Title

An Innovative Study Design to Assess the Community Effect of Interventions to Mitigate HIV Epidemics Using Transmission-Chain Phylodynamics.

Journal

American Journal of Epidemiology, 187(12)

Authors

Williams, Leslie
Mbisa, Jean
Hatzakis, Angelos
et al.

Publication Date

2018-12-01

DOI

10.1093/aje/kwy160

Peer reviewed

American Journal of Epidemiology

Vol. 187, No. 12
DOI: 10.1093/aje/kwy160
Advance Access publication:
August 7, 2018

Study Design

An Innovative Study Design to Assess the Community Effect of Interventions to Mitigate HIV Epidemics Using Transmission-Chain Phylodynamics

Gkikas Magiorkinis*, Timokratis Karamitros, Tetyana I. Vasylyeva, Leslie D. Williams, Jean L. Mbisa, Angelos Hatzakis, Dimitrios Paraskevis, and Samuel R. Friedman*

* Correspondence to Dr. Gkikas Magiorkinis, Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, 75 Mikras Asias Street, 11527 Athens, Greece (e-mail: gmagi@med.uoa.gr); or Dr. Samuel R. Friedman, National Development and Research Institutes, 71 West 23d Street, 4th floor, New York, NY 10010 (e-mail: friedman@ndri.org).

Initially submitted February 19, 2018; accepted for publication July 24, 2018.

Given globalization and other social phenomena, controlling the spread of infectious diseases has become an imperative public health priority. A plethora of interventions that in theory can mitigate the spread of pathogens have been proposed and applied. Evaluating the effectiveness of such interventions is costly and in many circumstances unrealistic. Most important, the community effect (i.e., the ability of the intervention to minimize the spread of the pathogen from people who received the intervention to other community members) can rarely be evaluated. Here we propose a study design that can build and evaluate evidence in support of the community effect of an intervention. The approach exploits molecular evolutionary dynamics of pathogens in order to track new infections as having arisen from either a control or an intervention group. It enables us to evaluate whether an intervention reduces the number and length of new transmission chains in comparison with a control condition, and thus lets us estimate the relative decrease in new infections in the community due to the intervention. We provide as an example one working scenario of a way the approach can be applied with a simulation study and associated power calculations.

community effect; HIV; human immunodeficiency virus; intervention; persons who inject drugs; phylodynamics; PWID; transmission chain

Abbreviations: HIV, human immunodeficiency virus; t_{MRCA}, time to most recent common ancestor; PWID, persons who inject drugs.

Over the last decade our understanding of how pathogens spread has greatly improved, but mitigating pathogen epidemics still remains an enormous challenge. Early mathematical models of infectious diseases predicted what reality confirmed: the introduction and spread of pathogens into modern human populations now occurs more easily than ever due to the fast-growing high-density human population, which may travel long distances more easily than ever (1, 2). Epidemics such as those caused by human immunodeficiency virus (HIV)-1, hepatitis C virus, hepatitis B virus, tuberculosis, or malaria are still holding, and we are still far from containing them. On multiple independent occasions, large and rapid socioeconomic deterioration or, in other cases, a rapid growth preceded epidemic outbreaks, suggesting that "big events" could be considered spatiotemporal hot spots for triggering new (3, 4) or fueling old epidemics (5–7). Although such epidemics will not stop happening, we endeavor to better understand when, and most importantly, how, we will be able to contain them.

A plethora of mathematical models aims to evaluate intervention strategies with respect to successful mitigation of pathogen epidemics (8, 9). It is thought that the evaluation of interventions and their success is dependent on randomized trials (10), which can be costly and unrealistic in many settings. In addition, randomized controlled trials measure the impact of an intervention only on those who directly participate in one of the trial arms (or their first degree contacts) and thus cannot assess the community impact of the intervention (11). In cases such as harm-reduction interventions (e.g., needle or syringe exchange programs), where randomized controlled trials cannot be applied

in practice, a substantial amount of data in support of certain interventions has resulted in considering them as well-supported. However, more research is required (12).

Here we propose a new approach that aims to measure and evaluate the effect of interventions to mitigate the spread of pathogens by studying their evolutionary dynamics. We are interested in measuring the "community effect" of interventions (i.e., the ability to prevent both the initiation and expansion of transmission chains from people that have received a given intervention).

