OPEN

AIDS, Publish Ahead of Print

DOI: 10.1097/QAD.0000000000003416

HIV transmission among acutely infected participants of a Dutch cohort study 2015-2021 is not associated with large, clustered outbreaks

Running head: Transmission of AEHI is mainly independent

Henrieke A.B. PRINS[1], Casper ROKX[1], Annelies VERBON[1], Ard van SIGHEM[2], Godelieve J. DE BREE[3], Maartje DIJKSTRA[3,4], Jan M. PRINS[3], Peter REISS[2,3,5], Jeroen J.A. van KAMPEN[6], David A.M.C. VAN DE VIJVER[6]

[1]Department of Medical Microbiology and Infectious Diseases and Department of Internal Medicine, Erasmus University Medical Centre, Rotterdam, The Netherlands

[2]Stichting hiv monitoring (SHM), Amsterdam, The Netherlands

[3]Department of Internal Medicine, Division of Infectious Diseases, and Amsterdam Institute for Infection and Immunity, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

[4]Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, Netherlands

[5]Department of Global Health, Amsterdam UMC, University of Amsterdam, and Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands

[6]Department of Viroscience, Erasmus University Medical Centre, Rotterdam, The Netherlands

Corresponding author:

Henrieke Prins, MD. Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Centre, Na901K, postbus 2040, 3000 CA Rotterdam, the Netherlands, e-mail address: h.prins@erasmusmc.nl, telephone: +31107033510

Abstract

Objective: Timely identification of acute or early HIV infection (AEHI) is important to help prevent onward transmission, and understanding the number of secondary infections resulting from individuals with AEHI is key to planning HIV prevention services and case finding.

Design: We performed a phylogenetic investigation of a dense sample of individuals with AEHI who took part in the Netherlands Cohort Study on Acute HIV infection (NOVA) in the Netherlands during 2015-2021.

Methods: Transmission clusters were identified using phylogenetic analyses based on HIV pol sequences. The Tamura-Nei model was used to estimate genetic distance. A number of 1,000 bootstraps was used to check the reliability of clustering using maximum likelihood. A cluster was defined as having a bootstrap value of at least 95% and a genetic distance of at most 1.5%. Sensitivity analyses using different values for the bootstrap and genetic distance were performed to study the reproducibility of the clustering.

Results: Of the 156 participants included in NOVA between July 2015 and April 2021, 134 individuals for whom baseline characteristics and genotypic resistance data at baseline were available could be included. We identified 10 clusters, but the majority of persons (111/134) were not part of a cluster, suggesting mainly independent transmission events.

Conclusions: Mainly independent transmission events among a study population consisting predominantly of MSM in a low-incidence high-resource setting is likely the result of active AEHI case finding and direct start of treatment, and the roll-out over recent years of preventive measures such as preexposure prophylaxis.

Key words: HIV; acute HIV infection; early HIV infection; phylogeny; transmission; cohort

Introduction

The acute stage of HIV infection is a brief, but highly infectious phase of infection (1). Individuals who are unaware of their status may transmit HIV to multiple individuals during this infectious phase. Timely identification of acute or early HIV infection (AEHI) (here defined as the first 6 months after infection) is therefore important to help prevent onward transmission. Understanding the number of secondary infections due to AEHI is also important, for the planning of HIV prevention services, such as immediate treatment initiation and pre-exposure prophylaxis (PrEP), and for case finding. Previous studies in low-incidence settings, conducted before an approach of "test and treat" was widely advocated, described substantial clustered transmission during AEHI (2-6). Mathematical modelling studies on the proportion of new HIV infections attributable to AEHI cases varied considerably, with estimates ranging from below 10% to over 80% (7-9). To understand the more contemporary transmission dynamics during AEHI, we performed a phylogenetic analysis among 134 therapy naïve

persons with AEHI participating in the Netherlands Cohort Study on Acute HIV infection (NOVA) in the Netherlands between July 2015 and April 2021 (10).

Methods

The NOVA is a cohort study that prospectively includes adults ≥18 years diagnosed with AEHI who are willing to start combination antiretroviral therapy (cART) within 24 hours of enrolment (10). AEHI is defined as the first six months after infection according to Fiebig staging based on the detection of plasma HIV-RNA by RT-PCR, HIV p24 antigen and anti-HIV antibodies by fourth generation ELISA, and western blot (11). Participants diagnosed in Fiebig VI could only be included if a negative HIV test less than six months prior to their diagnosis was performed. A sequence analysis to find genotypic resistance of HIV was performed using viral sequences from the participants using the Stanford HIV Drug Resistance database version 9.0 (12). The HIV subtype was identified using the REGA subtyping tool (13). Clinical and demographic data were collected in collaboration with Stichting hiv monitoring (SHM, HIV Monitoring Foundation), which coordinates the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort, a nationwide HIV cohort which prospectively captures data of approximately 98% of all people living with HIV (PLWH) in care (14). Clinical data in the ATHENA cohort are collected by trained data monitors using standardized data collection tools. Currently, the nine HIV treatment centers that participate in the NOVA serve 65% of all PLWH in care in the Netherlands, and cover the areas known to have the highest HIV prevalence (15).

