Platelet Decline

An Avenue for Investigation Into the Pathogenesis of Human Immunodeficiency Virus–Associated Dementia

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Background: The identification of biomarkers identifying onset of human immunodeficiency virus–associated dementia (HIV-D) is critical for diagnosis and the elucidation of pathophysiologic pathways.

Objective: To examine the association between platelet decline from baseline and HIV-D.

Design: Prospective cohort study within the North-East AIDS Dementia cohort.

Setting: Four participating referral centers in the United States.

Participants: A total of 396 subjects with advanced human immunodeficiency virus (HIV) infection recruited between 1998 and 2003 and undergoing serial neurologic assessments. Eligibility criteria required CD4 cell counts less than 200/µL or less than 300/µL with evidence of cognitive impairment. A cohort subset without prevalent HIV-D at baseline and without incident HIV-D at the visit immediately after baseline was analyzed (n=146).

Main Outcome Measure: Time to first diagnosis of HIV-D.

Results: After a median follow-up of 31.1 months, 40 subjects developed HIV-D. Platelet decline from baseline was associated with the development of HIV-D when examined as a time-dependent variable lagged by 6 to 12 months before outcome (multivariate hazard ratio [HR], 2.39; 95% confidence interval [CI], 1.14-5.02; P=.02). This association was stronger during the first 2 years of follow-up (multivariate HR, 6.76; 95% CI, 2.36-19.41; P<.001) than during later years (multivariate HR, 0.94; 95% CI, 0.33-2.67; P=.90).

Conclusions: These results suggest that individuals with declining platelet counts are at greater risk for HIV-D and that the dynamics of circulating platelets vary with respect to the temporal progression of HIV-D. This highlights an avenue to be explored in the understanding of HIV-D pathogenesis.

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An association between the magnitude of decline in circulating platelet counts and the development of CNS disease was recently identified in the simian immunodeficiency virus model for HIV CNS disease. Simian immunodeficiency virus–infected macaques with moderate to severe CNS disease developed a more precipitous decline from baseline platelet levels during the asymptomatic phase of infection than similarly infected macaques that developed minimal or no CNS lesions. Because this study was performed in an experimental animal model using standardized viral inocula and carefully controlling for potentially confounding factors, further investigation is necessary to determine whether the association between platelet decline and CNS disease holds true in a cohort of patients with HIV.

Thrombocytopenia is a common complication of HIV infection, occurring in 10% of HIV-infected individuals and one-third of patients with AIDS. Immune-mediated peripheral platelet destruction appears to play a primary role in HIV-associated thrombocytopenia. Although a biologic link between decline in platelets and development of HIV-associated CNS disease has yet to be identified, it is possible that the up-regulation of thrombopoietic growth factors in response to peripheral platelet destruction may adversely affect the CNS microenvironment. Platelets also play an integral role as immune effector cells, suggesting that platelet activation could influence both decline in platelet numbers and the development of HIV-D.

The North East AIDS Dementia (NEAD) cohort is a multicenter cohort of patients with advanced HIV disease who are neurologically vulnerable because of low CD4 counts and who have been followed up over time with serial neurologic, neuropsychological, functional, and psychiatric evaluations. This cohort is purposefully enriched to have a high incidence and prevalence of neurologic disease, and is therefore well suited to evaluate the hypothesis that platelet decline from baseline levels is linked to onset of HIV-D.

STUDY POPULATION

The NEAD cohort was formed in 1998 with the aim of evaluating risk factors associated with HIV-D or minor cognitive/motor disorder (MCMD) in individuals with advanced HIV infection. A total of 396 individuals were initially recruited from 4 sites: Columbia University, New York; The Johns Hopkins University, Baltimore, Maryland; University of Rochester, Rochester, New York; and Northwestern University, Chicago, Illinois. Study approval by respective institutional review boards was obtained, and all subjects provided informed consent. Inclusion criteria required HIV seropositivity and CD4 cell counts less than 200/µL. Subjects having CD4 cell counts less than 300/µL and evidence of cognitive impairment on neuropsychological testing were also eligible for inclusion. Exclusion criteria included current or past CNS infection, significant psychiatric disease, or other chronic neurologic disorders. Patients with a history of active substance abuse were not excluded.

PROCEDURES

Subjects were examined every 6 months and completed a standardized neurologic examination as well as neuropsychological, functional, and psychiatric evaluations. Blood samples were collected and assessed for the following variables: plasma HIV RNA levels (Nucleic assay, Organon-Teknika, Durham, North Carolina; limit of detection, 80 copies/mL), CD4 lymphocyte count, hemoglobin levels, and platelet count. Platelet counts and hemoglobin levels were measured in hospital laboratories by means of automated hematologic analyzers.