The method we describe here focuses on evaluating interventions among HIV-positive individuals. Our approach has been designed with HIV evolutionary dynamics in mind but can be adapted to evaluate interventions for other pathogens and for a broad range of settings. We specifically draw upon the example of HIV-preventive interventions among HIV-infected persons who inject drugs (PWID), where the community effect of an intervention in this group still remains a significant challenge. We focus on risk-network-based interventions such as the Transmission Reduction Intervention Program (13), which are designed not only to protect specific individuals recruited by the intervention arm but also to protect their risk network contacts.

METHODS

The problem

At time t an HIV epidemic has N_b infected individuals over a population with an overall number of individuals N_a (for a list of abbreviations used in equations see Table 1). At this time t an intervention is applied on n infected individuals and aims to mitigate the spread of HIV over the susceptible population. We believe this intervention has a community effect, so it reduces transmissibility not only by the recruited n individuals but also by the contacts in their risk networks. Importantly, the described approach can easily be expanded to include interventions that affect only the recruited individuals to whom the intervention was applied.

We collect blood samples at time t from both the intervention and defined (also infected) control groups, and we perform HIV genetic sequencing. After some later time t_i we revisit the population in order to evaluate the effect of the intervention. An important aspect of HIV natural history is that when someone gets infected they remain infected and carry HIV for life. This allows sequence sampling of the infected individuals many years after the transmission event.

To simplify calculations in our model we measure time t backward (as "time ago") from the last sampling date (e.g., when we revisit the location); this allows time to most recent common ancestor (t_{MRCA}) to be directly compared with t and t_i. Thus $t_i < t$ even though in calendar years the intervention occurred before the time point when we revisited the population (see Figure 1).

The "phylodynamic transmission chain" model

As indicated above we are interested in evaluating the ability of an intervention to prevent both the initialization and expansion of a transmission chain. We will evaluate these 2 properties of an intervention separately even though the expansion of a transmission chain is mechanistically dependent on the initialization.

At time t_i we will sample and sequence HIV from n_x individuals (we will call them incident individuals) who have been infected after the intervention (see Figure 1). To verify that a patient was infected within the intervention-resampling interval, we may use a combination of approaches and criteria including known time of seroconversion, an HIV-negative test result within the intervention-resampling interval, and/or an avidity test suggestive of recent infection and/or high-throughput deep-sequencing approaches (14).

Our first goal is to determine whether each one of these incident individuals is phylogenetically linked with the intervention group or with a nonintervention (or control) group. We consider that a phylogenetic link with the intervention group is established when the relationship of the t_{MRCA} for anyone in the intervention group and a given incident individual (t_{MRCAi}) as well as the t_{MRCA} for anyone in the control group (t_{MRCAg}) and any of the incident individuals is given by:

$$t_{MRCAi} < t_{MRCAg}$$

and

$$t_{MRCAi} < t + a,$$

where a is the phylogenetic assignment interval (see Figure 1); a is a nonnegative real number that defines the threshold time period before which the likelihood of attributing the transmission to either the control or the intervention group is unlikely.

A phylogenetic link with the control group is similarly verified (or resolved) when

$$t_{MRCAg} < t_{MRCAi}$$

and

$$t_{MRCAg} < t + a.$$

Thus, because both the intervention and control group participants are by design HIV-infected at time t, a link with the intervention group is established when the incident individual has a common ancestor with a participant in the intervention group that is within the phylogenetic assignment interval a; this common ancestor must also be more recent than the most recent ancestor of the incident individual with any participant in the control group. Likewise, a link with the control group is established when the incident individual has a common ancestor with the control group that is within a; this common ancestor needs also to be more recent than the most recent ancestor with the intervention group. Incident individuals that have t_{MRCA} with any of the groups larger than a are unlikely to be of interest because they are not considered to have been infected from either the control or the intervention group after the intervention took place. In Web Appendix 1 and Web Figures 2 and 3 (available at https://academic.oup.com/aje) we provide a generalized framework for taking into account phylogenetic uncertainty when establishing phylogenetic links.

