Preventing and Monitoring Infections

Getting more from heterogeneous HIV-1 surveillance data in a high immigration country: estimation of incidence and undiagnosed population size using multiple biomarkers

Federica Giardina,1,2,3* Ethan O Romero-Severson,2 Maria Axelsson,4 Veronica Svedhem,5,6 Thomas Leitner,2 Tom Britton1 and Jan Albert7,8

1Department of Mathematics, Stockholm University, Stockholm, Sweden, 2Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, USA, 3Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands, 4Department of Public Health Analysis and Data Management, Public Health Agency of Sweden, Solna, Sweden, 5Department of Medicine Huddinge, Karolinska Institute, Stockholm, Sweden, 6Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, 7Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden and 8Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden

*Corresponding author. University Medical Center Rotterdam, Doctor Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands. E-mail: f.giardina@erasmusmc.nl

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Abstract

Background: Most HIV infections originate from individuals who are undiagnosed and unaware of their infection. Estimation of this quantity from surveillance data is hard because there is incomplete knowledge about (i) the time between infection and diagnosis (TI) for the general population, and (ii) the time between immigration and diagnosis for foreign-born persons.

Methods: We developed a new statistical method for estimating the incidence of HIV-1 and the number of undiagnosed people living with HIV (PLHIV), based on dynamic modelling of heterogeneous HIV-1 surveillance data. The methods consist of a Bayesian non-linear mixed effects model using multiple biomarkers to estimate TI of HIV-1-positive individuals, and a novel incidence estimator which distinguishes between endogenous and exogenous infections by modelling explicitly the probability that a foreign-born person was infected either before or after immigration. The incidence estimator allows for direct calculation of the number of undiagnosed persons. The new methodology is illustrated combining heterogeneous surveillance data from Sweden between 2003 and 2015.

Results: A leave-one-out cross-validation study showed that the multiple-biomarker model was more accurate than single biomarkers (mean absolute error 1.01 vs ≥1.95).

We estimate that 816 [95% credible interval (CI) 775-865] PLHIV were undiagnosed in 2015, representing a proportion of 10.8% (95% CI 10.3-11.4%) of all PLHIV.

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Conclusions: The proposed methodology will enhance the utility of standard surveillance data streams and will be useful to monitor progress towards and compliance with the 90–90-90 UNAIDS target.

Key words: HIV-1, incidence estimation, undiagnosed HIV-1 infections, BED assay, pol sequences

Introduction
The majority of new HIV-1 infections originate from individuals who are undiagnosed and unaware of their infection, especially in countries with good access and adherence to antiretroviral therapy (ART), which greatly reduces infectiousness.1–4 Thus, knowledge about the size of the undiagnosed HIV-1 population is highly relevant for public health and HIV prevention. In 2014, the Joint United Nations Programme on HIV and AIDS (UNAIDS) launched the 90–90-90 target, which states that by 2020: (i) 90% of all persons living with HIV (PLHIV) should know their status; (ii) 90% of all diagnosed HIV cases should receive ART; and (iii) 90% of all people receiving ART should have achieved viral suppression.5 The European Center for Disease Prevention and Control (ECDC) estimates that one in seven (14%) PLHIV in Europe are unaware of their infection.6

Estimation of the size of the undiagnosed population is challenging, because the time of infection usually is unknown and the time until diagnosis (TI) is highly variable due to differences in testing behaviour, risk awareness and rate of disease progression. Until now, most estimates of HIV-1 incidence and undiagnosed population have been based on methods that classify patients as recently or long-term infected rather than estimate TI directly. Several such methods based on CD4+ T lymphocyte (CD4) counts, HIV-1 antibody tests and viral sequence diversity have been described. CD4 counts are commonly used,7 but their rate of decline is variable8–10 limiting their utility as a single biomarker. HIV-1 antibody concentrations increase over time from infection approaching an asymptote in long-term infections.11 The BED IgG-capture enzyme immunoassay (BED assay) has been used to estimate incidence12,13 by classifying recent and long-term infections. Sequence-based methods exploit the increase in intra-patient sequence diversity following infection, which can be approximated by the fraction of polymorphic nucleotides in the partial HIV-1 pol gene sequences that are used for detecting drug resistance mutations (referred to as pol polymorphisms).14,15

Fewer studies have attempted to estimate TI continuously, rather than distinguishing between recent and long-term infections, and to account for individual variations. Sommen et al.16 used antibody levels to two HIV-1 antigens (IDE and V3) to calculate the posterior distribution of HIV-1 infection times and incidence in France. Romero-Severson et al.11 used BED assay results to combine a model of within-host time-continuous IgG dynamics17 with a Bayesian estimator for the incidence of HIV-1 in Sweden in 2002–09. However, these studies neither explicitly accounted for immigration of infected persons, nor attempted to estimate the size of the undiagnosed HIV-1-infected population.