DEMOGRAPHIC INFORMATION AND HIV-RELATED HISTORY

Data on age, sex, ethnicity, education, drug use, self-reported duration of HIV infection, and route of infection were collected at baseline. Updated information on antiretroviral treatment, current alcohol or other drug use, analgesic use, and presence of concurrent HIV-related diagnoses (Kaposi sarcoma, cytomegalovirus, mycobacterial infections, lymphoma, cryptococcosis, candidiasis, cryptosporidiosis, wasting disease, and recurrent bacterial pneumonia) was collected at each visit. Patients were considered to have HIV-related illness if any of the foregoing syndromes were diagnosed. Antiretroviral therapy was classified as no treatment, treatment with 1 or 2 antiretroviral drugs, or treatment with HAART (≥3 antiretroviral agents).

NEUROLOGIC OUTCOME ASSESSMENT

Patients were categorized according to neurologic status by means of an algorithm based on data compiled from a battery of neurologic, neuropsychological, functional, and psychiatric assessments. Patients were classified as not impaired, having HIV-MCMD, or having HIV-D according to the 1991 American Academy of Neurology criteria. Neuropsychological examination was designed to capture information on verbal memory, visual memory, constructional skills, psychomotor skills, motor skills, reaction time, frontal systems, and general intellectual performance. Functional and psychiatric measures were designed to assess the effect of cognitive impairments on daily functions, as well as to measure stress, stamina, and depression. Readers are encouraged to refer to Sevigny et al and McArthur et al for details on neurologic testing.

STATISTICAL ANALYSIS

The outcome of interest was time from study enrollment to the first diagnosis of HIV-D. For those who did not develop HIV-D, follow-up time was censored at the last available visit. All individuals with prevalent HIV-D at recruitment were excluded from further analysis. Also excluded were individuals who developed HIV-D or were censored at the visit immediately following recruitment. This exclusion was necessary because the principal independent variable of interest, the change from baseline platelet count lagged by 1 study visit, was indeterminate in individuals who developed HIV-D or were censored at the visit immediately following recruitment. Baseline demographic, biological, and neuropsychological characteristics were compared between those who developed HIV-D and those who did not. Group comparisons were based on unpaired, 2-tailed t tests for continuous variables and Fisher exact tests for categorical variables. Associations between variables of interest and time to first diagnosis of HIV-D were examined by means of a Cox proportional hazards model. Platelet count change from baseline was the exposure of interest. Change from baseline was calculated...
by subtracting the value of platelet count at baseline from the value at each subsequent visit. This correction was performed at each time point and accounts for the fact that, although platelet levels are relatively constant in any one healthy individual, there is wide variation in normal platelet counts between individuals.22 All possible differences from baseline for all individuals were calculated. This variable was subsequently dichotomized at the 33rd percentile and examined categorically. Hemoglobin level was similarly examined. Exposures were examined as time-dependent variables at the visit at which transition to HIV-D was being assessed or as time-dependent variables at the visit before transition to HIV-D was being assessed, ie, the time-dependent covariate of interest (hemoglobin or platelet change from baseline) was lagged by 1 study visit (6-12 months before the visit at which transition to HIV-D was being assessed). The rationale for the lagged analysis was based on our hypothesis that changes in the periphery would precede the onset of clinical signs of dementia, as was previously demonstrated in the macaque model of HIV-D.13 Separate models were created for each exposure of interest. Multivariate models were created on the basis of understanding of disease pathogenesis, univariate analyses, and likelihood-ratio testing. Individuals were considered to have virologic control if plasma HIV RNA levels were below the level of detection on at least 50% of the individual’s visits. Models were stratified on the basis of site of enrollment. Goodness of fit for the final model was assessed by use of Cox-Snell residuals. The proportional hazards assumption was tested by means of scaled Schoenfeld residuals and {log [−log

Table 1. Summary Statistics for Demographic Information and Biological Variables at Baseline Stratified by Presence or Absence of HIV-D