Transmission clusters were identified using phylogenetic analyses based on HIV pol sequences. This involved either protease (PRO)-reverse transcriptase (RT)-integrase (INT) (n=26), PRO-RT (n=56), or RT (n=52). The HIV-1 pol sequences were aligned to 53 reference sequences from known subtypes from group M (subtype A-K and circulating recombinant forms). For each of the participants' sequences, ten mostly similar sequences were retrieved from Genbank and added to our dataset. All sequences were aligned and trimmed to equal length. After removal of duplicate sequences from Genbank and one erroneous NOVA sequence this resulted in 941 sequences (53 Los Alamos, 133 NOVA, 755 Genbank) with a length of 1319 nucleotides. Since INT was not always sequenced, the phylogenetic analysis included only PRO-RT. The analysis was based on the maximum likelihood method using the Tamura-Nei model to estimate genetic distance. A number of 1,000 bootstraps was used to check the reliability of the clustering. A cluster was defined as having a bootstrap value of at least 95% and a genetic distance of at most 1.5%. Sensitivity analyses using different values for the bootstrap and genetic distance were performed to study the reproducibility of the clustering.

The NOVA was approved by the medical ethical committee of the Amsterdam University Medical Centers (Academic Medical Center site) (NL51613.018.14) and all participants provided written informed consent.

Results

Of the 156 participants included in the NOVA between July 2015 and April 2021, 134 individuals for whom baseline characteristics and genotypic resistance data at baseline were available could be included in the phylogenetic analysis. Table 1 presents an overview of the baseline characteristics. Most of the participants acquired HIV through sex with other men (n=117, 87.3%), similar to the majority of new HIV diagnoses in the Netherlands (10). Diagnoses were predominantly in Fiebig stage IV-VI (n=95, 70.9%), and most individuals were infected with HIV-1 subtype B (n=98, 73.1%). Individuals had high plasma viral loads at enrollment with a median HIV-RNA of 5.8 (IQR 4.7-6.7) log10 copies/mL. The median CD4+ T cell count at baseline was 510 (IQR 355-683) cells/μL, and CD4+ T cell counts of below 200 cells/μL were uncommon (n=4, 3.0%, data not shown). Participants who were enrolled in Amsterdam accounted for two third of all NOVA participants (n=86, 64.2%). Over one fourth of all participants (n=37, 27.6%) were enrolled in the region of Rotterdam.

The analysis showed ten clusters, eight of which included two NOVA participants, and two which included three or more persons (Table 2). In total, 111 participants with AEHI were not part of a cluster. Apart from one cluster involving HIV-1 subtype CRF02_AG, all clusters were of subtype B. Of the 23 participants found to be part of a cluster, 12 (52.2%) enrolled in the region of Amsterdam. Over one third of the 23 participants involved in clusters (n=8, 34.7%) were enrolled in the region of Rotterdam. In 8/10 clusters found, participants enrolled in study sites in the same city. One cluster involved three participants from the same city who were diagnosed within four months from each other. The largest cluster involved four participants diagnosed in Fiebig 1, 5 and 6, all of whom started cART within 8 days from the date of their diagnosis, and three of whom were diagnosed in the same city over a period of three years. In 8/10 clusters, individuals received their diagnosis within a maximum of six months apart. Sensitivity analyses using different values for the bootstrap and genetic distance did not affect the clustering found in this study.

Transmitted drug resistance mutations found were not relevant to PrEP available in the Netherlands or current cART options, in line with the low and stable prevalence of transmitted drug resistance among the general population living with HIV in the Netherlands (15). Among the 26 participants sequenced for integrase, no mutations associated with resistance against integrase strand transfer inhibitor were found (data not shown).

