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Platelet decline as a predictor of brain injury in HIV infection

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Abstract An association between platelet decline and increased risk of progression to dementia has been observed in an advanced HIV infection cohort study. This investigation evaluated the prognostic significance of platelet decline for dementia, for psychomotor slowing, and for brain injury, as quantified in vivo, in a much larger population of HIV+ men. Platelet counts and neurocognitive data were available from biannual visits of 2,125 HIV+ men participating in the prospective, Multicenter AIDS Cohort Study from 1984 to 2009. Brain volumetric data were also available from an imaging substudy of 83 seropositive participants aged 50 and older. The association of platelet counts with neurocognitive outcome was assessed using Cox proportional hazard models where change in platelet count from baseline was a time-updated variable. Marked platelet decline was associated with increased risk of dementia in univariate analysis (hazard ratio [HR]=2.5, 95% confidence interval [CI]=1.8–3.5), but not after adjustment for CD4 cell count, HIV viral load, age, study site, hemoglobin, race, education, smoking, and alcohol use (HR=1.4, 95% CI=0.78–2.5). Platelet decline did not predict psychomotor slowing in either univariate (HR=0.79, 95% CI=0.58–1.08) or multivariate (HR=1.10, 95% CI=0.73–1.67) analysis. Analysis of brain volumetric data, however, indicated a relationship between platelet decline and reduced gray matter volume fraction in univariate (p=0.06) and multivariate (p<0.05) analyses. Platelet decline was not an independent predictor of dementia or psychomotor slowing, after adjusting for stage of disease. Findings from a structural brain imaging substudy of older participants, however, support a possible relationship between platelet decline and reduced gray matter.

Keywords HIV · HIV dementia · Hematologic · Volumetric MRI · Platelets

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Introduction

Cognitive deterioration is associated with reduced survival duration in HIV infection (Ellis et al. 1997; Farinpour et al. 2003; Sevigny et al. 2007); however, factors underlying neurological progression have not yet been determined. A large observational study of neurological outcome in advanced infection has identified a relationship between platelet decline and increased risk of HIV dementia (Wachtman et al. 2007). When examined as a time-dependent variable (lagged 6 to 12 months), participants with the largest platelet decline from baseline had more than twofold increased risk of dementia (multivariate hazard ratio [HR], 2.39; 95% confidence interval [CI], 1.14–5.02; adjusting for virologic control, antiretroviral therapy, concurrent HIV-related illness, duration of infection, baseline neurologic status, education, and recruitment site). The risk associated with platelet decline was higher in those with rapid deterioration to dementia (within 24 months of enrollment; multivariate HR=6.76; 95% CI 2.36–19.41; $p<0.001$).

These findings may be relevant to accelerated cognitive aging in HIV infection (e.g., Valcour et al. 2004). Platelets and alterations in chemotactic and adhesive characteristics of the endothelium are critical in the pathophysiology of atherosclerosis. Higher rates and progression of atherosclerosis have been reported in HIV infection (Depairon et al. 2001; Hsue et al. 2004). Decline in platelet count may reflect activation and aggregation (Jurk and Kehrel 2005). Under physiological conditions, platelets circulate in a quiescent state (for a review, see Angiolillo et al. 2010). Upon activation, platelets express receptors for adhesive proteins, adhere, aggregate, and recruit additional circulating platelets to sites of injury. This process is tightly regulated as premature or dysregulated activation may lead to arterial occlusion, ischemia, and injury to tissue (Brass 2010). Platelet activation has been associated with more rapid progression in other cognitive disorders, such as Alzheimer's disease (e.g., Stellos et al. 2010). Markers of platelet activation are elevated in HIV infection, including in asymptomatic periods (Mena et al. 2011). Moreover, markers of atherosclerotic risk, such as carotid artery intima-media thickness, have been associated with cognitive performance in infected subjects (Becker et al. 2009). To investigate the observed risk relationship between platelet decline and progression to dementia further (Wachtman et al. 2007), the prognostic significance for dementia and for psychomotor slowing was evaluated in the Multicenter AIDS cohort Study (MACS), a considerably larger prospective epidemiologic study of HIV-1 infection (Kaslow et al. 1987). In addition, the relationship was examined in a MACS imaging substudy of participants aged 50 and older.

Materials and methods

Standard protocol approvals, registrations, and patient consents

This study was approved by the IRB at each MACS site. Written informed consent was obtained from each subject prior to study participation.