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N=146)</th>
<th>Nondemented (n=106)</th>
<th>Demented (n=40)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>42.37 (7.35)</td>
<td>41.81 (7.48)</td>
<td>43.84 (6.88)</td>
<td>.14</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>110 (75.3)</td>
<td>83 (78.3)</td>
<td>27 (67.5)</td>
<td>.20</td>
</tr>
<tr>
<td>Female</td>
<td>36 (24.7)</td>
<td>23 (21.7)</td>
<td>13 (32.5)</td>
<td></td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>42 (28.8)</td>
<td>33 (31.1)</td>
<td>9 (22.5)</td>
<td>.28</td>
</tr>
<tr>
<td>Black</td>
<td>87 (59.6)</td>
<td>63 (59.4)</td>
<td>24 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>17 (11.6)</td>
<td>10 (9.4)</td>
<td>7 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Education, mean (SD), y</td>
<td>12.65 (2.53)</td>
<td>12.80 (2.47)</td>
<td>12.25 (2.69)</td>
<td>.24</td>
</tr>
<tr>
<td>Route of infection, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV needle use</td>
<td>24 (16.4)</td>
<td>14 (13.2)</td>
<td>10 (25.0)</td>
<td>.25</td>
</tr>
<tr>
<td>Sex with men or women</td>
<td>99 (67.8)</td>
<td>75 (70.8)</td>
<td>24 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Other (transfusion, unknown)</td>
<td>23 (15.8)</td>
<td>17 (16.0)</td>
<td>6 (15.0)</td>
<td></td>
</tr>
<tr>
<td>Antiretroviral therapy, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>32 (21.9)</td>
<td>22 (20.8)</td>
<td>10 (25.0)</td>
<td>.17</td>
</tr>
<tr>
<td>1 or 2 ARVs</td>
<td>9 (6.2)</td>
<td>9 (8.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>105 (71.9)</td>
<td>75 (70.7)</td>
<td>30 (75.0)</td>
<td></td>
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<tr>
<td>Plasma HIV RNA level, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Undetectable</td>
<td>28 (21.2)</td>
<td>21 (22.3)</td>
<td>7 (18.4)</td>
<td>.90</td>
</tr>
<tr>
<td>80-10 000 copies/mL</td>
<td>36 (27.3)</td>
<td>26 (27.7)</td>
<td>10 (26.3)</td>
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</tr>
<tr>
<td>&gt;10 000-100 000 copies/mL</td>
<td>45 (34.1)</td>
<td>32 (34.0)</td>
<td>13 (32.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;100 000 copies/mL</td>
<td>23 (17.4)</td>
<td>15 (16.0)</td>
<td>8 (21.1)</td>
<td></td>
</tr>
<tr>
<td>CD4 lymphocyte count/µL, mean (SD)</td>
<td>129.79 (81.15)</td>
<td>129.40 (79.02)</td>
<td>130.82 (87.57)</td>
<td>.92</td>
</tr>
<tr>
<td>Presence of HIV-related disease, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>131 (89.7)</td>
<td>97 (91.5)</td>
<td>34 (85.0)</td>
<td>.34</td>
</tr>
<tr>
<td>Yes</td>
<td>15 (10.3)</td>
<td>9 (8.5)</td>
<td>6 (15.0)</td>
<td></td>
</tr>
<tr>
<td>Daily NSAID use, No. (%)</td>
<td>(n=98)</td>
<td>(n=72)</td>
<td>(n=26)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (15.3)</td>
<td>9 (12.5)</td>
<td>6 (23.1)</td>
<td>.21</td>
</tr>
<tr>
<td>No</td>
<td>83 (84.7)</td>
<td>63 (87.5)</td>
<td>20 (76.9)</td>
<td></td>
</tr>
<tr>
<td>Narcotic analgesia, No. (%)</td>
<td>(n=98)</td>
<td>(n=72)</td>
<td>(n=26)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (14.3)</td>
<td>13 (18.1)</td>
<td>1 (3.8)</td>
<td>.10</td>
</tr>
<tr>
<td>No</td>
<td>84 (85.7)</td>
<td>59 (81.9)</td>
<td>25 (96.2)</td>
<td></td>
</tr>
<tr>
<td>Nonnarcotic analgesia, No. (%)</td>
<td>(n=98)</td>
<td>(n=72)</td>
<td>(n=26)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (19.4)</td>
<td>13 (18.1)</td>
<td>6 (23.1)</td>
<td>.57</td>
</tr>
<tr>
<td>No</td>
<td>79 (80.6)</td>
<td>59 (81.9)</td>
<td>20 (76.9)</td>
<td></td>
</tr>
<tr>
<td>History of drug use, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>136 (93.2)</td>
<td>97 (91.5)</td>
<td>39 (97.5)</td>
<td>.29</td>
</tr>
<tr>
<td>Never</td>
<td>10 (6.8)</td>
<td>9 (8.5)</td>
<td>1 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current use</td>
<td>43 (29.4)</td>
<td>32 (30.2)</td>
<td>11 (27.5)</td>
<td>.84</td>
</tr>
<tr>
<td>No current use</td>
<td>103 (70.6)</td>
<td>74 (69.8)</td>
<td>29 (72.5)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, mean (SD), g/dL</td>
<td>13.33 (1.82)</td>
<td>13.41 (1.86)</td>
<td>13.11 (1.71)</td>
<td>.37</td>
</tr>
<tr>
<td>Platelets, mean (SD), x 10^3/µL</td>
<td>192.74 (73.49)</td>
<td>191.22 (73.94)</td>
<td>196.74 (73.09)</td>
<td>.69</td>
</tr>
</tbody>
</table>