We select a depending on the natural course of the infection; a reflects the fact that each infected patient has a population of nonidentical viruses, sometimes referred as quasispecies. The role of this genetic diversity in HIV transmission dynamics has been studied intensively and thus we already have a fairly good

Table 1. Description of Symbols Used in an Analysis Evaluating the Community Effect of an Epidemic Intervention

Symbol	Description
N_a	Size of the population where the intervention will take place
N_b	Size of the background (infected) population
N_c	Size of the control group
N_i	Size of the intervention group
n	Overall number of individuals recruited by the intervention
n_x	Number of incident individuals
n_b	Number of transmission chains generated by the background (infected) population
n_c	Number of transmission chains generated by the control group
n_i	Number of transmission chains generated by the intervention group
t	Time point of the intervention
t_i	Time point of the resampling
t_{MRCA}	Time to most recent common ancestor
t_{MRCAi}	Time to most recent common ancestor for anyone in the intervention group and a given incident individual
t_{MRCAg}	Time to most recent common ancestor for anyone in the control group and a given incident individual
t_{MRCAgi}	Time to most recent common ancestor between one person from the control group and its closest member of the intervention group
a	Phylogenetic assignment interval
r_a	Transmission rate
r_b	Transmission rate of the background population
r_c	Transmission rate of the control group
r_i	Transmission rate of the intervention group
l	Relative size of background (infected) population compared with the intervention groups
l_b	Length of transmission chains generated by the background (infected) population
l_c	Length of transmission chains generated by the control group
l_i	Length of transmission chains generated by the intervention group
π_c	Proportion of transmitters in the control group
π_i	Proportion of transmitters in the intervention group

understanding of how HIV genetic diversity is passed in transmitter-acceptor couples (15). These studies have shown that at the time of transmission there is a quasispecies population bottleneck suggesting that only a minority of the genetic diversity within the "donor" can be transmitted. Due to within-patient evolution and stochasticity, the transmitted strains may or may not contain exactly the same genomic sequence that was isolated from the transmitter at the time of the intervention. This means that the t_{MRCA} between the donor and the receiver viral strains is likely to predate the transmission event, which in turn creates the necessity of nonzero a. The phenomenon, known since 1999, that has been described as pretransmission interval (16) explains why $t_{MRCA}$and actual transmissions may not coincide (17).

Sampling at time t_i: power calculations

How should we sample at time t_i to have sufficient power to evaluate the effect of an intervention?

Our intuitive expectation is that if the intervention has reduced transmissibility, then the individuals who received the intervention would have generated a smaller number of transmission chains; a random sample of incident cases should result in finding fewer individuals from the intervention group having phylogenetic links with any of the incident individuals than the control group.

Another intuitive expectation is that if the intervention has reduced transmissibility in the risk contacts of the intervention arm, then a random sample of incident cases should result in reconstructing shorter transmission chains having phylogenetic links with the intervention rather than the control group.

One of the major questions that we want to explore is how many individuals we would need to sample at time t_i given that we have performed an intervention on n individuals at time t. If the intervention has no effect, then the transmission rate (r_a) would be equal among background population (r_b), control (r_c), and intervention groups (r_i):

$$r_b = r_c = r_i\,(H_0) \ \text{(null hypothesis)}.$$

If the intervention has the anticipated effect, then the transmission rates of the background population and control population

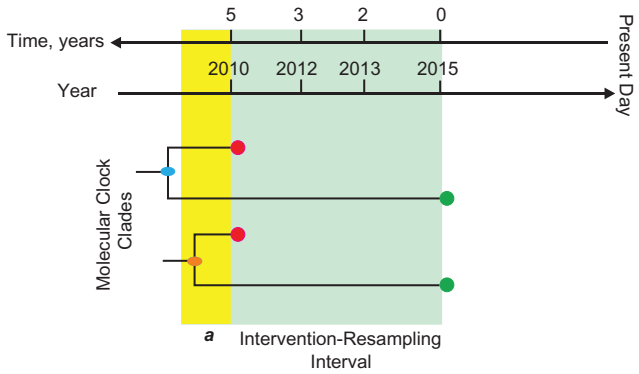

Figure 1. Theoretical representation of the phylogenetic assignment process as used in the phylodynamics transmission-chain model. Calendar years are shown in the lower scale, while reverse chronological years are shown in the upper scale (measured backward from the second time point in 2015). Red circles indicate viral strains isolated at the time of the intervention ($t = 5$, year 2010). Green circles indicate viral strains isolated from incident cases at the second time point (resampling at $t = 0$, year 2015). With light-blue ellipses we show a common ancestor before the phylogenetic assignment window (*a*) (i.e., not phylogenetically assigned to the viral strains isolated in 2010) while with light-orange ellipses we show a common ancestor within the phylogenetic assignment window (thus phylogenetically assigned to the strain isolated in 2010).

