Title:
Viral loads within 6 weeks after diagnosis of HIV infection in early and later stages

Short running title:
Viral loads in early HIV infection

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ABSTRACT

Background: Early (including acute) HIV infection has been reported to be associated with viral loads higher than those in later stages. We examined this relationship near the time of diagnosis, using data reported to the US National HIV Surveillance System.

Methods: We analyzed data on infections diagnosed in 2012-2016 and reported through December 2017. Diagnosis and staging were based on the 2014 US surveillance case definition for HIV infection. We divided early HIV-1 infection (stage 0) into two subcategories. In subcategory 0α, a negative or indeterminate HIV-1 antibody test was ≤60 days after the first confirmed positive HIV-1 test; or a negative/indeterminate antibody test or qualitative HIV-1 nucleic acid test (NAT) was ≤180 days before the first positive test, the latter being a NAT or detectable viral load. In subcategory 0β, a negative/indeterminate antibody test or qualitative NAT was ≤180 days before the first positive test, the latter being an HIV antibody or antigen/antibody test. We compared median earliest viral loads for each stage and subcategory in each of the first 6 weeks after diagnosis, using only the earliest viral load for each individual.

Results: Of 203,392 infections, 115,297 (57%) were reported with a quantified earliest viral load within 6 weeks after diagnosis and criteria sufficient to determine the stage at diagnosis. Among 5,081 infections in stage 0, the median earliest viral load fell from 694,000 copies/mL in week 1 to 125,022 in week 2, and 43,473 by week 6. Among 30,910 infections in stage 1, the median earliest viral load ranged from 15,412 to 17,495. Among 42,784 infections in stage 2, the median viral load declined from 44,973 in week 1 to 38,497 in week 6. Among 36,522 infections in stage 3 (AIDS), the median viral load dropped from 205,862 in week 1 to 119,000 in week 6. The median earliest viral load in stage-0 subcategory 0α fell from 1,344,590 copies/mL in week 1 to 362,467 in week 2, and 47,320 in week 6, while that in subcategory 0β was 70,114 copies/mL in week 1, and then ranged from 32,033 to 44,067 in week 6.
weeks 2-6. The median viral load in subcategory 0α was higher than that in subcategory 0β in each of the first 6 weeks after diagnosis ($P < .001$).

**Conclusion:** In the 1st week after diagnosis, viral loads in early infections are generally much higher than those in infections in later stages at diagnosis. By the 3rd week, however, they are generally lower than those in stage 3. High viral loads in early infection are much more common in subcategory 0α than in subcategory 0β, consistent with 0α comprising mostly acute infections, and 0β comprising mostly post-acute early infections. These findings may inform prioritization of interventions for prevention.

**KEY WORDS**

early HIV infection; acute HIV infection; primary HIV infection; viral load; HIV testing
Introduction

The 2014 revision of the US surveillance case definition for human immunodeficiency virus (HIV) infection added “stage 0” to its staging system to represent early infection (assumed to last about 6 months after the start of infection). HIV infections are classified in stage 0 if they have evidence of being early—negative or indeterminate HIV test results near the time of diagnosis. Otherwise, they are classified in later stages—1, 2, or 3 (acquired immunodeficiency syndrome [AIDS]) [1]. Prompt recognition of infections in stage 0 can provide a critical opportunity to prevent transmission of HIV infection during acute (or primary) infection (part of stage 0). Acute infection is often associated with very high viral loads [2-7], which increase the risk of transmission [8]. Intervention would include antiretroviral treatment to suppress the viral load, and the provision of “partner services,” in which public health workers interview the patient to identify sex or needle-sharing partners in the past 12 months, trace the partners, and offer them HIV testing, counseling, and linkage to care, as appropriate [9,10]. If infected, such partners are likely also to have early infection.

This analysis was intended primarily to document the high viral loads that justify giving priority to stage-0 infections for intervention to prevent transmission. For that purpose, we compared median earliest viral loads by week in the first 6 weeks after diagnosis for each stage (0, 1, 2, or 3). In addition, to explore the possibility that the highest priority for intervention should be given to the subcategory of stage 0 that approximates acute infection (expected to have the highest viral loads), rather than given equally to the subcategory that consists mostly of post-acute early infection (with lower viral loads), we compared median viral loads among those two subcategories.
Methods

Data

We used data for the 203,392 HIV infections diagnosed during 2012-2016 and reported to the National HIV Surveillance System (NHSS) of the Centers for Disease Control and Prevention (CDC) through December 2017 from the 50 US states, the District of Columbia, Puerto Rico, and the US Virgin Islands. In the software of the NHSS database, the stage at diagnosis can be automatically classified as stage 0 only for HIV infections diagnosed in or after 2014 (when the definition of stage 0 was published [1]). For this analysis, however, we extended the application of the definition of stage 0 retroactively to infections diagnosed in 2012 and 2013 to increase the number of stage-0 diagnoses available for analysis.