Abbreviations: ARVs, antiretroviral drugs; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HIV-D, HIV-associated dementia; IV, intravenous; NSAID, nonsteroidal anti-inflammatory drug.

a Obtained by unpaired, 2-tailed t test for continuous variables and Fisher exact test for categorical variables.
Of the 396 patients recruited into the cohort, 141 were categorized with prevalent HIV-D at baseline and were excluded from further analysis. Forty-nine individuals had no follow-up beyond baseline. Of the remaining 206 patients, an additional 60 individuals were excluded from further analysis because of incident HIV-D or censoring at the visit immediately following recruitment. This group of 60 was composed of 36 who developed dementia and 24 who were censored in the first 6 months (34 with incident dementia and 17 censored individuals) to 12 months (2 with incident dementia and 7 censored individuals) after recruitment. The final data set contained a total of 146 individuals, of whom 40 patients (27.4%) developed HIV-D. Of these 146 individuals, 65 (44.5%) were diagnosed as having MCMD at baseline. The remaining subjects were considered not cognitively impaired. Median follow-up time was 31.1 months, with a range of 10.9 to 65.3 months. Estimated cumulative incidence of HIV-D was 2.8% at 1 year, 17.4% at 2 years, and 31.0% at 3 years from baseline.

Baseline demographic and biological data, categorized by development of HIV-D, are presented in Table 1. Significant differences between those developing HIV-D and those remaining dementia-free were not evident. Individuals who eventually developed HIV-D had a trend toward longer duration of HIV infection and were older. Platelet levels and hemoglobin levels were comparable between the 2 groups at baseline.

Univariate and multivariate analyses for primary exposures of interest are presented in Table 3. For the model