Here we present a statistical method to estimate HIV-1 incidence and the number of undiagnosed PLHIV. The method is based on: (i) a model of the joint dynamics of multiple-biomarker levels from the time of infection that allow a more accurate estimation of individual TIs; and (ii) the estimation of the time from immigration to diagnosis for exogenous infections using unlinked surveillance data.
The TI model uses BED, CD4 and \textit{pol} polymorphisms, but can be easily generalized to include other or additional biomarkers. The estimation of the time from immigration to diagnosis allows the assessment of how endogenous and exogenous infections contribute to the undiagnosed fraction.

In the application of the model to surveillance data from Sweden, we estimated that endogenous HIV-1 infections have decreased over 2010–15 and that 10.8% (95% CI, 10.3–11.4%) of all infected persons in Sweden were undiagnosed in 2015. This estimate is in line with previously published results, and adds uncertainty quantification.

**Methods**

The methodology to estimate HIV incidence and number of undiagnosed PLHIV was developed for countries like Sweden, i.e. countries with reliable HIV surveillance systems, availability of biomarker data on (a subset of) newly diagnosed patients, and non-negligible immigration.

**Data**

Multiple surveillance data sources in Sweden were collated for this work. We used published data on 1357 HIV-1 infected patients diagnosed in Sweden between 2003 and 2010\cite{11,17,19,20} with complete biomarker data on CD4 counts, BED levels and \textit{pol} polymorphism counts at diagnosis. These patients represented 39% of all patients diagnosed in Sweden during this period. These data included likely country of infection, transmission route, last negative and first positive HIV-1 test, laboratory evidence of primary HIV-1 infection and plasma HIV-1 RNA levels, but not data on time of arrival in Sweden for foreign-born persons. A subset of 31 treatment-naive patients, having longitudinal biomarker data and known infection times, was used to build and validate the multiple biomarker model.

Data on the yearly number of diagnosed HIV-1 cases in Sweden between 2003 and 2015 were collected as part of mandatory national case reporting, and was stratified by reported transmission route, place of infection (Sweden or abroad) and AIDS at HIV diagnosis. In total 5777 patients were diagnosed in Sweden during 2003–15. Data on linkage to care (diagnosed HIV-1 patients regularly attending scheduled visits) were obtained from the Swedish National Quality Assurance Registry InfCareHIV, as previously described. For 2466 of 2978 (83%) of the foreign-born patients, data on the time between first arrival and diagnosis in Sweden were available; however, these data were anonymized and could not be linked to the other data. For details on the patients and laboratory methods, see Supplementary Table S1 and Section 1, available as Supplementary data at IJE online.

**Modelling approach**

Our modelling approach to estimate the incidence of new HIV-1 infections and the size of the undiagnosed population consists of the following four main steps: (i) estimate parameters of our novel multiple-biomarker model using longitudinal biomarker data on 31 individuals with known infection dates; (ii) apply the multiple-biomarker model to estimate TI of the HIV-1 infected population, stratified by transmission group and country of origin using data from case reports, and use bootstrap for patients with missing biomarkers; (iii) estimate HIV incidence trends over the period 2000 to 2015, using the time distribution from TI to diagnosis obtained in the previous step and including the probabilistic allocation of foreign-born infected individuals to endogenous or exogenous infections; and (iv) calculation of the undiagnosed fraction with its uncertainty using bootstrap.

**Multiple-biomarker model**

The K biomarkers \(Y^k_i\), \(k = 1,\ldots,K\) are modelled jointly as a function of TI as follows:

\[
Y^k_i = f^k(t_{ij} - u_i) + \epsilon^k_i
\]

where \(i\) denotes the individual with biomarkers measured at calendar time \(t_{ij}\) after infection date \(u_i\). The dynamics are described by biomarker specific curves \(f^k(\cdot)\), fixed and random effects \(\beta^k_i\) and \(\epsilon^k_i\), as i.i.d. error terms such that \(\epsilon^k_i \sim N(0, \sigma_\epsilon^k)\). The model is formulated in a Bayesian framework and fitted using Markov chain Monte Carlo (MCMC) to longitudinal data with known TI. Full specification on the prior distributions assigned to the unknown parameters can be found in Supplementary Section 2.1, available as Supplementary data at IJE online. The biomarker model used data from three biomarkers and was trained on a subset of 31 treatment-naive patients having longitudinal biomarker data and known infection times. For comparison, the three biomarkers were also modelled separately. To assess the representativeness of the dataset, we compared the estimated parameter values with values reported in the literature.