We excluded the 48,880 (24.0%) infections for which the stage at diagnosis could not be determined. Their reported data included neither the negative/indeterminate HIV test results required to meet the criteria for stage 0, nor a CD4 T-lymphocyte test result or opportunistic illness diagnosis required to meet the criteria for stage 1, 2, or 3 within 3 months after diagnosis.

We assumed that specimens for earliest viral loads would generally have been collected before antiretroviral therapy started. Therefore, to reveal the natural trend of viral loads before their suppression by antiretroviral drugs, we restricted our analysis to the viral load with the earliest specimen-collection date within 6 weeks after diagnosis for each infection. This restriction removed another 32,047 infections from the analysis because they had no viral load within 6 weeks after diagnosis. The date when antiretroviral drugs were first received was missing for most infections, but was reported to have preceded the date of the first viral load for 2,125 of the remaining infections, so we excluded those too.
To try to avoid erroneous data on earliest viral loads, we excluded another 4,711 cases in which the first viral load was reported to be undetectable or with a value of 0 – 19 copies/mL. Such low values would be unlikely in the absence of antiretroviral prophylaxis or therapy started on the basis of a diagnosis earlier than the date of the reported first positive HIV test. In addition, viral loads reported as 0 – 9 copies/mL may actually have been logarithmically transformed values that could not be compared with the untransformed values on which our analysis was based. Enumerated viral loads reported to be undetectable probably represented the lower limit of the test’s ability to quantify the viral load, rather than its actual value. We also excluded another 167 cases with viral loads for which no numerical value was reported. To calculate the number of days between the diagnosis date and viral load date accurately, we also excluded 165 cases in which data for one or both of these dates were incomplete (e.g., missing the day component). The final analytic file had data on 115,297 HIV infections (each corresponding to the first viral load within 6 weeks after a diagnosis), representing 56.7% of the 203,392 total reported cases diagnosed in 2012-2017.

Definitions

Stage 0: The HIV surveillance case definition published in 2014 says that stage 0 may be recognized based either on testing history—“a negative or indeterminate HIV test … result within 180 days before the first confirmed positive HIV test result”—or on “a testing algorithm: a sequence of tests performed as part of a laboratory testing algorithm that demonstrate the presence of HIV-specific viral markers such as … nucleic acid (RNA or DNA) 0–180 days before or after an antibody test that had a negative or indeterminate result” [1]. Unfortunately, this definition is impractical to apply strictly to NHSS data because our data do not state whether multiple reported tests belong to the same diagnostic algorithm or are from unrelated testing events. Therefore, the definition of stage 0 used for our analysis of NHSS data is based only on the sequence of test HIV results, not on whether they were intended to constitute a
diagnostic algorithm. We classified the stage of disease at diagnosis as stage 0 based on any of the following possible sequences of positive (or reactive) and negative (or nonreactive) or indeterminate HIV test results, diagrammed in Figure 1:

A. 1) The first positive HIV test result was from an antibody or antigen/antibody test, 2) it was accompanied (on the same date) or followed within ≤ 60 days by a negative or indeterminate result from an HIV antibody test or the antibody component of a combination antigen/antibody test, and 3) a positive HIV-1 nucleic acid test (NAT, qualitative or viral load) result was within ≤ 180 days after (or on the same date as) the negative/indeterminate test;

or

B. 1) The first positive HIV test result was an HIV-1 NAT (and there was no positive antibody test on the same date), and 2) the NAT was accompanied or followed within ≤ 60 days by a negative or indeterminate result from an HIV antibody test or the antibody component of a combination antigen/antibody test (regardless of whether there was a later positive antibody test);

or

C. 1) The first positive HIV test result was from an antibody or antigen/antibody test, 2) it was accompanied or followed by a positive NAT, and 3) both positive tests were followed by (were not on the same date as) a negative or indeterminate result from an HIV antibody test or the antibody component of a combination antigen/antibody test that was within ≤ 60 days after the first positive test;