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**Table 2. Univariate Analyses of Relationships Between Select Demographic and Biological Variables and Risk of HIV-D**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time Varying</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>No</td>
<td>1.03 (0.99-1.07)</td>
<td>.14</td>
</tr>
<tr>
<td>Female sex</td>
<td>No</td>
<td>1.47 (0.76-2.85)</td>
<td>.25</td>
</tr>
<tr>
<td>Race</td>
<td>No</td>
<td>1.39 (0.65-2.99)</td>
<td>.40</td>
</tr>
<tr>
<td>White</td>
<td>No</td>
<td>1.94 (0.72-5.21)</td>
<td>.19</td>
</tr>
<tr>
<td>Black</td>
<td>No</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>No</td>
<td>1.94 (0.72-5.21)</td>
<td>.19</td>
</tr>
<tr>
<td>Education level</td>
<td>No</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>No</td>
<td>1.94 (0.39-2.33)</td>
<td>.91</td>
</tr>
<tr>
<td>College</td>
<td>No</td>
<td>1.96 (0.99-1.15)</td>
<td>.10</td>
</tr>
<tr>
<td>Duration of HIV infection (n=145)</td>
<td>No</td>
<td>1.06 (0.99-1.15)</td>
<td>.10</td>
</tr>
<tr>
<td>Route of infection</td>
<td>No</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Sex men or women</td>
<td>No</td>
<td>1.84 (0.88-3.86)</td>
<td>.10</td>
</tr>
<tr>
<td>Female</td>
<td>No</td>
<td>0.95 (0.39-2.33)</td>
<td>.91</td>
</tr>
<tr>
<td>Race</td>
<td>No</td>
<td>0.92 (0.43-1.94)</td>
<td>.82</td>
</tr>
<tr>
<td>White</td>
<td>Yes</td>
<td>1.07 (0.23-5.06)</td>
<td>.93</td>
</tr>
<tr>
<td>Black</td>
<td>Yes</td>
<td>0.57 (0.26-1.26)</td>
<td>.17</td>
</tr>
<tr>
<td>Other</td>
<td>Yes</td>
<td>0.52 (0.25-1.09)</td>
<td>.08</td>
</tr>
<tr>
<td>Education level</td>
<td>Yes</td>
<td>1.39 (0.65-2.99)</td>
<td>.40</td>
</tr>
<tr>
<td>High school</td>
<td>Yes</td>
<td>1.94 (0.72-5.21)</td>
<td>.19</td>
</tr>
<tr>
<td>College</td>
<td>Yes</td>
<td>1.94 (0.39-2.33)</td>
<td>.91</td>
</tr>
<tr>
<td>Duration of HIV infection (n=145)</td>
<td>Yes</td>
<td>1.94 (0.39-2.33)</td>
<td>.91</td>
</tr>
<tr>
<td>Route of infection</td>
<td>Yes</td>
<td>1.94 (0.39-2.33)</td>
<td>.91</td>
</tr>
<tr>
<td>Sex men or women</td>
<td>Yes</td>
<td>0.72 (0.30-1.72)</td>
<td>.46</td>
</tr>
<tr>
<td>Female</td>
<td>Yes</td>
<td>0.79 (0.27-2.25)</td>
<td>.66</td>
</tr>
<tr>
<td>Race</td>
<td>Yes</td>
<td>0.69 (0.35-1.33)</td>
<td>.27</td>
</tr>
<tr>
<td>White</td>
<td>Yes</td>
<td>1.98 (0.93-4.21)</td>
<td>.08</td>
</tr>
<tr>
<td>Black</td>
<td>Yes</td>
<td>1.82 (0.41-8.00)</td>
<td>.43</td>
</tr>
<tr>
<td>Other</td>
<td>Yes</td>
<td>0.38 (0.04-3.39)</td>
<td>.38</td>
</tr>
<tr>
<td>CSF HIV RNA level (n=86)</td>
<td>Yes</td>
<td>0.69 (0.35-1.33)</td>
<td>.27</td>
</tr>
<tr>
<td>CD4 lymphocyte count ≥ 200 cells/mL</td>
<td>Yes</td>
<td>1.98 (0.93-4.21)</td>
<td>.08</td>
</tr>
<tr>
<td>Presence of HIV-related disease</td>
<td>Yes</td>
<td>2.01 (1.06-3.78)</td>
<td>.03</td>
</tr>
<tr>
<td>MCDM at recruitment</td>
<td>Yes</td>
<td>0.97 (0.94-4.16)</td>
<td>.07</td>
</tr>
<tr>
<td>Current daily NSAID use (n=136)</td>
<td>Yes</td>
<td>0.96 (0.39-2.32)</td>
<td>.92</td>
</tr>
<tr>
<td>Current nonnarcotic analgesia use (n=136)</td>
<td>Yes</td>
<td>1.20 (0.62-2.32)</td>
<td>.60</td>
</tr>
<tr>
<td>Current narcotic analgesia use (n=136)</td>
<td>Yes</td>
<td>0.66 (0.30-1.42)</td>
<td>.29</td>
</tr>
<tr>
<td>Current alcohol use</td>
<td>Yes</td>
<td>0.66 (0.30-1.42)</td>
<td>.29</td>
</tr>
</tbody>
</table>

Abbreviations: ARVs, antiretroviral drugs; CI, confidence interval; CSF, cerebrospinal fluid; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HIV-D, HIV-associated dementia; HR, hazard ratio; IV, intravenous; MCDM, minor cognitive/motor disorder; NSAID, nonsteroidal anti-inflammatory drug.

aCases were considered virologically controlled if plasma HIV RNA was at undetectable levels at greater than 50% of visits.
in which the variable platelet count change from baseline was lagged by 1 study visit, individuals with a decrease in platelet count of \(21 \times 10^{9}/\mu L\) or greater had a 2-fold increased risk of dementia (HR, 2.09; \(P = .02\)) compared with individuals with stable or increased platelet levels. For the model in which the variable platelet count change from baseline was not lagged by 1 study visit, a decrease in platelet count was not significantly associated with dementia (HR, 1.13; \(P = .71\)). This contrasted with low hemoglobin level, which was not associated with an increased risk of dementia when measured at the lagged time points (HR, 0.83; \(P = .59\)) but was significantly associated with an increased risk of dementia when measured contemporaneously with outcome (HR, 2.14; \(P = .02\)). Results were similar after adjustment for duration of HIV infection, education, baseline neurologic status (MCMD or nonimpaired), presence of HIV-related illness (time dependent), level of virologic control, and antiretroviral therapy (time dependent) (Table 3).

Statistical tests of the proportional hazards assumption indicated that the lagged platelet model did not fulfill this assumption and that the association between decrease in platelet count and hazard of dementia likely changed over time. To account for this, separate HRs for decrease in platelet count were estimated for the first 2 years of follow-up and beyond 2 years of follow-up by adding a time-dependent covariate representing the interaction of platelet change from baseline and time. Durations of HIV infection, education level, and site of recruitment were stratified by site of recruitment.