**Estimation of TI and handling of foreign-born cases**

The Bayesian formulation allows us to use our model to estimate directly the unknown time of infection of new...
individuals, by treating their TI as a ‘latent’ variable, described by a prior distribution. This approach was used in a leave-one-out cross-validation analysis using the 31-patient longitudinal dataset (where TI was represented by any of the $t_i - u_i$), as well as in the prediction of TI for newly infected individuals (1357 patients between 2003 and 2010), with biomarkers measured only at time of diagnosis, i.e. $j = 1$.

To estimate HIV-1 incidence and undiagnosed PLHIV, foreign-born persons infected before first arrival should only be counted after arrival to the country of investigation, e.g. Sweden. As immigration dates were not available for the above-mentioned 1357 patients, we first estimated a typical distribution for the time interval between immigration e and HIV diagnosis using independent and unlinked surveillance data, and then applied this distribution to foreign-born patients among the 1357 patients.

**Incidence estimation**

We extended the model proposed by Sommen et al.\textsuperscript{16} to estimate HIV-1 incidence, defined as the number of new infections. Our modifications allow us: (i) to consider all reported cases within a moving window of size $m$ (i.e. $[t, t + m]$) in the incidence estimation at time $t$, denoted as $I_t$ (rather than only cases reported at time $t$); and (ii) to account for imported infections. Typically, the time period of interest is 1 year, and we denote the incidence as $I_{[\tau_1, \tau_2]}$ where $\tau_2 = \tau_1 + 1$. The incidence is expressed as a weighted sum of posterior densities of infection (or entry) times:

$$I_{[\tau_1, \tau_2]} = \int_{[\tau_1, \tau_2]} f^{endo}(t) + f^{exo}(t) = \sum_{i \in N_{endo}} \int_{\min(\tau_1, t_i)}^{\min(\tau_2, t_i)} \omega^{endo}(u_i) p(u_i | t_i) du_i + \sum_{i \in N_{exo}} \int_{\min(\tau_1, t_i)}^{\min(\tau_2, t_i)} \omega^{exo}(e_i) g(e_i | t_i) de_i$$

(1)

where $N_{endo}$ and $N_{exo}$ denote the number of endogenous and exogenous infections diagnosed in $[\tau_1, \tau_1 + m]$, respectively, and $\omega^{endo}$ and $\omega^{exo}$ are equal to the inverse of the probability of being diagnosed in $[\tau_1, \tau_1 + m]$ for individuals infected at $u_i$ or immigrated at $e_i$, respectively. Here, $p(u_i | t_i)$ represents the posterior distribution of the infection time for an individual $i$ (diagnosed at $t_i$) obtained using observed biomarkers, and $g(e_i | t_i)$ is the ‘backward’ distribution used to generate an entry time $e_i$ for a foreign-born case given diagnosis at $t_i$ in the country (by year of diagnosis). The time of the latest negative test that individual $i$ may have is denoted by $l_i$. The second term in (1) is evaluated only when the generated immigration time $e_i$ is more recent than the infection $u_i$ time as estimated by the biomarkers. This comparison allows for the definition of the sets $N_{endo}$ and $N_{exo}$. For unobserved years (i.e. upper end of the moving window falls after the present time) the number of diagnoses is assumed to follow a Poisson distribution with mean equal to the reported cases in the last observed year.

**Estimation of undiagnosed fraction**

The calculation of the HIV-infected undiagnosed individuals in a specific year follows directly from the incidence estimation (both endogenous and exogenous). Here, we define more generally the incidence of infection at time $t$ as $I_t$ and the number of undiagnosed individuals $U$ at time $t$ can be written as:

$$U_t = \int_{t}^{t+1} f^{endo} \left[ P\left[ t' - t > (\tau_3 - t) \right] \right] dt$$

$$+ \int_{t}^{t+1} f^{exo} \left[ P\left[ t' - t > (\tau_3 - t) \right] \right] dt$$

where $t'$ are the diagnosis times for individuals estimated to have been infected (or immigrated) at time $t$. That is, the number of undiagnosed patients in year $\tau_3$ is the cumulative sum of the product of the number of endogenous/exogenous infections that occurred/were imported $t$ years ago and the probability of being not yet diagnosed after $\tau_3 - t$ years. This calculation is stratified by transmission route.