or

D. 1) The first positive HIV test result was from an HIV-1 NAT, 2) it was followed by (was not on the same date as) a positive result from an HIV antibody or antigen/antibody test, and 3) both positive tests were followed by a negative or indeterminate result from an HIV antibody test or the antibody component of a combination antigen/antibody test that was within ≤ 60 days after (not on the same date as) the first positive test (the NAT), but that could have been on the same date as the second positive test (the antibody or antigen/antibody test);
E. 1) A negative result from an HIV-1 qualitative NAT or a negative/indeterminate result from an HIV antibody or antigen/antibody test was followed within ≤ 180 days by (and was not on the same date as) 2) the first positive HIV test result, which was from an HIV-1 NAT; or

F. 1) A negative result from an HIV-1 qualitative NAT or a negative/indeterminate result from an HIV antibody or antigen/antibody test was followed within ≤ 180 days by (and was not on the same date as) 2) the first positive HIV test result, which was from an HIV antibody or antigen test that was confirmed by 3) a positive result from a second (“supplemental”) HIV test of a different (“orthogonal”) type.
Figure 1. Test sequences defining stage-0 preliminary subgroups

A: 0 to 60 days 0 to 180 days
- First positive antibody\(^a\) test date ≤ Negative antibody\(^b\) test date ≤ First positive NAT\(^c\) date

B: 0 to 60 days no time limit
- First positive NAT\(^c\) date ≤ Negative antibody\(^b\) test date < First positive antibody\(^a\) test date (if any)

C: 0 to 60 days
- First positive antibody\(^a\) test date ≤ First positive NAT\(^c\) date < Negative antibody\(^b\) test date

D: 0 to 60 days
- First positive NAT\(^c\) date ≤ First positive antibody\(^a\) test date ≤ Negative antibody\(^b\) test

E: 0 to 180 days
- Date of last negative\(^bd\) test before first positive < First positive test date (NAT\(^c\))

F: 0 to 180 days
- Date of last negative\(^bd\) test before first positive < First positive test date (antibody\(^a\) test)

\(^a\)Positive “antibody” test results include positive results from the antigen component or the antibody component of combination antigen/antibody tests.

\(^b\)“Negative antibody” test results include indeterminate results from supplemental IgG-only supplemental antibody tests and negative results from antigen/antibody tests in which only the antibody component is negative or in which both the antigen and antibody components are negative.

\(^c\)Positive nucleic acid tests (NATs) include qualitative and quantitative (viral load) tests.

\(^d\)Negative nucleic acid tests (NATs) include only qualitative NATs.
We defined preliminary subcategories of stage 0 (0A through 0F) based on each of the above sequences (A through F). Subcategory 0A includes infections recognized as acute based on results from a testing algorithm recommended by the Association of Public Health Laboratories [APHL] and the US Centers for Disease Control and Prevention) [11]. However, it also includes a small proportion of sequences that did not conform exactly to recommendations. For example, in a small percentage of those, the first positive result was from an antibody test that could detect only IgG antibody, which would have been more appropriate as a supplemental test rather than the initial test. In others, the negative test result was from an antigen/antibody test that could detect both IgM and IgG antibodies, which would have been more appropriate as an initial test. Subcategory 0B includes infections recognized as acute based on results from a testing algorithm recommended for populations with a high incidence of HIV infection, in which a specimen for a pooled NAT is collected on the same date as the specimen for an initial HIV antibody immunoassay that had a negative result [12].

Subcategories 0C and 0D would have been the same as 0A and 0B, respectively, except that the negative/indeterminate antibody or antigen/antibody test follows both the positive NAT and the positive antibody or antigen/antibody test, rather than preceding or being between them (Fig. 1). Subcategories 0C and 0D seem not to fit the criteria for stage 0 in the published case definition [1], because their test sequences do not conform to any recommended algorithm and they do not fit a testing history of a negative result within 180 days before the first positive result. We included them in stage 0 for this analysis because we found them to be associated with high viral loads characteristic of acute infection.

Subcategories 0A through 0D are mutually exclusive, but their test sequences could overlap those of subcategories 0E or 0F in some cases. If there was such an overlap, we classified the cases in subcategories 0A through 0D rather than in 0E or 0F, because the former are based on a more recent
negative test result than the latter, and therefore are more likely to represent acute infection.