### Table 3. Univariate and Multivariate Analyses of Relationship Between Platelet Change From Baseline and Hemoglobin Levels and the Risk of HIV-D

<table>
<thead>
<tr>
<th>Category</th>
<th>Unadjusted HR (95% CI) P Value</th>
<th>Adjusted HR (95% CI) P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lagged Time-Dependent Exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, (\times 10^{9}/\mu L) (n=140/138)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/no decline from baseline</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Moderate/large decline from baseline</td>
<td>2.09 (1.10-3.96) (P = .02)</td>
<td>2.39 (1.14-5.02) (P = .02)</td>
</tr>
<tr>
<td>Hemoglobin levels, g/dL (n=146/144)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.13 (0.59-2.20) (P = .71)</td>
<td>1.22 (0.60-2.48) (P = .57)</td>
</tr>
<tr>
<td>Low</td>
<td>0.83 (0.42-1.64) (P = .59)</td>
<td>0.88 (0.43-1.81) (P = .73)</td>
</tr>
<tr>
<td><strong>Immediate Time-Dependent Exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, (\times 10^{9}/\mu L) (n=142/141)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/no decline from baseline</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Moderate/large decline from baseline</td>
<td>5.67 (2.27-14.11) (P &lt; .001)</td>
<td>6.76 (2.36-19.41) (&lt; .001)</td>
</tr>
<tr>
<td>Hemoglobin levels, g/dL (n=146/145)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Low</td>
<td>1.13 (0.59-2.20) (P = .71)</td>
<td>1.22 (0.60-2.48) (P = .57)</td>
</tr>
<tr>
<td><strong>Unadjusted HR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate Time-Dependent Exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, (\times 10^{9}/\mu L) (n=142/141)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/no decline from baseline</td>
<td>1 [Reference]</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Hemoglobin levels, g/dL (n=146/145)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Low</td>
<td>1.13 (0.59-2.20) (P = .71)</td>
<td>1.22 (0.60-2.48) (P = .57)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HIV-D, HIV-associated dementia; HR, hazard ratio.

### Table 4. Univariate and Multivariate Analysis of Relationship Between Platelet Change From Baseline and Risk of HIV-D With and After the First 2 Years of Follow-up

<table>
<thead>
<tr>
<th>Platelet Change</th>
<th>Unadjusted HR (95% CI) P Value</th>
<th>Adjusted HR (95% CI) P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early development of HIV-D or censoring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/no decline from baseline</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Moderate/large decline from baseline</td>
<td>5.67 (2.27-14.11) (P &lt; .001)</td>
<td>6.76 (2.36-19.41) (&lt; .001)</td>
</tr>
<tr>
<td>Late development of HIV-D or censoring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/no decline from baseline</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Moderate/large decline from baseline</td>
<td>0.79 (0.30-2.06) (P = .63)</td>
<td>0.94 (0.33-2.67) (P = .90)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HIV-D, HIV-associated dementia; HR, hazard ratio.

**Footnotes:**

- Exposures were examined as time-dependent variables at the visit at which HIV-D was assessed (immediate) and lagged by 1 visit prior to the visit at which HIV-D was assessed (lagged). All individuals who developed the dementia endpoint were seen 6 to 12 months before failure. Of 106 censored individuals, 98 (92.4%) were seen 6 to 12 months before censoring. Seven individuals were seen within 18 months of censoring, and 1 individual was seen within 2 years of censoring.
- Full models were adjusted for virologic control, antiretroviral therapy, concurrent HIV-related illness, duration of HIV infection, education level, and site of recruitment.
- Total number of subjects included in univariate model/multivariate model.
- Reported normal range for hemoglobin, 11.5 to 15.5 g/dL.
in platelet counts from baseline and risk of dementia was not evident after 2 years of follow-up (HR, 0.79; \( P = .63 \)). These findings were confirmed after adjustment for covariates.

To examine the variability of platelet change from baseline for an individual from visit to visit, the standard deviation over all visits for each participant was calculated and averaged to 30.74 \( \times 10^3/\mu L \) for all participants. The trajectories of platelet change were compared between individuals developing dementia and those who remained unimpaired by plotting the decline from baseline in a retrograde fashion over time. The thick black line represents the median platelet decline from baseline at each time point.