Study population and measures

The MACS is a prospective multicenter study of the natural history of HIV infection among gay/bisexual men. The cohort includes 6,972 participants enrolled in staggered waves from 1984 to 2009 at four study centers (Chicago, Pittsburgh, Baltimore, and Los Angeles) followed with biannual examinations (over 100,000 total visits). While the MACS cohort also includes seronegative men, only seropositive men are included in this analysis of HIV-associated neurocognitive decline and brain injury. Each visit includes testing for HIV status, CD4 cell count, HIV viral load, hematologic variables, and neuropsychological screening. Highly active antiretroviral therapy (HAART) use is documented at each visit and defined according to the US Department of Health and Human Services Kaiser Panel guidelines (Brown et al. 2005). Alcohol and tobacco use is also documented at each visit, with current use defined as one or more drinks per week for alcohol and as any use in the past 6 months for tobacco. Detailed information concerning MACS study design and data collection, sample composition, questionnaires, and specimen repositories is presented at the website: http://www.statepi.jhsph.edu/macs/macs.html.

Neurological outcome measures

Cognitive outcome measures included dementia status and presence of psychomotor slowing, defined on the basis of neuropsychological test findings. Dementia diagnoses were made by MACS study neurologists and neuropsychologists after reviewing all available neuropsychological, neurological, and medical information. Consensus diagnoses were derived using the 1991 American Academy of Neurology criteria (Janssen 1991). Psychomotor slowing was determined based on biannual administration of the symbol digit modalities test (Smith 1982) and trail-making tests parts A and B (Reitan 1979). The symbol digit modalities test (Smith 1982) evaluates speed of visual information processing and attention. Trail-making A is a test of attention, motor speed, and visuospatial tracking. Trail-making B, which includes an additional set-shifting component, evaluates executive functioning to a greater extent than symbol digit modalities or trail-making A tests. For this study, psychomotor slowing was determined using norma-

tive values from HIV-negative participants of the MACS cohort. Specifically, presence of psychomotor slowing was defined as: two or more standard deviations below the mean performance of HIV-negative MACS participants on one or more tests (symbol digit modalities, trail-making A, or trail-making B) *or* one standard deviation below the mean on both symbol digit modalities and trail-making part B or on both trail-making parts A and B.

Magnetic resonance (MR) brain volumetric measures, which were available for one recent visit in 83 HIV-seropositive participants in a MACS cardiovascular substudy, were used to evaluate the relationship of platelet decline to brain injury (i.e., atrophy). Participants in this substudy were restricted to those age 50 years and older and weighing less than 300 lb, with no self-reported history of heart disease (heart attack, heart surgery, other heart illness) or cerebrovascular disease. Imaging data were acquired on a Siemens 3T Trio scanner at three centers (a Siemens Allegra scanner was used at one center) using Siemens phase-array head coil (maximum gradient slew rate, 200 mT/m/s; maximum gradient strength, 40 mT/m). Parameters used for implementation of the sagittal Magnetization-Prepared Rapid Acquisition Gradient Echo sequence were as follows: FOV=256 mm, slices=160, TR=2,300 ms, TE=2.91 ms, TI=900 ms, flip angle=9°, thickness=1.2 mm. All image post-processing was conducted at a single site (Pittsburgh) using semi-automated segmentation algorithms that require minimal operator interaction. Non-brain tissue was removed from T2 and PD images with BET2 (brain extraction tool). Semi-automated segmentation algorithms were used to calculate volumes of gray matter, white matter, and CSF, which were then expressed as volume percentages relative to the sum of these tissues within the individual intracranial cavity to adjust for individual differences in head size. Further details concerning the imaging protocol and derivation of the volumetric measurements have been published elsewhere (Becker et al. 2011).

Statistical methods

Analysis was limited to HIV-infected MACS participants with at least two platelet measures available after HIV seroconversion (302 men excluded). Participants developing dementia before seroconversion or within 180 days of study enrollment were excluded. Fifty-one participants with a report of dementia after their last study visit were excluded from the analysis of dementia. For psychomotor slowing, the analysis was further restricted to those with at least two visits with trails A, trails B, and symbol digit test results (1,114 men excluded). Differences in characteristics of participants with and without dementia were tested with chi-square for categorical variables and with the Wilcoxon rank sum and test of medians for continuous variables. Platelet decline was calculated as the change from first available until last available measurement, and current platelet measure was lagged by 6 months as in the previous Northeast AIDS (NEAD) study (Wachtman et al. 2007). The association of change in platelet count from baseline with dementia and psychomotor slowing was assessed by Cox proportional hazard models with last observed change from baseline platelet count as a time-dependent covariate. Associations with brain volume fractions were evaluated using multivariate linear regression. Both univariate and adjusted models were used.