Discussion

Testing, immediate treatment and preventative measures for HIV are interventions that require major public health commitment. A better understanding of spatiotemporal clustering during AEHI can provide information about transmission networks in specific populations and as such can help make evidence-informed decisions about targeted prevention measures for distinct demographic groups in specific regions. In this large sample of persons with AEHI in the Netherlands, we found multiple small AEHI clusters in mostly separate geographic locations, often occurring within six months, but the majority of participants (111/134) were not part of

a cluster. This suggests that transmission during AEHI among MSM during our study period largely resulted from independent onward transmission events, rather than from large clustered outbreaks. This implies that these independent transmission events likely represent transmission from an index case with a still undiagnosed HIV infection, or from someone on ART who is not virally suppressed. Concerning the former, identifying such individuals may benefit from optimized partner notification, availability of self-testing as well as enhanced surveillance by medical specialists in hospitals, at local sexual health clinics, and by general practitioners. Emphasis should be placed on the extension of strategies to test and immediately treat acute HIV infection that have proven to be effective in a Dutch setting (16).

By comparison, most (2-5) but not all (17) previously reported studies in similar settings did describe substantial clustered transmission during AEHI. Importantly, these studies were conducted at a time when immediate start of cART following diagnosis, improved diagnosis of AEHI, and the roll-out of PrEP, were not yet widely implemented. These factors likely contributed to the reduced clustering of infections during AEHI which we observed in the NOVA, which is a more recently established prospective cohort study. In terms of public health commitment, the findings of this study therefore underline the importance of continuing such preventive efforts. However, if transmission events during AEHI are indeed mainly independent, this would preclude the opportunity of concentrating public health efforts on specific networks, and would support spending of public health resources on overall high PrEP coverage of at-risk populations.

A strength of this analysis is that this study sample is likely to be representative of the Dutch HIV epidemic, since we included approximately one fourth of all individuals diagnosed with AEHI in the Netherlands during the overlapping study inclusion period (18). However, since we did not systematically sequence the general larger HIV population, we were not able to assess the contribution of AEHI to overall HIV transmission occurring in the Netherlands between 2015 and 2021. Moreover, the small clusters that we found in this study may have contained individuals diagnosed with AEHI who did not take part in the NOVA and whose sequence data could therefore not be included, or persons diagnosed outside AEHI who could thus not take part in the NOVA, or undiagnosed individuals.

Conclusion

In this study population consisting predominantly of MSM in a low-incidence high-resource setting, transmission among individuals with AEHI appeared to largely result from independent transmission events rather than large outbreaks resulting from a single AEHI index case. This limited clustering of AEHI cases that we observed is likely the result of active AEHI case finding and direct start of cART, as well as the roll-out over recent years of preventive measures such as PrEP, and provides hope that with such efforts we can eliminate linked transmissions and achieve a future free of HIV transmission.

Author contributions

HP, CR, AV, JvK and DvdV designed the phylogenetic study. AvS and JvK provided baseline and sequence data. HP and DvdV performed the study, analyzed the data, and wrote the first draft. HP, CR, AV, AvS, GdB, MD, JP, PR, JvK, and DvdV interpreted the results and critically revised subsequent versions of the manuscript. All authors approved the final version of the manuscript.

Funding

The NOVA is part of the H-TEAM Initiative which is supported by Aids Fonds (grant number: 2013169); Amsterdam Dinner Foundation, Bristol-Myers Squibb International Corp (study number: AI424-541); Gilead Sciences Europe Ltd (grant number: PA-HIV-PREP-16-0024); Gilead Sciences (protocol numbers: CO-NL-276-4222, CO-US-276-1712); Janssen Pharmaceuticals (reference number: PHNL/JAN/0714/0005b/1912fde); M.A.C. AIDS Fund, ViiV Healthcare (PO numbers: 3000268822, 3000747780); and ZonMw (grant number: 522002003). The ATHENA cohort is managed by Stichting HIV Monitoring and supported by a grant from the Dutch Ministry of Health, Welfare and Sport through the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment.

Transparency declarations

The following authors have received grants and honoraria: GdB from Bristol-Meyer Squibbs, Gilead Sciences, ViiV Healthcare, Exevir, Aidsfonds and Takeda, all paid through institution; PR from Gilead Sciences, Janssen Pharmaceutica, ViiV Healthcare and Merck, Gilead Sciences, all paid to his institution; CR from AIDSfonds, ZonMW, Dutch Federation Medical Specialists, Health Holland, Merck, Janssen-Cilag, Gilead and ViiV Healthcare; JvK from NIH, ZonMW, Health Holland and Aidsfonds. All other authors declared no conflicts of interest.