Finally, raw platelet counts were examined at 6 to 12 months before development of HIV-D or censoring and were not associated with an increased risk of HIV-D (data not shown). Normalization to baseline platelet count was necessary to correct for interindividual variability and to demonstrate the associations presented. For the group developing HIV-D, 25.6% (10 of 39) of subjects had platelet levels below normal range (< 150 \( \times 10^3/\mu L \)), while, for those not developing HIV-D, 27.2% (28 of 103) had low platelet counts at this time point.

**Comment**

This study demonstrates that a decline in circulating platelet numbers is associated with the development of HIV-D within 6 to 12 months of the platelet decline. Those HIV-infected individuals with a decline in platelets from baseline values at this lagged time point had a 2-fold increased risk of dementia on univariate and multivariate analysis. Hemoglobin level was not predictive at the lagged time point, although it was associated with dementia when examined coincident with transition to HIV-D. Lower baseline hematocrit levels have previously been linked to increased risk of dementia in the NEAD cohort, and hemoglobin levels up to 1 year before AIDS onset were significantly associated with higher dementia hazards after AIDS diagnosis in the Multicenter AIDS Cohort.11,24

The link between platelets and HIV-D is less well characterized. A single report in the pre-HAART era found a 70% increased risk of HIV-D associated with baseline platelet counts less than 100 \( \times 10^3/\mu L \).25 The temporal variation in the association of platelets and hemoglobin with subsequent onset of dementia is interesting and may relate to the varied pathogenesis of the respective hematologic abnormalities. Anemia associated with HIV, although multifactorial, is primarily linked to reduced erythrocyte production in the bone marrow,26-29 Autoimmune hemolytic anemia is a rare occurrence in HIV infection.28 In contrast, thrombocytopenia is reported to occur earlier in the course of HIV infection, often independent of other AIDS-related cytopenias, and is frequently linked to a peripheral destruction of platelets.26,30 Reduced marrow thrombopoiesis is considered a feature of advanced AIDS.28 This difference between a peripheral and a marrow cause of the respective cytopenias may explain the precedent nature of
the platelet association and the more terminal nature of the hemoglobin association.

An investigation of the cause of platelet decline may shed light on the fact that the platelet decline appears to precede HIV-D. We propose that fluctuations in hematopoietic growth factors and inflammatory mediators in the periphery before manifestation of dementia affect both the change in platelets and the later onset of CNS disease. Interleukin 6 is a proinflammatory cytokine and platelet growth factor with potential for action in the periphery and the CNS. Elevated serum interleukin 6 levels have been linked to thrombocytopenia in patients with HIV, whereas elevated plasma and CSF interleukin 6 levels have been associated with simian immunodeficiency virus CNS disease.28,43 Thrombopoietin, a stimulus of megakaryocyte maturation and thrombopoiesis, is a second such cytokine. Although it is not clear that thrombopoietin crosses an intact blood-brain barrier, this cytokine has received attention for its role in inducing neuronal apoptosis.31,32 This is in contrast to the neuroprotection offered by erythropoietin.33 It would be of interest to assess the variation of these cytokines over time in patients with HIV at risk for CNS disease to determine whether they are coordinately regulated and linked to the temporal variation in the relationship between platelets, hemoglobin, and dementia.

Activation of platelets resulting in aggregation or sequestration can also lead to measured decreases in platelet numbers.34,35 Platelets have been shown to circulate in an activated state in the setting of HIV infection, with the degree of activation correlated with severity of disease.36 The ability to release bioactive mediators and up-regulate surface expression of CD40 ligand and Fas ligand allows activated platelets to modulate immune effector cells, stimulate release of cytokines from monocytes, and mediate attachment and rolling along endothelial surfaces.38-42 Activated monocytes are also important in the pathogenesis of HIV-D.43 Activation within the marrow is thought to enhance ingress of circulating monocytes to the CNS and contribute to the development of HIV-D.44,45 It is possible that changes in the peripheral inflammatory milieu during the 6 to 12 months before detection of HIV-D similarly affect monocytes and platelets, contributing to the decline in platelet numbers and increasing the risk of HIV-D.

Further analyses indicated that decline from baseline platelet levels was associated with a 5- to 6-fold increased risk of dementia during the first 2 years of follow-up, but it was not associated with an increased risk of dementia after 2 years. It is possible that individuals who do not progress rapidly to neurologic compromise differ in respect to immune activation, treatment adherence, or virologic control relative to those who develop dementia more rapidly. Since the advent of HAART, distinct subtypes of HIV-D have been reported, which include a subacute progressive dementia similar to the syndrome observed in the pre-HAART era, chronic active dementia as seen in patients with poor adherence and virologic resistance, and chronic inactive dementia in patients with effective virologic control and who are neurologically stable or improved.5 Further investigation is necessary to determine whether our results reflect the existence of one or more of these subgroups.

