/LS assay but not with the Avidity assay, indicating that viral suppression affects the performance of cross sectional incidence assays based on antibody titer. Similar results were obtained for four long-term non-progressors/elite controllers from Brazil, who gave results compatible with recent infection based on the competitive BED-CEIA assay, whereas only 2 out of 4 were misclassified when using the avidity assay [66].

The application of four available tests (BED-CEIA, Vironostika, Avidity index, and IDE-V3) to estimate HIV-1 incidence in West Africa, where high prevalence is observed (10%) in a virologi-

cal scenario of non-subtype B infections, generated very discrepant results, varying from 1.2% (IDE-V3) to 11.2% (AI) [40]. In this study they tested a panel of samples of known seroconversion dates and assessed misclassification, showing that incident cases were more likely to be observed among patients infected for more than 180, but less than 360 days.

Recently (April 22-24/2009), the World Health Organization convened a meeting in order to standardize the terminology and statistical methods used to adjust (or not adjust) the different HIV assays. Although consensus was not reached respecting the statistical methods, a brand new terminology was advanced in the sense that "*the meaning of 'recently infected'* [would be] *defined by the assay itself* (...). *This removes the need to speak of sensitivity and specificity of the assay in the usual diagnostic sense, as the assay is no longer a proxy for any other condition*" [67] (p. 19). The WHO Working Group suggested to standardize the terminology, beginning with a new, broader definition of the very techniques and methods used in the estimation of recent infection as RITA (Recent Infection Testing Algorithm), defined as follows: "*A combination of specific laboratory and/or other methods that is intended to classify individuals as positive or negative for the state of 'recent infection', for the purposes of estimating HIV incidence*" [67] (p. 7).

A brand new development was recently proposed by Karon et al. [68], in a paper coauthored by Ron Brookmeyer, the leading proponent of the use of back-calculation as a key tool for estimating HIV incidence from reported AIDS cases, a strategy later compromised by the change in surveillance AIDS and the widespread dissemination of anti-retrovirals, in both high-income countries and middle-income countries such as Brazil and Thailand. As mentioned before, Brookmeyer was also the originator of the methods using biomarkers to estimate HIV incidence in the context of cross-sectional studies (as dis-

cussed at length in a former paper [15]). The new method advanced by Karon et al. [68] profit from both previous insights, and propose a new surveillance strategy to be adopted at the macro-level (for regions or even countries), "*in order to estimate the probability that an infected person will be detected as recently infected*" [68] (p. 4617), accounting from the intrinsic variability in terms of his/her testing behaviors (e.g. testing frequency, the availability of previous tests and the reporting of results to those people). In sum, the method estimates "*the number of persons detected as recently infected divided by the estimated probability of detection*" [68] (p. 4617). Notwithstanding the inventiveness of this new strategy, old phantoms still haunt it, such as the accuracy of the window periods defined by the different methods/algorithms, as discussed by WHO [67], and the very availability of information about biomarkers at the population level, which could be partially addressed by multiple imputations. It is still precocious to say whether such new strategy will be found to be a valid method in concrete situations.

In sum, methods aiming to estimate HIV incidence have progressed a lot in recent years, but are unfortunately still far from optimal, with no consensus among the experts in terms of a gold standard either respecting the methods themselves or the best way to adjust (or not adjust) them. As observed by the person responsible for the original statistical reasoning informing all such methods, Ron Brookmeyer [61], there is also no gold standard in the context of longitudinal studies, which are subjected to different but relevant biases.

Paraphrasing the philosopher of science Hacking [69] (p. 11), "*the taming of chance*" is an essential feature of the contemporary world, constituting the "*very stuff of the fundamental processes of nature and of society*". However, the very attempt of taming chance is far from straightforward and simple.

Resumo

O artigo revisa os métodos sorológicos utilizados na estimação da incidência da infecção pelo HIV baseada em estudos seccionais, assim como os principais achados de estudos conduzidos no Brasil que utilizaram estes métodos. Cada um dos métodos foi descrito sucintamente, assim como suas vantagens e limitações. Os diferentes métodos foram ainda analisados enquanto um conjunto de estratégias complementares, por vezes, contraditórias, sob permanente crítica e revisão, ainda longe de um padrão-ouro. Finalmente, uma questão adicional — essencial a um monitoramento acurado da epidemia por meio desses métodos — é discutida: devem tais métodos ser ajustados ou não? O debate segue em aberto e deve ser visto como um desdobramento inevitável de um campo de pesquisa particularmente dinâmico, informado pelos avanços de ciências as mais diversas, como a epidemiologia, a estatística e a modelagem matemática, além das várias áreas da ciência básica, como imunologia, virologia e biologia molecular.

HIV-1; Infecções por HIV; Incidência

Contributors

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