Acknowledgements

We would kindly like to thank all participants of the NOVA cohort study. We thank Ellsworth Campbell and Bill Switzer from the CDC and Dmitry Kireev for their kind help with the analysis. We would also like to thank the members of the Erasmus MC HIV Eradication Group (EHEG), all medical doctors, research nurses, and laboratory personnel involved in the NOVA cohort study at the Amsterdam University Medical Centre, the Erasmus University Medical Centre, Maasstad Hospital Rotterdam, Onze Lieve Vrouwe Gasthuis, DC Klinieken, UMC Utrecht, Radboud UMC, Leiden UMC, and Rijnstate Hospital for enrolment, follow up and (handling of) sampling of NOVA participants, including Karin Grintjes, Reinout van Crevel, Janette Rahamat-Langendoen, Henk Scheper, Jutte de Vries, Annouschka Weijsenfeld, Frank Pijnappel, Agnes Harskamp, Neeltje Kootstra, Suzanne Jurriaans, Marc van der Valk, Hans-Erik Nobel, Arne van Eeden, Loek Elsenburg, Kees Brinkman, Imke Hooijenga, Janneke Stalenhoef, Mark Claassen, Petra van Bentum, Jan den Hollander, Jannigje Smit, Yvonne

Muller, Peter Katsikis, Tokameh Mahmoudi, Rob Gruters, Andy Hoepelman, Monique Nijhuis, Anne Wensing. We would also like to thank Stichting HIV Monitoring for providing data collection and management support, including Mariska Hillebregt, and Leonie de Groot – Berndsen.

References

1. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis. 2005;191(9):1403-9.

2. Bezemer D, van Sighem A, Lukashov VV, van der Hoek L, Back N, Schuurman R, et al. Transmission networks of HIV-1 among men having sex with men in the Netherlands. AIDS. 2010;24(2):271-82.

3. Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al. High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis. 2007;195(7):951-9.

4. Pao D, Fisher M, Hue S, Dean G, Murphy G, Cane PA, et al. Transmission of HIV-1 during primary infection: relationship to sexual risk and sexually transmitted infections. AIDS. 2005;19(1):85-90.

5. Yerly S, Vora S, Rizzardi P, Chave JP, Vernazza PL, Flepp M, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS. 2001;15(17):2287-92.

6. Bezemer D, Cori A, Ratmann O, van Sighem A, Hermanides HS, Dutilh BE, et al. Dispersion of the HIV-1 Epidemic in Men Who Have Sex with Men in the Netherlands: A Combined Mathematical Model and Phylogenetic Analysis. PLoS Med. 2015;12(11):e1001898; discussion e.

7. Miller WC, Rosenberg NE, Rutstein SE, Powers KA. Role of acute and early HIV infection in the sexual transmission of HIV. Curr Opin HIV AIDS. 2010;5(4):277-82.

8. Powers KA, Ghani AC, Miller WC, Hoffman IF, Pettifor AE, Kamanga G, et al. The role of acute and early HIV infection in the spread of HIV and implications for transmission prevention strategies in Lilongwe, Malawi: a modelling study. Lancet. 2011;378(9787):256-68.

9. Ratmann O, van Sighem A, Bezemer D, Gavryushkina A, Jurriaans S, Wensing A, et al. Sources of HIV infection among men having sex with men and implications for prevention. Sci Transl Med. 2016;8(320):320ra2.

10. Dijkstra M, Prins H, Prins JM, Reiss P, Boucher C, Verbon A, et al. Cohort profile: the Netherlands Cohort Study on Acute HIV infection (NOVA), a prospective cohort study of people with acute or early HIV infection who immediately initiate HIV treatment. BMJ Open. 2021;11(11):e048582.

11. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. 2003;17(13):1871-9.

12. Stanford University HIV Drug Resistance Database. Available online at https://hivdb.stanford.edu/hivdb/by-sequences/. Accessed on August 30.

13. de Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, et al. An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics. 2005;21(19):3797-800.

14. Boender TS, Smit C, Sighem AV, Bezemer D, Ester CJ, Zaheri S, et al. AIDS Therapy Evaluation in the Netherlands (ATHENA) national observational HIV cohort: cohort profile. BMJ Open. 2018;8(9):e022516.

15. van Sighem A, Wit F, Boyd A, Smit C, Matser A, Reiss P. Monitoring Report 2019. Human Immunodeficiency Virus (HIV) Infection in the Netherlands. Amsterdam: Stichting HIV Monitoring, 2020. Available online at www.hiv-monitoring.nl.

16. Dijkstra M, van Rooijen MS, Hillebregt MM, van Sighem A, Smit C, Hogewoning A, et al. Decreased Time to Viral Suppression After Implementation of Targeted Testing and Immediate Initiation of Treatment of Acute Human Immunodeficiency Virus Infection Among Men Who Have Sex With Men in Amsterdam. Clin Infect Dis. 2021;72(11):1952-60.