Credible intervals are obtained by generating 100 bootstrap samples of size 1000 from the distributions of infection times. All statistical analyses were carried out using R version 3.3.21 and JAGS.\textsuperscript{22} For further details on the model and parameters, see the Supplementary Section 2 and Section 3, available as Supplementary data at IJE online.

**Results**

The multiple-biomarker model improved estimation of TI

The multiple-biomarker model for estimating TI based on BED levels, CD4 counts and $pol$ polymorphisms, was trained on longitudinal data from 31 HIV-1 patients with known infection times. The parameter values describing the growth or decline of the biomarkers for the training data (Supplementary Figures S1–S3 and Table S2, available as Supplementary data at IJE online) agreed with published values\textsuperscript{7,14,23–25} which justified the use of the model on biomarker data from other HIV-1 patients. A leave-one-out cross-validation analysis showed that the multiple biomarker model gave more accurate estimates of TI than each of the single biomarkers according to four different measures of precision (Table 1). TI estimates were more
precise if biomarkers were measured sooner after infection (MAE = 0.91 at <1 year; 1.20 at 1–2 years; 1.40 at >2 years, see Supplementary Table S3, available as Supplementary data at IJE online). Biomarker measurements at two or three time points, rather than a single time point, only slightly improved TI estimation (Supplementary Table S4, available as Supplementary data at IJE online). This motivated the use of single time point biomarker measurements in the application to newly diagnosed patients in Sweden.

Table 1. Mean predictive performance of single and multiple biomarker models assessed with a leave-one-out cross-validation analysis evaluated by four measures of precision

| Model   | Bias  | MAE   | RMSE  | Coverage |
|---------|-------|-------|-------|----------|
| CD4     | −2.62 | 3.12  | 3.77  | 0.81     |
| BED     | −1.80 | 1.95  | 2.21  | 0.90     |
| POL     | −2.16 | 2.22  | 3.20  | 0.81     |
| MBM     | −0.68 | 1.01  | 1.38  | 0.93     |

CD4, CD4+ T lymphocyte count; BED, antibody levels measured using the BED IgG-capture enzyme immunoassay; POL, fraction of polymorphisms in HIV-1 pol gene sequences; MBM, multiple biomarker model based on CD4, BED and pol; MAE, mean absolute error; RMSE, root mean square error. All values are in years.

Proportion of undiagnosed HIV-1-infected persons close to 10% UNAIDS target

Our model also provided estimates on the number of undiagnosed PLHIV in Sweden at the end of the years 2010 to 2015. Table 2 shows that number of undiagnosed PLHIV in Sweden was estimated to have decreased from 907 (95% CI, 880-934) persons in 2010 to 816 (95% CI, 775-865) in 2015. During the same period, the number of diagnosed patients linked to care increased from 5281 to 6747, which means that the proportion of undiagnosed PLHIV decreased from 14.7% (95% CI, 14.3-15.0%) in 2010 to 10.8% (95% CI, 10.3-11.4%) in 2015. Of the undiagnosed PLHIV in 2015, 341 persons were estimated to have been infected while living in Sweden (78 MSM, 8 IDU and 255 HET) and 475 persons were estimated to have been infected before first entering Sweden (165 MSM, 11 IDU and 299 HET).

Discussion

In this manuscript we present a new method for estimating HIV incidence and size of undiagnosed population which differentiates between exogenous and endogenous infection of foreign-born cases. Our method builds on (i) a Bayesian model to estimate time of infection (TI) using multiple biomarkers, and (ii) an extension of the incidence estimator proposed by Sommen et al. to explicitly model the probability that an HIV-1 infection in an migrating person occurred before or after immigration. Our methodology was designed for use on heterogeneous and unlinked surveillance data.