would be approximately equal (for a placebo control group), and the transmission rate of the intervention group would be lower:

$$r_b = r_c > r_i(H_1) \quad \text{(alternative hypothesis)}.$$

The expected number of transmission chains generated between time t and t_i from the background population (n_b), the control group (n_c), and the intervention group (n_i) will be provided through functions of the transmission rate and the size of the population belonging to each one of these groups at time t (N_b, N_c, and N_i, respectively). The same parameters will also contribute to the expected length of transmission chains generated between time t and t_i from the background population (l_b), the control group (l_c), and the intervention group (l_i).

By linking the transmission chains with either the control or the intervention group, we estimate the proportion of transmitters that is the number of individuals who eventually transmitted the virus. We are thus interested in comparing the proportion of the transmitters between the intervention and control group given by the respective ratios ($\pi_c = \frac{n_c}{N_c}$ and $\pi_i = \frac{n_i}{N_i}$) . We can also measure and compare the length of the generated transmission chains.

Simulation parameters

We use a simulator that implements stochastic models of structured and unstructured population dynamics (18). The simulator generates transmission chains, which we then transform into molecular-clock trees by simulating the sequence evolution of pathogens (19).

From each infected individual we generate transmission chains based on: 1) the "accessible" population, that is, the size of the risk network (or the population "bubble") upon which the pathogen may spread from a single individual within the locality of the study and within a specific time frame; and 2) pathogen transmission assumptions that are based mainly on our expectation of how large an epidemic a pathogen could create in a completely susceptible population of 10,000 people without structure.

More details on our methods can be found in Web Appendix 1.

RESULTS

Size of intervention and postintervention sampling

When assessing the effect of an intervention within a community, we intuitively expect that the size of the intervention compared with the size of the existing epidemic is an important factor: The larger the epidemic, the more "diluted" will be the role of the intervention in controlling further spread of HIV. At the time of the design we need to identify which of the following scenarios applies to our sample:

1. An epidemic with a very small intervention group relative to the background infected population: $N_i \ll N_b$. This might be the case in well-established epidemics in large at-risk populations (e.g., Durban, South Africa, or St. Petersburg, Russia) where an evaluation of the intervention is required, but the funds may not yet be sufficient for large-scale interventions. Data on incidence rates and on the proportion of the infected on antiretroviral therapy (and therefore unlikely to generate many new transmission cases) may affect the power analyses for determining which cities fall into this category.
2. An epidemic (typically small) with an intervention group that would be comparable to the total number of infected individuals with $N_i = N_c = N_b/l$ (where l is a natural nonzero number showing the relative size of the background epidemic compared with the intervention groups). Interventions that aim to mitigate outbreaks in situations with small at-risk populations (e.g., Athens Metropolitan Area (20) or Indiana rural outbreaks (21)) fit this sampling scenario.

Scenario 1 is a special case of scenario 2 when $l \to \infty$. Therefore, under the null hypothesis (H_0) we would have $n_i = n_c \ll n_b$, and the ratio $n_i/n_b = n_c/n_b \cong 0$. This suggests that for the well-established epidemics (scenario 1), through random sampling it is highly unlikely to sample incident cases within a reasonable $t_i - t$ period that could be attributable to either the intervention or control arms. On the other hand, as scenario 2 suggests, in smaller outbreaks random sampling might allow us to recruit a sufficient number of incident individuals. Under the null hypothesis, for every $l + 2$ incident cases we would sample l from the background, 1 from the intervention group, and 1 from the control group.