17. Dennis AM, Pasquale DK, Billock R, Beagle S, Mobley V, Cope A, et al. Integration of Contact Tracing and Phylogenetics in an Investigation of Acute HIV Infection. Sex Transm Dis. 2018;45(4):222-8.

18. Number of AHI diagnoses in the Netherlands between 15 July 2015 and 15 April 2021 based on data from Stichting HIV Monitoring, 18 March 2022. Personal communication. .

Table 1. Baseline characteristics of 134 NOVA cohort study participants

	n or median	% or IQR
Age in years	36	28-47
Gender		
Male	130	97.0%
Female	4	3.0%
Region of birth[a]		
Europe	103	77.4%
Caribbean	10	7.5%
South America	10	7.5%
Other	10	7.5%
MSM		
Yes	117	87.3%
No	6	4.5%
Unknown	11	8.2%
Site of HIV diagnosis		
STI clinic	65	48.5%
General practice	39	29.1%
Hospital	18	13.4%
Other[b]	8	6.0%
Unknown	4	3.0%
Subtype		
B	98	73.1%
Non-B	36	26.9%
A	10	

CRF02_AG	7	
C	6	
CRF01_AE	5	
F	4	
CRF06_cpx	2	
CRF47_BF	1	
G + CRF02_AG[c]	1	
Fiebig stage		
I	3	2.2%
II	17	12.7%
III	5	3.7%
IV	37	27.6%
V	39	29.1%
VI[d]	19	14.2%
Unknown	14	10.4%
Plasma viral load (log10 copies/ml)[e]	5.8	4.7-6.7
CD4+ T cell count (cells/µL)[f]	510	355-683
CD8+ T cell count (cells/µL)[g]	941	550-1485
CD4/CD8 T cell ratio[f]	0.5	0.4-0.9

ART, antiretroviral therapy; MSM, men who have sex with men; STI; sexually transmitted infection.

[a]1 value missing, [b]community-based testing (n=4), own initiative (n=4); [c]G + CRF02_AG was based on a best match (4.52%) with this subtype according to the Stanford University HIV Drug Resistance Database (12); [d]Participants diagnosed in Fiebig VI could only be included in case of a negative test result available in the previous six months; [e]4 values missing; [f]4 values missing; [g]5 values missing; [h]5 values missing.

Table 2. HIV transmission clusters between 2015-2021 involving NOVA participants and their baseline characteristics

Sex	Subtype	HIV diagnosis	Region enrolled	Fiebig at diagnosis	CD4+ count at diagnosis	Plasma HIV-RNA at diagnosis	Mutations at baseline	Drug class
Male Male	B B	09/2017 07/2019	Rotterdam Rotterdam	F6 F5	700 300	22600 19400000	T215S T215S	NRTI NRTI
Male Male	B B	08/2019 11/2019	Rotterdam Amsterdam	F2 Unknown	270 808	33100000 2263455	T215S T215S	NRTI NRTI
Male Male	B B	10/2015 12/2015	Amsterdam Amsterdam	F4 F6	530 590	84675 33862	None None	None None
Male Male	B B	10/2018 04/2019	Amsterdam Amsterdam	F2 F3	580 340	8356721 10000000	None None	None None
Male Male	B B	10/2018 12/2018	Rotterdam Rotterdam	F4 F5	200 1200	10000000 46700	None None	None None
Male Male	B B	06/2018 03/2018	Amsterdam Amsterdam	F5 F5	750 530	4379 69028	None None	None None
Male	B B	03/2016 08/2016	Amsterdam	F5 F1	250 510	1420000 98700	None None	None

Male Male Male	B B	03/2019 08/2017	Amsterdam Amsterdam Leiden	F6 F5	270 269	1195913 41400	None None	None None None
Male Male	B B	08/2017 01/2018	Leiden Leiden	F5 F6	438 680	16000 88400	None None	None None
Male Male	B B	10/2016 04/2017	Amsterdam Amsterdam	F5 F6	470 705	650000 775502	None None	None None
Male Male Male	CRF02_AG CRF02_AG CRF02_AG	05/2017 09/2017 08/2017	Rotterdam Rotterdam Rotterdam	F5 F5 F6	270 420 570	1060000 376000 43300	None None None	None None None

NRTI, nucleoside reverse transcriptase inhibitors. Participants diagnosed in Fiebig VI could only be included in case of a negative test result available in the previous six months; CD4+ T cell counts are presented in cells/μL; HIV-RNA is presented in RNA copies/mL.