Results

Among the men meeting criteria for inclusion in this analysis, there were 3,184 HIV+ men without dementia at study entry into the MACS of whom 250 (7.9%) developed dementia while in the study. Men who developed dementia were similar in age, baseline CD4 cell count, tobacco use, and length of follow-up to those who did not develop dementia. Men who developed dementia were significantly less likely to be African–American, were more educated, more likely to drink alcohol, more likely to develop AIDS, and had larger decreases in platelets over time than those who did not develop dementia (Table 1).

Clinical diagnosis of dementia In univariate analysis, platelet decline of 100,000/μL or more was associated with a significant, more than twofold increase in risk of dementia (HR=2.5, 95% CI=1.8–3.5), and there was a significant trend of increased dementia risk with increasing platelet loss ($p<0.0001$). Change in platelet count, however, was no longer associated with dementia when adjusted for stage of HIV disease. In multivariate analysis adjusting for CD4 cell count, HIV viral load, age, study site, hemoglobin, race, education, and tobacco and alcohol use, platelet decline was not significantly associated with dementia risk (Table 2, HR=1.4, 95% CI=0.78–2.5). Significant predictors of dementia in the adjusted model included: older age, lower CD4 cell count, higher viral load, and study site (Table 2). Alcohol use of 4–13 drinks per week was associated with decreased hazard of dementia compared to less frequent drinkers. When examined in a model in which platelet decline was lagged by 18 instead of 6 months, a similar pattern was observed, with a significant increase in risk of dementia in univariate analysis (HR=1.9, 95% CI=1.3–2.7) and not in adjusted analysis (HR=1.09, 95% CI=0.59–2.01).

Psychomotor slowing Platelet decline was also evaluated as a predictor of presence of psychomotor slowing (see Materials and methods for definition). Of the 2,068 HIV+ men in MACS who had at least two visits with neuropsy-

Table 1 Comparison of characteristics of men who developed dementia while in the MACS, 1984–2009

Baseline risk factors	All, %	Dementia (ever during follow-up), %		Chi-squared *p* value
		No	Yes	
	N=3,235	*N*=2,985	*N*=250	
Race				<0.0001
White non-Hispanic	70	69	86	
White Hispanic	6	6	6	
Black non-Hispanic	19	20	7	
Other	5	5	1	
Education				
≤High school	20	21	13	0.02
Some college	54	54	57	
Post-graduate	25	25	30	
Missing/unknown	<1	<1	<1	
Smoking				0.15
Never	36	37	33	
Former	19	19	20	
Current	43	43	47	
Missing/unknown	1	1	0	
Alcohol use				
None	4	5	<1	0.0002
3 or fewer drinks/week	33	33	31	
Up to 13 drinks/week	39	39	37	
>13 drinks/week	17	17	20	
Missing/unknown	7	6	12	
AIDS				
No	54	57	17	
Yes, at baseline	<1	<1	0	0.28
Yes during follow-up	46	43	83	<0.0001
Platelets, mean (SD) (thousands/mm^3)		240 (64)	241 (67)	
Platelet decline from baseline/μL				
≤20,000	43	44	31	<0.0001
20,001–100,000	41	41	44	
>100,000	16	15	25	
		Median (IQR)	Median (IQR)	Median test *p* value
Age at enrollment (years)		33 (28, 38)	33 (29, 39)	0.26
Baseline CD4 count (cells/μL)		581 (404, 803)	562.50 (425, 745.50)	0.21
Baseline HIV viral load (copies/ml)		1,011.0 (40, 20,817)	2,784.5 (300, 32,577)	0.65
Years from first to last platelet measure		5.71 (3.59, 12.16)	6.23 (4.33, 8.62)	0.11
Baseline hemoglobin		15.1 (143, 158)	15.2 (144, 160)	0.18

chological testing, there were 698 (33.7%) participants who met the criteria for psychomotor slowing at some time during the 25-year follow-up. Participants with psychomotor slowing were more likely to be African–American, less educated, have lower baseline CD4 cell count, to smoke, to be nondrinkers, and less likely to develop AIDS during follow-up than those without psychomotor slowing (each $p<0.05$, data not shown). Platelet decline did not predict psychomotor slowing in either univariate (HR=0.79, 95% CI=0.58–1.08) or multivariate (HR=1.10, 95% CI=0.73–1.67) analysis (Table 2). In multivariate analysis, other factors associated with increased risk of psychomotor