Depending on the expected reduction of transmissibility and the relative size of the background epidemic l, we can estimate the minimum number of incident cases that we would need to sample in order to test our alternative hypothesis. The required number of incident cases, however, needs to be adjusted depending on the amount of phylogenetic information contained in the resulting phylogenies: If we can resolve all the phylogenetic links between the intervention or control group and the incident cases, then the minimum number to be sampled for

evaluating the impact of the study will be lower than if we cannot. If we cannot resolve whether a given incident case came from the control group, the intervention group, or the background, then we would consider that case as censored (i.e., rendered missing). The number of censored incident cases reduces the power of our estimation. Intuitively, in the ideal case we could minimize the number of censored individuals if we could maximize the phylogenetic distance between members of each one of the groups at the time point of the intervention.

A guide for power calculations

By means of stochastic modeling and empirical estimates of transmissibility, we can estimate the proportion of individuals that we expect to produce infections (and thus transmission chains) within the time frame $(t - t_i)$. With standard proportion power calculations we can then estimate the minimum number of transmission chains that we need to sample when we visit the site after $(t - t_i)$ years.

How can we use the estimated necessary number of transmission chains to extrapolate the number of incident cases that we would need to sample in order to get a sufficiently powered study? At the very least a transmission chain can be attributed to 1 incident case. Thus, the absolutely minimum number of incident cases would be equal to the minimum number of transmission chains. By simulating the sampling strategy of incident cases over a population of simulated (under the null and the alternative hypotheses) transmission chains, we can then inflate the number of additional incident cases needed to be sampled to get the required minimum number of transmission chains. To illustrate how this framework operates we provide an example of a power calculation below.

Example of power calculation

Sizes of control and intervention groups. We have simulated transmission chains ($n = 10{,}000$) for the control and intervention groups (see Methods). The proportion of individuals that generate a transmission chain is 0.75 and 0.50 for the control and intervention groups, respectively (assuming that the intervention reduces transmissibility by 50% compared with the control group). Based on the power calculation test (P value = 0.02, power = 0.95, 2-tailed) in order to show that 75% is different compared with 50% in independent populations, we would need to have 115 participants assigned both to the intervention group and to the control group. We will slightly inflate this number and we will assign 120 participants per group. We expect these to generate 150 transmission chains (90 for the control and 60 for the intervention). If we relax the power calculation (P value = 0.05, power = 0.80, 2-tailed), then we could perform the intervention with as few as 55 persons per group.

Number of incident cases to sample at revisit. We need to multiply the number of incident cases linked with any of the 2 arms ($n = 150$) by 2, given that the simulation shows that on average 2 randomly sampled incident cases would be part of the same transmission chain. We need to inflate this number further because not all incident cases would be linked with the intervention group or the control group—a significant number would be unlinked (or considered to be produced from the background infected population). If we assume that 50% of the incident cases would be linked to either the control group or the intervention group, then the minimum sampling number is 600 incident cases. If the proportion of the incident sample that can be phylogenetically linked with either the control group or the intervention group is higher, then the number of incident cases sampled at follow-up can be reduced. Such enriched sampling effects can be reached through network-based approaches using the people recruited during the intervention as seeds. For example in Athens, through a network-based sampling approach, we have shown that approximately 50% of the phylogenetically related HIV-1 infected PWIDs reported a social network relationship (first or second degree) (22).

Proof of concept: simulation of an HIV-preventive intervention among PWID. Based on the above-stated power estimation, we have designed an intervention. We posit that in 2010 we visited a place that had an ongoing HIV epidemic for at least 10 years, with at least 1,000 infected individuals. We randomly selected 120 to participate in the intervention and 120 to participate in the control group. In 2015, we revisited the place and sampled 620 individuals who had been infected after the intervention (i.e., we further inflated the number from our power calculation by 20 cases). These 620 people were sampled in 2015 through an approach that we expected to link 50% of the incident cases with either the control group or the intervention group (specifically, targeting the risk networks of the control and intervention groups). These 620 incident cases could be part of transmission chains coming from the study groups or from unsampled people (background infected population). We assumed that our sampling approach would be able to retrieve transmissions from our study groups with equal probability to that of the transmissions coming from the background. The sampling approach was performed in a way that was unbiased with respect to recovering transmissions from the intervention or the control groups. Thus, whether the sampled transmissions were the result of the control group or the intervention group is proportional to the number of incident individuals generated from each one of these groups.