Subcategories 0E and 0F differ only by the fact that in 0E the first positive test was a NAT, while in 0F the first positive test was an antibody or antigen/antibody test. Since the interval between the negative/indeterminate test and the first positive test in 0E or 0F could be up to 180 days, these two tests would generally not belong to the same testing algorithm, and most likely represent two separate testing events, of which the first received the interpretation that HIV infection was absent, and the second, that HIV was present.

We considered subcategories 0A through 0F as “preliminary” because, after our preliminary analysis showed which of them were associated with high viral loads soon after diagnosis (Table 2), we combined those into a larger subcategory named “0α” (assumed to approximate acute infection), and named the remainder “0β” (assumed to consist mostly of post-acute early infection).

For our analysis, an undetectable viral load before the first positive test result was not accepted as the negative HIV test result indicative of the earliness of the infection in subcategories 0E or 0F. An unpublished investigation (personal communication from Galang RR and Peters PJ, December 2014) found that such undetectable viral loads were not reliable evidence of early infection, but were instead often due to therapy for established infections diagnosed on the basis of earlier test results that had not been reported to the surveillance system.

We did not classify the stage at diagnosis as stage 0 if a test result was reported that contradicted the first positive HIV test result or contradicted the negative or indeterminate HIV test result that would have been the indicator of earliness (i.e., the contradictory test and the test that it contradicted were on same date and of the same type, but had opposite results), because such contradictory results imply that one of
them was erroneous. This happened in about 5% of infections that would otherwise have met the criteria for stage 0; these were instead classified in other stages (1, 2, or 3) and kept in the analysis unless removed for other reasons described above.

Stages 1, 2, or 3 at diagnosis: The criteria for stage 0 took precedence over criteria for more advanced stages. If the criteria for stage 0 were not met, the stage at diagnosis was defined by the earliest criteria for stage 1, 2, or 3 met within 3 months after diagnosis of HIV infection. These criteria were based on a CD4 T-lymphocyte count or percentage indicative of stage 1, 2, or 3, or diagnosis of an opportunistic illness indicative of stage 3 [1]. If earliest criteria were met for different stages (other than stage 0) in the same month, the stage at diagnosis was selected as the most advanced of those stages.

Test date: The date on which the test specimen was collected. This could pertain to the dates of the positive and negative tests used for diagnosis of stage 0 or the date of the earliest viral load after or on the same date as the diagnostic tests. In some cases, the first viral load could function as a diagnostic test.

Diagnosis date for HIV infection: We defined the “diagnosis date” as the earliest date of objective evidence of HIV infection, selected from the earliest of the following 4 possible events: 1) the first positive HIV test (this was the earliest objective evidence for 96% of diagnoses; rarely it was several days earlier than the confirmatory test date in a multi-test algorithm); 2) the first diagnosis of an opportunistic illness indicative of stage 3; 3) the first CD4 T-lymphocyte count or percentage low enough to indicate stage 3; or 4) the first “clinical” diagnosis of HIV infection documented in a medical record, but for which a prior positive HIV test result on which the diagnosis was based could not be found by surveillance staff (accounting for <1% of diagnoses).
Statistical Methods

We calculated the median, 25\textsuperscript{th} percentile, and 75\textsuperscript{th} percentile for the earliest viral loads by week after HIV infection diagnosis for each stage of disease at diagnosis and for each subcategory of stage 0. To assess the possible effect of unreported antiretroviral drugs on the speed of the decline in the viral load by week after diagnosis among all infections diagnosed in stage 0, we compared results for viral loads that had missing information on when antiretroviral drugs were started with results for viral loads reported to have been on or before the date when antiretroviral drugs were started (assumed not to have been affected by such drugs).

To test the statistical significance of differences between median viral loads in two different stages, we did pairwise two-sample Wilcoxon comparisons, using PROC NPAR1WAY in SAS\textsuperscript{®} software, version 9.4 for Windows (SAS Institute; Cary, North Carolina). We used the Dwass, Steel, Critchlow-Fligner (DSCF) option to generate multiple comparisons (e.g., 6 combinations of pairs from 4 categories [stages 0, 1, 2, and 3], or 10 combinations of pairs from 5 categories [stage-0 subcategories 0\textalpha{} and 0\textbeta{} and stages 1, 2, and 3]) for each family of comparisons (one family per week) [13]. We used Holm’s method for stepwise adjustment of the significance threshold for each comparison to account for the number of comparisons in each family [14,15]. This adjustment was needed only if the \( P \) value was \(<.05 \) but \( \geq .001 \).