Table 2 Longitudinal analysis of predictors of dementia and of psychomotor slowing among 3,184 men in the MACS followed between 1984 and 2009

Parameter	Dementia				Psychomotor slowing			
	Univariate		Multivariate		Univariate		Multivariate	
	Hazard ratio	95% CI	Hazard ratio	95% CI	Hazard ratio	95% CI	Hazard ratio	95% CI
Platelet decline from baseline/μL								
≤20,000	1		1.00		1		1.00	
>20,000–100,000	1.33	[0.98, 1.80]	1.39	[0.86, 2.26]	0.85	[0.70, 1.03]	0.92	[0.70, 1.21]
>100,000	2.51	[1.78, 3.54]	1.40	[0.78, 2.51]	0.79	[0.58, 1.08]	1.10	[0.73, 1.67]
Hemoglobin g/dl								
>15.2	1		1		1		1	
14.2–15.2	0.67	[0.41, 1.11]	0.68	[0.35, 1.35]	1.02	[0.79, 1.31]	1.12	[0.79, 1.58]
<14.2	2.74	[1.90, 3.94]	1.53	[0.88, 2.66]	1.61	[1.29, 2.00]	1.29	[0.93, 1.80]
Age per 5-year increase	1.31	[1.21, 1.42]	1.28	[1.11, 1.47]	0.86	[0.81, 0.92]	0.97	[0.89, 1.06]
Current tobacco use	0.90	[0.68, 1.19]	1.23	[0.80, 1.89]	1.43	[1.20, 1.71]	0.99	[0.75, 1.29]
Current alcohol use								
3 or fewer drinks/week	1		1		1		1	
4–13 drinks/week	0.36	[0.28, 0.48]	0.62	[0.41, 0.96]	0.78	[0.63, 0.97]	0.80	[0.59, 1.07]
>13 drinks/week	0.34	[0.19, 0.61]	0.65	[0.27, 1.59]	0.80	[0.55, 1.15]	0.95	[0.57, 1.58]
CD4 per 100-cell increase	0.69	[0.64, 0.74]	0.87	[0.79, 0.97]	0.92	[0.89, 0.96]	0.99	[0.94, 1.04]
HIV RNA per $\log_{10}$	2.03	[1.63, 2.53]	1.47	[1.15, 1.88]	1.26	[1.14, 1.39]	1.17	[1.03, 1.32]
Study site								
Baltimore	1		1		1		1	
Chicago	0.78	[0.47, 1.29]	0.55	[0.22, 1.37]	0.90	[0.71, 1.14]	0.78	[0.56, 1.09]
Pittsburgh	0.66	[0.38, 1.15]	1.86	[0.79, 4.37]	0.77	[0.60, 0.99]	0.87	[0.58, 1.30]
LA	3.14	[2.17, 4.56]	4.84	[2.46, 9.49]	0.53	[0.42, 0.67]	0.58	[0.41, 0.83]
Minority race	0.66	[0.46, 0.94]	1.38	[0.82, 2.35]	2.48	[2.06, 2.97]	2.59	[1.96, 3.43]
Education								
High school or less	1		1		1		1	
Some college	1.15	[0.77, 1.71]	1.27	[0.62, 2.59]	0.43	[0.35, 0.53]	0.48	[0.36, 0.66]
Post-graduate	1.09	[0.71, 1.69]	1.07	[0.50, 2.30]	0.35	[0.27, 0.45]	0.38	[0.26, 0.56]

slowing included minority race, less education, and higher viral load, and performance varied significantly by study site (Table 2). When examined in a model in which platelet decline was lagged by 18 instead of 6 months, results were similarly null (adjusted HR=0.76, 95% CI=0.53–1.08).

When examined as a continuous variable, the effect of platelets, change in platelets, lagged change in platelets, and last location carried forward change in platelets on development of dementia or psychomotor slowing; the hazard ratio was either 1.0 or .99 in all cases.

MR-quantified brain atrophy When brain volume fractions of gray matter, white matter, and CSF were examined in a subset of 83 seropositive MACS participants using linear regression, marked platelet decline (>100,000/μL) was nearly significantly associated with reduced gray matter volume fraction in univariate analysis ($p=0.06$) and significantly associated with reduced gray matter volume fraction in multivariate analysis (adjusting for CD4 cell count, HIV viral load, age, study site, hemoglobin, race, education, smoking, and alcohol use) (Table 3). Gray matter volume fractions were 0.026 (univariate) and 0.044 (multivariate) lower in those with largest platelet decline compared to those with no or modest platelet decline (Table 3). Platelet decline was not predictive of white matter or CSF percent volumes in either univariate or multivariate adjusted analysis (Table 3). Higher CD4 cell count was associated with increased CSF volume fraction.