Estimating the proportion of transmitters. In Web Figure 1 we see the molecular-clock tree of the above-mentioned simulation scenario. The scale is in years. We consider that someone from each of the groups has generated a transmission chain when the t_{MRCA} from any of the 620 people sampled in 2015 and any of the control or the intervention groups falls after 2009.5 (allowing thus for a window of $a = 0.5$ year before 2010, which is the gray shaded box on the tree). We recovered 130 transmission chains that could be linked with either the control group or the intervention group. Seventy-four transmission chains were phylogenetically linked to the control arm while 56 transmission chains were phylogenetically linked to the intervention arm, suggesting a statistically significant reduction of the transmission rate (74/120 versus 56/120 with a $P = 0.025$, 2-tailed χ^2 test). The rate of false phylogenetic links was 2/120 for the control group and 5/120 for the intervention group. We found similar results in 4 independent simulations of the same scenario (Table 2). The role of the phylogenetic assignment interval a on evaluating on the proportion of transmitters is analyzed in Web Appendix 2 and Web Table 1.

Table 2. Statistics and Characteristics of 5 Independent Simulations of the Proof of Concept Scenario Evaluating the Community Effect of an Epidemic Intervention

Simulation	Characteristics						
	Root Height (years)	Burn-in (× 1,000 Generations)	Control Transmitters	Intervention Transmitters	*P* Value (Control vs. Intervention)	False Control Transmitters	False Intervention Transmitters
1	12.2	5,000	74	56	0.025	2	5
2	12.01	2,000	85	61	0.0015	2	5
3	12.03	2,500	81	62	0.0128	1	6
4	11.79	3,000	83	59	0.0017	3	8
5	11.92	2,000	81	42	<0.0001	2	9

Evaluating the wider community effect by measuring the "length" of transmission chains. The number of generated transmission chains is a measure of the direct or "first-wave" community effect of an intervention. To evaluate an intervention's long-term effect at the community level, another approach is required. As stated above, we are interested in evaluating interventions that are designed to have wider community effects. Such interventions are designed to reduce transmissibility even among persons who are socially or distally related to the intervention "seeds." Here we propose to assess this wider community effect by comparing the length of the produced transmission chains, which are phylogenetically linked to either the intervention or control seeds. By size or length of transmission chains, we mean the number of the incident individuals linked with a specific person belonging to the intervention or the control group.

Again using the same scenario as described previously, we are looking at transmission dynamics where a point introduction of HIV in a population of 10,000 susceptible persons could produce an epidemic of approximately 5,000 within 5 years. This scenario when stochastically simulated will produce a range of transmission chains (see Figure 2). When transmissibility is reduced by 50%, the length of the simulated transmission chains in a newly introduced epidemic will be significantly shorter than in the full transmissibility scenario. This suggests that the size of the transmission chains that are phylogenetically linked to the intervention group is expected to be smaller than the transmission chains that are phylogenetically linked to the control group. We note that the length of the transmission chains recovered after sampling is much smaller than the actual full length; length is sensitive to sampling effects. However, the relative length between the intervention and control groups should be different, even after sampling.

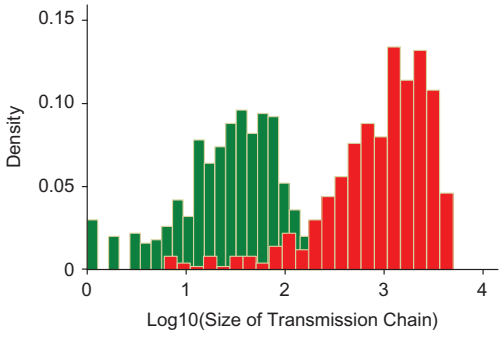

Figure 2. Distribution of the length of the transmission chains when simulating the spread of the pathogen in population "bubbles" of 10,000 under transmission parameters described in Methods. Red signifies the control arms and green signifies the intervention arms.

Indeed when analyzing Web Figure 1, we see that the average size of the control-linked transmission chains was 2.8, while the average size of the intervention-linked transmission chains was 1.9, suggesting that under the suggested sampling scheme we observed at least 30% reduction in the transmission chain's length ($P = 0.01$, Mann-Whitney rank-sum test).