Results

Among the 115,297 infections that remained in our analysis after we applied the exclusion criteria described above, the stage of disease at diagnosis was stage 0 for 5,081 (4.4\%), stage 1 for 30,910 (26.8\%), stage 2 for 42,784 (37.1\%), and stage 3 for 36,522 (31.7\%). Among the infections in stage 0, the median earliest viral load fell from 694,000 copies/mL in week 1 to 125,022 in week 2, and 43,473
by week 6 (Table 1). In stage 1, the median earliest viral load alternated weekly between increasing and
decreasing, ranging from a high of 17,495 copies/mL in week 2 to a low of 15,412 in week 5. In stage 2,
the viral load declined from 44,973 copies/mL in week 1 to 38,497 in week 6. In stage 3, the viral load
dropped from 205,862 copies/mL in week 1 to 167,297 in week 2, and to 119,000 by week 6. In week 1,
the median earliest viral load for diagnoses in stage 0 was much higher than that for diagnoses in stages
2, or 3 (P < .001 for comparison of each pair of results), but by week 2 it did not differ significantly
from that for diagnoses in stage 3 (P = .053), and by week 3, it had fallen below that for diagnoses in
stage 3 (P < .001). The median viral load for diagnoses in stage 0 was higher than that for diagnoses in
stage 2 in weeks 1 to 4 (P < .001), but did not differ from it in week 5 or 6 (P > .58).
Table 1. Median earliest viral load (in copies/mL), by week after diagnosis and stage of
disease at diagnosis of HIV infection, comparing stages 0, 1, 2, and 3

<table>
<thead>
<tr>
<th>Stage</th>
<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>2,635</td>
<td>694,000</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>934</td>
<td>125,022</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>629</td>
<td>70,886</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>408</td>
<td>55,734</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>277</td>
<td>52,067</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>198</td>
<td>43,473</td>
</tr>
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<table>
<thead>
<tr>
<th>Stage</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>17,098</td>
<td>44,973</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>8,968</td>
<td>42,892</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>6,357</td>
<td>39,800</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4,523</td>
<td>40,045</td>
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<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3,326</td>
<td>41,549</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2,512</td>
<td>38,497</td>
</tr>
</tbody>
</table>
In the first week after diagnosis, infections in stage-0 preliminary subcategories 0A, 0B, 0C, 0D, and 0E had median viral loads exceeding 1.2 million copies/mL, compared with only 70,114 copies/mL for infections in subcategory 0F (Table 2). In week 2, median viral loads for subcategories 0A through 0E remained higher (>290,000 copies/mL) than the 44,467 copies/mL for subcategory 0F, as well as higher than the median viral loads for stages 1 (17,495), 2 (42,892), and 3 (167,297) \((P < .001\) for each of these comparisons). Subcategories 0E and 0F were both defined by a negative test result within 180 days before the first positive test result (a positive NAT for 0E and a positive antibody test for 0F), but their earliest viral loads in the first 2 weeks after diagnosis differed greatly (Table 2). This difference may be explained in part by the interval between the stage-0-defining negative and first positive tests being short (1 or 2 weeks) for a much greater proportion of infections in subcategory 0E (51% [130 of 257]) than for infections in subcategory 0F (9% [139 of 1,541]), and by the median value for this interval being only 9 days for subcategory 0E, as compared with 98 days for subcategory 0F. Due to the small number of observations per week after week 2 for subcategories 0D and 0E, we combined weeks 3 through 6 into a single time period in Table 2. Because of the similarity of findings for preliminary subcategories 0A through 0E evident in Table 2, we combined them into a larger subcategory named “0α” for further analysis by week, and renamed subcategory 0F as “0β” (Table 3).
Table 2. Median earliest viral load (in copies/mL), by week after diagnosis and stage of disease at diagnosis of HIV infection, comparing stage-0 preliminary subcategories 0A through 0F