We applied the method to data from Sweden and estimated TI using three biomarkers (CD4 counts, BED assay results and proportion of polymorphic sites in HIV-1 pol sequences) as well as data on main transmission route, presence of a last negative test, AIDS at diagnosis and
diagnosed primary HIV-1 infection (PHI). We treated TI as a continuous random variable rather than as a simple binary state of being recently or long-term infected, which increases power and reduces bias. We found that a combination of the three biomarkers gave more precise TI estimates than one or two of these biomarkers. Each of the three biomarkers has certain advantages and limitations and shows considerable inter-individual variability. A well-known disadvantage of the BED assay is that it can give false-positive recent results for patients with low CD4 counts. This problem is reduced by our model because the inclusion of CD4 counts and pol polymorphisms partially corrects false-recent BED results, and because persons with AIDS at diagnosis were assigned a prior distribution with an average time from infection to diagnosis of 8 years (Supplementary Figure S4, available as Supplementary data at IJE online). Furthermore, low BED results in AIDS patients were rare in our dataset (Supplementary Figure S7 and Table S5, available as Supplementary data at IJE online).
We observed a decrease in the estimated incidence of HIV-1 infections and number of undiagnosed PLHIV in Sweden. The proportion of undiagnosed PLHIV was estimated to be 10.8% in 2015, which is close to the 10% UNAIDS 90–90-90 target. We estimated that the incidence of HIV-1-infections among persons residing in Sweden decreased by almost two-thirds from 2010 to 2015. The decrease was more pronounced in MSM and IDU than among heterosexually infected persons. This agrees with the fact that 87% of persons with diagnosed HIV-1-infection

Figure 3. Estimated HIV-1 incidence in Sweden 2003-15, per year and transmission route. The model explicitly accounts for exogenous infections. Thus, persons estimated to have been infected before first entry in Sweden only contribute to incidence in Sweden from the estimated date of entry. The upper panels show estimated incidence of HIV-1-infection among persons residing in Sweden. The lower panels show the incidence of HIV-1-infected persons entering Sweden for the first time. MSM, men who have sex with men; IDU, intravenous drug users; HET, heterosexual transmission route. Note the scale on the y-axis is different for IDU.
Table 2. Estimated number and proportion of undiagnosed HIV-1-infected persons in Sweden 2010–15. The number undiagnosed in 2015 was calculated using the estimated incidence divided by main transmission route. The number undiagnosed in previous years (2014–10) was calculated assuming the incidence in the years before 2003 constant and equal to that of 2003

| Year | No. of PLHIV linked to care | No. of undiagnosed PLHIV (95% CI) | Proportion undiagnosed PLHIV (95% CI) |
|------|-----------------------------|-----------------------------------|---------------------------------------|
| 2010 | 5281                        | 907 (880-935)                     | 14.7% (14.3-15.0%)                    |
| 2011 | 5616                        | 894 (863-925)                     | 13.8% (13.3-14.1%)                    |
| 2012 | 5918                        | 877 (837-916)                     | 12.9% (12.3-13.4%)                    |
| 2013 | 6205                        | 865 (826-912)                     | 12.2% (11.7-12.8%)                    |
| 2014 | 6469                        | 845 (802-896)                     | 11.5% (11.0-12.1%)                    |
| 2015 | 6747                        | 816 (775-865)                     | 10.8% (10.3-11.4%)                    |

The strengths of our approach are that it relies on routine or easy-to-collect data and provides estimates of the size of undiagnosed population, stratified by HIV transmission group, explicitly accounting for exogenous infections. In addition, the method considers the several sources of uncertainty involved, such as differences in HIV-1 testing behaviour, differences in disease progression and biomarker measurement error. Because the method estimates TI for each investigated person, it allows for detailed HIV-1 epidemiological investigations, such as the causes and consequences of late presentation of HIV infection and the effectiveness of HIV-1 prevention (e.g. pre-exposure prophylaxis). The method can easily be adapted to other biomarkers like the LAg avidity assay and viral diversity.

Our study suggests a way forward for generating up-to-date reports on the key parameters (incidence and number of undiagnosed persons) needed to understand the effectiveness of HIV control methods, the application of public health triage and the progress towards the UNAIDS 90–90–90 targets. The global movement of people in response to a changing world means that health systems will be challenged with increasing numbers of foreign-born persons; surveillance methods will need to correctly account for infections in those populations which occur both before and after migration. Integrating computational modules into public health surveillance streams that can account for complex, heterogeneous data with missing values will greatly increase the utility of surveillance, not only as a passive monitoring tool but as an active intervention aid.

Supplementary Data
Supplementary data are available at IJE online.

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