DISCUSSION

We have introduced a methodological approach to assess the direct and wider community effect of interventions aiming to control the spread of viral pathogens. The method is based on establishing molecular-clock-based phylogenetic links of newly infected patients to individuals having received the intervention or a control condition. The most important advantage of the suggested method is that it can assess the wider community effect without the necessity of follow-up among an at-risk group. Thus it remains strong even in circumstances where retaining participants for follow-up is a major challenge (e.g., among PWID communities). Our method provides a new tool for evaluating interventions that previously could be evaluated only with community-randomized controlled trials, with probability sampling of communities, or with (for a narrow subset of interventions like antiretroviral treatment) couples studies (23, 24) (for more examples, see Web Appendix 3).

Another strength of the suggested method is that a control group can be formed retrospectively (e.g., by identifying individuals who were infected at the time of intervention and were not part of the risk network of the intervention arm). This is feasible as long as we know that these people were indeed infected at the time of the intervention (thus the risk of transmission is equal between the intervention group and the control group), and nucleotide sequences of such people can be retrieved at any time point. This feature can be particularly important in the event that many interventions have already taken place without the formation of control groups, when assessing their wider community effect can be particularly challenging. Such ongoing or recent interventions are unlikely to have collected blood

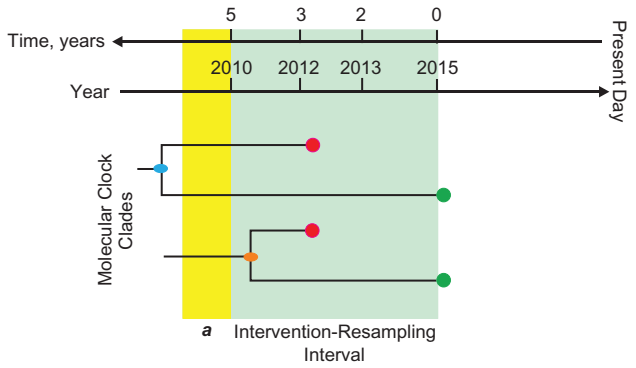

Figure 3. Theoretical representation of the phylogenetic assignment process as used in the phylodynamics transmission-chain model after relaxing assumption to include samples collected after the intervention (modification of Figure 1). The molecular sequences are sampled after the intervention. Phylogenetic assignment may take place after the intervention, but never before the earlier point of the phylogenetic assignment window *a*.

samples at the time point of the intervention, but such early samples are not necessarily required. We may collect samples after the intervention and, by virtue of molecular-clock phylogenetics, still assess phylogenetic links. One obvious adjustment will be on the use of the window *a* for phylogenetic assignment: The common ancestor might be dated even after the time of the intervention (Figure 3).

In conclusion, we have presented a new approach that can evaluate the community effect of interventions that aim to mitigate the spread of pathogens among susceptible populations. The approach exploits molecular evolutionary dynamics of pathogens in order to track new infections as having arisen from either a control group or an intervention group. It enables us to evaluate whether an intervention reduces the number and length of new transmission chains in comparison with a control condition, and thus lets us estimate the relative decrease in new infections in the community due to the intervention. We have also described a framework to calculate sample size and power of suggested intervention designs. This new approach provides a novel formal framework to design and evaluate interventions in settings and situations in which traditional approaches such as randomized controlled trials cannot be applied.

ACKNOWLEDGMENTS

Author affiliations: Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece (Gkikas Magiorkinis, Angelos Hatzakis, Dimitrios Paraskevis); Hellenic Pasteur Institute, Athens, Greece (Timokratis Karamitros); Department of Zoology, University of Oxford, Oxford, United Kingdom (Tetyana I. Vasylyeva); National Development and Research Institutes, New York, New York (Leslie D. Williams, Samuel R. Friedman); and Virus Reference Department, Public Health England, London, United Kingdom (Jean L. Mbisa).

This work was funded by the National Institute on Drug Abuse, National Institutes of Health (grant DP1DA034989, "Preventing HIV Transmission by Recently-Infected Drug Users" to S.R.F).

Conflict of interest: none declared.