<table>
<thead>
<tr>
<th>Subcategory of stage 0</th>
<th>0A</th>
<th>0B</th>
</tr>
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<tbody>
<tr>
<td>Week</td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1st</td>
<td>1,083</td>
<td>1,258,925</td>
</tr>
<tr>
<td>2nd</td>
<td>426</td>
<td>338,888</td>
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<table>
<thead>
<tr>
<th>Subcategory of stage 0</th>
<th>0C</th>
<th>0D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1st</td>
<td>187</td>
<td>1,780,000</td>
</tr>
<tr>
<td>2nd</td>
<td>76</td>
<td>635,690</td>
</tr>
<tr>
<td>3rd – 6th</td>
<td>71</td>
<td>75,300</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subcategory of stage 0</th>
<th>0E</th>
<th>0F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1st</td>
<td>224</td>
<td>1,346,776</td>
</tr>
<tr>
<td>2nd</td>
<td>17</td>
<td>294,798</td>
</tr>
<tr>
<td>3rd – 6th</td>
<td>16</td>
<td>135,540</td>
</tr>
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</table>
Table 3. Median earliest viral load (in copies/mL), by week after diagnosis and stage of disease at diagnosis of HIV infection, comparing stage-0 subcategory 0α with subcategory 0β

<table>
<thead>
<tr>
<th>Week</th>
<th>Subcategory of stage 0</th>
<th>Subcategory of stage 0β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0α (= 0A + 0B + 0C + 0D + 0E)</td>
<td>0β (= 0F)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>1st</td>
<td>2,027</td>
<td>1,344,590</td>
</tr>
<tr>
<td>2nd</td>
<td>598</td>
<td>362,467</td>
</tr>
<tr>
<td>3rd</td>
<td>375</td>
<td>122,970</td>
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<tr>
<td>4th</td>
<td>243</td>
<td>77,100</td>
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<tr>
<td>5th</td>
<td>177</td>
<td>61,414</td>
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<tr>
<td>6th</td>
<td>120</td>
<td>47,320</td>
</tr>
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</table>
Median viral loads in stage-0 subcategory 0α were higher than those in subcategory 0β in each of the first 4 weeks after diagnosis ($P < .001$ for weeks 1, 2, and 3, and $P = .008$ for week 4 [significant compared to a Holm-adjusted threshold of $P = .017$]), but did not differ significantly from them in weeks 5 or 6 ($P > .39$) (Table 3). The median viral load for stage-0 subcategory 0α was also much higher than that for stage 3 in weeks 1 and 2 ($P < .001$), but did not differ from it in week 3 ($P = .088$, and was lower than that for stage 3 in weeks 4 – 6 ($P < .001$) (Fig. 2). Median viral loads for subcategory 0α were higher than those for stage 2 in weeks 1 through 4 ($P < .001$), but did not differ from them in weeks 5 or 6 ($P > .14$). The median viral load for subcategory 0β was greater than that for stage 2 in week 1 ($P < .001$), but did not differ from it in later weeks ($P > .88$). In every week, the median viral load for stage-0 subcategory 0β was greater than that for stage 1, and the median viral loads for stages 1, 2 and 3 differed significantly from one another in the same direction as the order of their names (i.e., $1 < 2 < 3$) ($P < .001$).
Figure 2. Median earliest viral load (in copies/mL), by week after diagnosis and stage of disease at diagnosis of HIV infection, comparing stages 0, 1, 2, and 3, and stage-0 subcategories 0α (= 0A + 0B + 0C + 0D + 0E) and 0β (= 0F)

Copies/mL

Stage 0α

Stage 0

Stage 3

Stage 0β
Although high viral loads were much more common among infections diagnosed in subcategory 0α than among those in subcategory 0β or stages 1, 2, or 3, a small percentage of infections subcategory 0β and those other stages did have high viral loads. Earliest viral loads exceeding 500,000 copies/mL occurred in 11.6% (179/1,541) of infections in subcategory 0β, 4.2% (1,310/30,910) of those in stage 1, 7.1% (3,039/42,784) of those in stage 2, and 24.2% (8,831/36,522) of those in stage 3, as compared with 50.0% (1,754/3,540) of those in subcategory 0α. Among infections in subcategory 0β having a viral load of >500,000 copies/mL, the interval between the first positive test and the last prior negative test ranged from 1 to 176 days (with a median of 66 days); it could have been up to 180 days by the definition of 0β.

