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

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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 1000 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.

Conclusion: 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.

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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 preexposure 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-naive 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 diagnosed with AEHI who are willing to start combination antiretroviral therapy (cART) within 24 h of enrolment [10]. AEHI is defined as the first 6 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 6 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 with HIV (PWH) 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 PWH 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 reverse transcriptase \( (n = 52) \). The HIV-1 pol sequences were aligned to 53 reference sequences from known subtypes from group M (subtypes A–K and circulating recombinant forms). For each of the participants’ sequences, 10 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. As 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 1000 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 \( (\text{IQR} \ 4.7–6.7) \) log10 copies/ml. The median CD4+ T-cell count at baseline was 510 \( (\text{IQR} \ 355–683) \) cells/μl, and CD4+ T-cell counts of below...
Table 1. Baseline characteristics of 134 NOVA cohort study participants.

| Characteristic                  | n or median | Percent or IQR |
|--------------------------------|-------------|----------------|
| Age in years                   | 36          | 28–47          |
| Gender                         |             |                |
| Male                           | 130         | 97.0%          |
| Female                         | 4           | 3.0%           |
| Region of birth*               |             |                |
| 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†                         | 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           |                |
| CRF07_BF                       | 1           |                |
| G + CRF02_AG†                  | 1           |                |
| Fiebig stage                   |             |                |
| I                              | 3           | 2.2%           |
| II                             | 17          | 12.7%          |
| III                            | 5           | 3.7%           |
| IV                             | 37          | 27.6%          |
| V†                             | 39          | 29.1%          |
| VI†                            | 19          | 14.2%          |
| Unknown                        | 14          | 10.4%          |
| Plasma viral load (log10 copies/ml)\(^{†}\) | 5.8 | 4.7–6.7 |
| CD4\(^+\) T-cell count (cells/μl)\(^\text{a}\) | 510 | 355–683 |
| CD8\(^+\) T-cell count (cells/μl)\(^\text{a}\) | 941 | 550–1485 |
| CD4\(^+\)/CD8\(^+\) T-cell ratio\(^\text{a}\) | 0.5 | 0.4–0.9 |

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 10 clusters, eight of which included two NOVA participants, and two of 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; 34.8%) were enrolled in the region of Rotterdam. In 8 of 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 4 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 3 years. In 8 of 10 clusters, individuals received their diagnosis within a maximum of 6 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 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 preventive 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 6 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, as we included approximately one-fourth of all individuals diagnosed with AEHI in the Netherlands during the overlapping study inclusion period [18]. However, as 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.

In 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.

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| Sex | Subtype | HIV diagnosis | Region enrolled | Fiebig at diagnosis | CD4<sup>+</sup> count at diagnosis | Plasma HIV-RNA at diagnosis | Mutations at baseline | Drug class |
|-----|---------|---------------|-----------------|--------------------|-----------------------------------|---------------------------|----------------------|------------|
| Male | B       | 09/2017       | Rotterdam       | F6                 | 700                               | 22 600                    | T215S                | NRTI       |
| Male | B       | 07/2019       | Rotterdam       | F5                 | 300                               | 19 400 000               | T215S                | NRTI       |
| Male | B       | 08/2019       | Rotterdam       | F2                 | 270                               | 33 100 000               | T215S                | NRTI       |
| Male | B       | 11/2019       | Amsterdam       | Unknown            | 808                               | 2 263 455                | T215S                | NRTI       |
| Male | B       | 10/2015       | Amsterdam       | F4                 | 530                               | 84 675                   | None                 | None       |
| Male | B       | 12/2015       | Amsterdam       | F6                 | 590                               | 33 862                   | None                 | None       |
| Male | B       | 10/2018       | Amsterdam       | F2                 | 580                               | 8 356 721                | None                 | None       |
| Male | B       | 04/2019       | Amsterdam       | F3                 | 340                               | 10 000 000               | None                 | None       |
| Male | B       | 10/2018       | Rotterdam       | F4                 | 200                               | 10 000 000               | None                 | None       |
| Male | B       | 12/2018       | Rotterdam       | F5                 | 1200                              | 46 700                   | None                 | None       |
| Male | B       | 06/2018       | Amsterdam       | F5                 | 750                               | 43 79                    | None                 | None       |
| Male | B       | 03/2018       | Amsterdam       | F5                 | 530                               | 69 028                   | None                 | None       |
| Male | B       | 03/2016       | Amsterdam       | F5                 | 250                               | 1 420 000                | None                 | None       |
| Male | B       | 08/2016       | Amsterdam       | F1                 | 510                               | 98 700                   | None                 | None       |
| Male | B       | 03/2019       | Amsterdam       | F6                 | 270                               | 1 195 913                | None                 | None       |
| Male | B       | 08/2017       | Leiden          | F5                 | 269                               | 41 400                   | None                 | None       |
| Male | B       | 08/2017       | Leiden          | F6                 | 438                               | 16 000                   | None                 | None       |
| Male | B       | 01/2018       | Leiden          | F6                 | 680                               | 88 400                   | None                 | None       |
| Male | B       | 10/2016       | Amsterdam       | F5                 | 470                               | 650 000                  | None                 | None       |
| Male | B       | 04/2017       | Amsterdam       | F6                 | 705                               | 775 502                  | None                 | None       |
| Male | CRF02_AG| 05/2017       | Rotterdam       | F5                 | 270                               | 1 060 000                | None                 | None       |
| Male | CRF02_AG| 09/2017       | Rotterdam       | F5                 | 420                               | 376 000                  | None                 | None       |
| Male | CRF02_AG| 08/2017       | Rotterdam       | F6                 | 570                               | 43 300                   | None                 | None       |

Participants diagnosed in Fiebig VI could only be included in case of a negative test result available in the previous 6 months; CD4<sup>+</sup> T-cell counts are presented in cells/m<sup>3</sup>; HIV-RNA is presented in RNA copies/ml. NRTI, nucleoside reverse transcriptase inhibitors.
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Conflicts of interest
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