REFERENCES

1. Anderson RM, May RM. Population biology of infectious diseases: Part I. *Nature*. 1979;280(5721):361–367.
2. May RM, Anderson RM. Population biology of infectious diseases: Part II. *Nature*. 1979;280(5722):455–461.
3. Nikolopoulos GK, Sypsa V, Bonovas S, et al. Big events in Greece and HIV infection among people who inject drugs. *Subst Use Misuse*. 2015;50(7):825–838.
4. Faria NR, Rambaut A, Suchard MA, et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science*. 2014;346(6205):56–61.
5. Paraskevis D, Nikolopoulos G, Fotiou A, et al. Economic recession and emergence of an HIV-1 outbreak among drug injectors in Athens metropolitan area: a longitudinal study. *PLoS One*. 2013;8(11):e78941.
6. Magiorkinis G, Angelis K, Mamais I, et al. The global spread of HIV-1 subtype B epidemic. *Infect Genet Evol*. 2016;46: 169–179.
7. Vasylyeva TI, Liulchuk M, Friedman SR, et al. Molecular epidemiology reveals the role of war in the spread of HIV in Ukraine. *Proc Natl Acad Sci U S A*. 2018;115(5):1051–1056.
8. Anderson RM, May RM. Vaccination and herd immunity to infectious diseases. *Nature*. 1985;318(6044):323–329.
9. Halloran ME, Auranen K, Baird S, et al. Simulations for designing and interpreting intervention trials in infectious diseases. *BMC Med*. 2017;15:223.
10. Goswami ND, Pfeiffer CD, Horton JR, et al. The state of infectious diseases clinical trials: a systematic review of ClinicalTrials.gov. *PLoS One*. 2013;8(10):e77086.
11. Friedman SR, Perlman DC, Ompad DC. The flawed reliance on randomized controlled trials in studies of HIV behavioral prevention interventions for people who inject drugs and other populations. *Subst Use Misuse*. 2015;50(8–9):1117–1124.
12. Fernandes RM, Cary M, Duarte G, et al. Effectiveness of needle and syringe Programmes in people who inject drugs—an overview of systematic reviews. *BMC Public Health*. 2017; 17:309.
13. Nikolopoulos GK, Pavlitina E, Muth SQ, et al. A network intervention that locates and intervenes with recently HIV-infected persons: the Transmission Reduction Intervention Project (TRIP). *Sci Rep*. 2016;6:38100.
14. Puller V, Neher R, Albert J. Estimating time of HIV-1 infection from next-generation sequence diversity. *PLoS Comput Biol*. 2017;13(10):e1005775.
15. Kariuki SM, Selhorst P, Ariën KK, et al. The HIV-1 transmission bottleneck. *Retrovirology*. 2017;14:22.
16. Leitner T, Albert J. The molecular clock of HIV-1 unveiled through analysis of a known transmission history. *Proc Natl Acad Sci U S A*. 1999;96(19):10752–10757.
17. Romero-Severson E, Skar H, Bulla I, et al. Timing and order of transmission events is not directly reflected in a pathogen phylogeny. *Mol Biol Evol*. 2014;31(9):2472–2482.

18. Vaughan TG, Drummond AJ. A stochastic simulator of birth-death master equations with application to phylodynamics. *Mol Biol Evol*. 2013;30(6):1480–1493.
19. Rambaut A, Grassly NC. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Comput Appl Biosci*. 1997;13(3):235–238.
20. Paraskevis D, Nikolopoulos G, Tsiara C, et al. HIV-1 outbreak among injecting drug users in Greece, 2011: a preliminary report. *Euro Surveill*. 2011;16(36):19962.
21. Conrad C, Bradley HM, Broz D, et al. Community outbreak of HIV infection linked to injection drug use of oxymorphone—Indiana, 2015. *MMWR Morb Mortal Wkly Rep*. 2015;64(16):443–444.
22. Kostaki EG, Nikolopoulos GK, Pavlitina E, et al. Molecular analysis of HIV-1 infected individuals in a network-based intervention (TRIP): phylogenetics identify HIV-1 infected individuals with social links. *J Infect Dis*. 2018;218(5):707–715.
23. Boily MC, Mâsse B, Alsallaq R, et al. HIV treatment as prevention: considerations in the design, conduct, and analysis of cluster randomized controlled trials of combination HIV prevention. *PLoS Med*. 2012;9(7):e1001250.
24. Shrier I, Boivin JF, Steele RJ, et al. Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? A critical examination of underlying principles. *Am J Epidemiol*. 2007;166(10):1203–1209.