Among the 3,540 infections diagnosed in subcategory 0α, the decline in the median earliest viral loads in the subgroup of 2,834 cases with missing information on when antiretroviral drugs were started was similar to that in the subgroup of 706 cases in which antiretroviral drugs were reported to have been started no earlier than the date on which the viral load specimen was collected. In each of these subgroups, the median earliest viral load decreased by more than half from week 1 to week 2, and did so again from week 2 to week 3. By week 4, the median viral load in each subgroup had decreased >95% compared with its value for week 1 (from 1,298,413 to 41,687 copies/mL among the 2,834 cases with missing antiretroviral information, and from 1,485,669 to 73,809 copies/mL among the 706 cases reported to have started antiretroviral drugs no earlier than the viral load date).

Discussion

Principal Results

Our findings confirmed that early HIV infection, represented by stage 0, is associated with higher viral loads than those in infections diagnosed in later stages of disease. However, this was true mainly in the
first week after diagnosis, when the median viral load among infections diagnosed in stage 0 was more than three times that among infections diagnosed in stage 3.

We also found that stage-0 preliminary subcategories 0A, 0B, 0C, 0D, and 0E, which we combined as subcategory 0α, were associated with higher viral loads than those in preliminary subcategory 0F, which we renamed subcategory 0β (stage 0 = 0α + 0β). This difference should be expected, because most of 0α (preliminary subcategories 0A, 0B, 0C, and 0D) is limited to diagnoses in which a negative/indeterminate antibody test result indicative of the earliness of infection was either on the same date as or ≤60 days after the first positive HIV test date, and the median interval between the first positive HIV test and the last prior negative test was much shorter for infections in preliminary subcategory 0E (9 days) than for those in subcategory 0F (98 days). Thus, a negative/indeterminate test result was closer in time to viral loads in the first week after diagnosis for most infections in subcategory 0α than it was for most infections in subcategory 0β. This allowed most infections in subcategory 0β to have enough time for complete seroconversion and a decline in the viral load before it was first measured. This difference between subcategories 0α and 0β could justify more urgent intervention to suppress the viral load and to provide partner services to prevent transmission if infections are diagnosed in subcategory 0α than if they are diagnosed in subcategory 0β. If so, the NHSS software should be upgraded to distinguish automatically between stage-0 subcategories 0α and 0β, to help health departments to account for this difference when they prioritize prevention efforts.

On the other hand, 12% of infections in subcategory 0β had viral loads greater than 500,000 copies/mL, suggesting that they might be acute infections. This happened despite an interval of as long as 176 days between the first positive HIV test and the last prior negative test, because the infection could have started long after that last negative test, and much nearer to the first positive test date. In addition, a
small percentage of infections in stages 1 and 2 had viral loads exceeding 500,000 copies/mL. These might actually have been acute infections that were misclassified in stages 1 or 2 because they were missing negative or indeterminate HIV test results required to be classified in stage 0. Therefore, when deciding which infections should receive the highest priority for prevention of transmission, consideration should be given not only to whether the criteria are met for stage-0 subcategory 0α, but also to whether other evidence, such as a high viral load near the time of diagnosis, suggests acute infection.

If the restriction of our analysis to each patient’s earliest detectable viral load succeeded in excluding viral loads influenced by antiretroviral drugs, then our findings may accurately reflect the natural trend of viral loads before suppression by drugs. Our success in excluding the influence of antiretroviral drugs is suggested by the similarity of the decline in median earliest viral loads in the subgroup of subcategory 0α in which antiretroviral therapy was reported to have started no earlier than the viral load specimen collection date (when the earliest viral load was assumed not to have been affected by antiretroviral drugs) and those in the larger subgroup with missing information about antiretroviral drugs. It is also consistent with the similarity of the median viral loads we found for subcategory 0α and those found in a cohort of 19 untreated high-risk persons in Thailand [16]. In that cohort, the median viral load peaked at about 2,500,000 copies/mL in week 3 after diagnosis of acute infection, dropped to 63,000 copies/mL in week 6, and remained about the same level up to 144 weeks later in the absence of treatment. Similarly, in our study, the median earliest viral loads for subcategory 0α dropped from about 1,250,000 copies/mL in week 1 to about 40,000 copies/mL in week 6 after diagnosis. In contrast, our findings for subcategory 0α differed from the lower viral loads found in a cohort of 71 persons who received antiretroviral therapy promptly after diagnosis of acute infection, in which the median viral load peaked at about 450,000 copies/mL in week 2 and dropped to about 2,500 copies/mL in week 4, and to less than 100
24 copies/mL after week 12 [16]. If our findings reflect viral loads in the absence of antiretroviral therapy, then they imply that the interval after diagnosis of acute HIV infection in which most viral loads exceed 750,000 copies/mL lasts less than 2 weeks. Thus, urgent antiretroviral therapy to suppress the high viral loads of acute infection may be too late to make much difference if not started very shortly after diagnosis. By the third week after diagnosis, the viral load would probably have declined spontaneously to a level similar to that found in stage 2.