Although this study shows an association between platelet decline and risk of dementia, it is not yet clear what changes in clinical management a drop in platelets should trigger. Although there is a clear decline in median platelet count change from baseline just before diagnosis of HIV-D, individual trends in platelet change indicate weak specificity and sensitivity. Contributing to this may be the fact that preinfection platelet levels were not available. Examination of platelet decline by normalizing to the baseline platelet count was necessary to demonstrate the associations noted in this study. A comparison with preinfection platelet levels, which are likely to be greater than those measured in the presence of low CD4 counts, may strengthen the utility of platelet decline as a biomarker. Alternatively, an investigation of platelet dynamics may indicate that changes in mean platelet volume, thrombopoietin levels, or platelet activation may hold greater prognostic utility.

Other factors demonstrating trends toward increased risk of dementia in this cohort included presence of concurrent HIV-induced disease and presence of MCM at recruitment. The presence of opportunistic infections and HIV-related neoplastic or metabolic syndromes likely reflect HIV progression and would be expected to be associated with an increased risk of dementia. Similarly, individuals with MCM at baseline would be considered more likely to have neurologic progression. Studies have now shown that minor cognitive impairment is likely a harbinger of frank HIV-D and HIV encephalitis.11,46 Individuals who have pursued college education were 48% less likely to develop HIV-D than individuals who did not complete high school. This observation may reflect the value of “cerebral reserve” in abrogating CNS decline.47 This trend remained pertinent in the multivariable models for hematologic exposures (data not presented).

This study represents data collected from a prospective cohort with a minimal amount of incomplete data. Nonetheless, an important limitation lies in the fact that exclusion of early failures was necessary because a change in circulating platelet numbers from baseline could not be calculated for the visit immediately before dementia. This reduction in sample size is responsible for the variations in cumulative incidence and median follow-up time from that previously reported in NEAD studies.11 Individuals who develop HIV-D within 1 year of recruitment may differ systematically from the sample analyzed, although, because the onset of HIV-D has been reported to occur insidiously over 3 to 9 months,2 this analysis may serendipitously avoid a prevalence-incidence bias.

Although numerous potential confounders were addressed in the study design, there remains the potential for unmeasured confounding. The effects of medications on platelets must be considered. Antiretroviral drugs, such as zidovudine and indinavir, are known to affect platelet levels.48-50 Although controlling for antiretroviral therapy in a categorical sense likely corrects for this, the selected models did not control for specific drug therapies. Antipsychotic drugs, occasionally prescribed to patients with HIV-D, have also been associated with blood dyscrasias.50,51 Although thrombocytopenia associated
with these medications is rare, data on the administration of such medications were not captured in a standardized manner. Selection bias must also be addressed. Although the majority (68%) of those censored were done so administratively, it is possible that individuals lost to follow-up may not be lost at random. Also of concern would be the neurologic status of individuals during missed visits. Generalizability of data from the NEAD cohort has previously been addressed.11

Because CD4 cell counts and HIV RNA levels have proven not to be predictive of HIV-D,11 it is important to investigate alternative serum and hematologic markers. Should these markers be routinely measured in a clinical setting, such as platelet counts, they may prove useful for patient management. This study identifies a significant association between platelet decline and incident HIV-D. Further characterization of the dynamics of platelet decline may point to sensitive and specific biomarkers for the onset of HIV-D. Elucidation of the mechanism of the mechanism of the relationship to HIV-D represents an avenue for further research that is likely to identify pathways common to the peripheral circulation, bone marrow, and CNS.

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Author Contributions: Dr McArthur had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Schifitto, Marder, Cohen, Mankowski, and McArthur. Acquisition of data: Esposito, Schifitto, Marder, Cohen, Epstein, and McArthur. Analysis and interpretation of data: Wachtman, Skolasky, Tarwater, Schifitto, McDermott, Nath, Sacktor, Mankowski, and McArthur. Drafting of the manuscript: Wachtman, Skolasky, Esposito, Mankowski, and McArthur. Critical revision of the manuscript for important intellectual content: Tarwater, Schifitto, Marder, McDermott, Cohen, Nath, Sacktor, Epstein, Mankowski, and McArthur. Statistical analysis: Wachtman, Skolasky, Tarwater, and McDermott. Obtained funding: Schifitto, Marder, Epstein, and McArthur. Administrative, technical, or material support: Esposito and Schifitto. Study supervision: Schifitto, Marder, Sacktor, and McArthur.

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