Our analysis was intended to assess the extent to which early infections have extremely high viral loads, as had been reported among acute infections, because such high viral loads demand urgent intervention to prevent transmission. However, such intervention in early infection should perhaps receive high priority even after the acute phase, when the viral load has declined to a more stable lower level, as some studies suggest that the viruses in early infections may be more infectious than those in later infections with similar viral loads. This may be due to a partial immune response that develops in older infections, which might favor viruses having mutations that resist the immune response, but at the expense of reducing their transmissibility [17-19]. In addition, even if an infection is no longer acute by the time of diagnosis, a diagnosis in stage 0 implies that the infection was recently acute and the viral load was therefore probably very high (even if only briefly), so transmission to recent sex partners would be more likely than if the infection had been diagnosed in a later stage. Therefore, such post-acute early infections should also receive high priority for partner services. Another reason to give priority to intervention in stage 0, even after viral loads have declined, is that treatment of HIV infection within 6 months after the start of infection (approximately the time frame of stage 0) could reduce the patient’s risk of morbidity and mortality. Such early treatment is associated with a smaller HIV reservoir size, lower levels of immune activation, and a higher probability of restoration of CD4 T-lymphocyte counts to normal levels [16,20-24].
Limitations

Our analysis was limited by its dependence on the NHSS receiving from health departments the reports of negative/indeterminate HIV test results needed to meet criteria for stage 0. Health departments may be unable to report these results to the NHSS if laboratories and healthcare providers do not report them to health departments. It may also be impossible for healthcare providers to recognize most early HIV infections, because most HIV-infected patients may not present themselves for diagnostic evaluation until after complete seroconversion (when HIV tests would no longer have negative or indeterminate results), and because even patients who arrive during acute HIV infection may not be tested for it until after seroconversion if physicians do not suspect it because its symptoms are nonspecific. Early infections may then be misclassified as later infections, including some as stage 3 (AIDS) because low CD4 T-lymphocyte counts and opportunistic illnesses meeting criteria for stage 3 sometimes occur transiently in acute infection [25, 26].

Our analysis was also limited by the fact that data from the NHSS are incomplete (e.g., missing the day-component of some dates, some test results, information about antiretroviral drugs), and sometimes of questionable quality (e.g., contradictory test results). We compensated for these limitations of NHSS data by cleaning them in various ways, such as by excluding observations with incomplete dates, or supposedly earliest viral loads that were reported to be undetectable or extremely low (0 – 19 copies/mL), and excluding from stage 0 those cases that had contradictory results (positive and negative) from apparently the same type of test on the same date. Even after the exclusion of these observations, our use of NHSS data brought the advantage of a much larger number of observations than could have been obtained from a study limited to patients receiving care from a small number of providers.
We interpreted the decreasing trends in earliest viral loads by week after diagnosis as if they represented results from individuals followed weekly in the absence of therapy, but actually each individual was observed at only one point in time (the date of his earliest viral load). Longitudinal data on weekly viral loads for individuals who were not receiving antiretroviral drugs would have provided a scientifically sounder basis for analyzing viral load trends during the first several weeks after diagnosis, but such a study would not have been practical or ethically feasible, as current treatment guidelines recommend starting therapy soon after diagnosis [27].

Conclusions

In summary, we confirmed that viral loads among infections in early infection (stage 0) are generally much higher than those in later stages at diagnosis, particularly during the first week after diagnosis. By the 4th week, however, they are generally lower than those in stage 3. Viral loads are also higher for subcategory 0α of stage 0 than for subcategory 0β in the first 4 weeks after diagnosis. These findings may be useful in allocating prevention resources by indicating which infections should receive the highest priority for urgent intervention. Where health departments do not have the resources to intervene immediately for all persons with a new diagnosis of HIV infection to ensure linkage to care, counseling to prevent transmission, and partner services, they should give higher priority to those with infections diagnosed in stage 0, especially those in subcategory 0α or known to have recently had a high viral load, and they should try to do so within the first 2 weeks after diagnosis.
References