Discussion

This investigation evaluated decline in platelet count as a predictor of neurological outcome in the MACS. Platelet decline was associated with dementia in univariate analysis.

Table 3 Predictors of brain volume fractions among 83 HIV-infected men, univariate and multivariate regression results

Covariate	Brain volume fractions								
	Gray matter			White matter			CSF		
	Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Univariate model									
Intercept	0.52	0.01		0.28	0.01		0.30	0.02	
Platelet decline from baseline/μL									
≤20,000	Ref			Ref			Ref		
>20,000–≤100,000	−0.002	0.009	0.87	0.006	0.011	0.59	−0.032	0.028	0.25
>100,000	−0.026**	0.014	0.06	0.010	0.020	0.41	−0.013	0.042	0.76
Adjusted model									
Intercept	0.65	0.07		0.33	0.09		0.01	0.22	
Platelet decline from baseline/μL									
≤20,000/	Ref			Ref			Ref		
>20,000–≤100,000	−0.001	0.011	0.99	−0.002	0.013	0.91	−0.003	0.035	0.93
>100,000	−0.044*	0.018	0.02	0.020	0.015	0.51	0.020	0.060	0.73
Hemoglobin, g/dl									
>15.2	Ref			Ref			Ref		
14.2–15.2	0.002	0.012	0.90	0.027	0.022	0.21	−0.054	0.038	0.16
<14.2	0.010	0.011	0.40	−0.005	0.014	0.75	−0.009	0.035	0.79
Current smoking	−0.005	0.012	0.97	0.004	0.015	0.79	−0.014	0.038	0.72
Alcohol use level									
3 or fewer drinks/week	Ref			Ref			Ref		
4 to 13 drinks/week	−0.007	0.012	0.56	0.009	0.015	0.53	0.020	0.040	0.50
>13 drinks/week	−0.008	0.017	0.96	0.010	0.021	0.54	−0.015	0.051	0.78
CD4 (per 100 cell increase)	0.001	0.002	0.50	−0.004	0.002	0.08	0.010*	0.010	0.02
HIV RNA per $\log_{10}$	−0.010	0.006	0.09	0.001	0.007	0.92	0.010	0.021	0.57
HAART use	−0.016	0.013	0.20	−0.008	0.016	0.96	0.021	0.043	0.56
Study site									
Baltimore	Ref			Ref			Ref		
Chicago	0.020	0.01	0.21	−0.023	0.018	0.71	0.045	0.050	0.35
Pittsburgh	0.001	0.02	0.94	−0.008	0.021	0.80	0.015	0.051	0.97
LA	0.020	0.01	0.08	−0.004	0.017	0.64	−0.028	0.041	0.51
Age (per 5-year increase)	−0.010	0.006	0.09	−0.004	0.008	0.64	0.021	0.019	0.33

*$p<0.05$; **$p=0.06$

When the analysis was adjusted for CD4 cell count, HIV viral load, age, study site, hemoglobin, race, education, and tobacco and alcohol use, the relationship was no longer significant. Nor did platelet decline predict psychomotor slowing determined by neuropsychological testing. A significant association with reduced gray matter was identified, however, in adjusted analysis based on objective, brain volumetric measurements from a neuroanatomic imaging substudy of older MACS participants (aged 50 and older).

Results for dementia outcome differ from those of the NEAD. The NEAD identified a predictive relationship between platelet decline and dementia in AIDS participants followed only a few years (Wachtman et al. 2007). This may be due to differences in the cohorts. The NEAD included men and women in advanced infection (CD4 cell counts less than 200/μL or less than 300/μL with cognitive impairment) whereas the MACS included only men and at any stage of infection. The MACS follow-up duration was also considerably longer (up to 25 years). Some of the covariates included in the analyses differed (e.g., hemoglobin). Whether hemoglobin was included or not, the prognostic significance of platelet decline in the MACS did not change (data not shown). Importantly, while participants in the NEAD were in advanced infection and may have been older, the adjusted model in that study did not include age. Accelerated aging and a relation to cognitive deterioration have been shown in

HIV infection (e.g., Valcour et al. 2004). Platelet decline may herald imminent cognitive decline associated with active CNS injury in older, rapidly deteriorating patients. The MACS imaging substudy of older participants (aged 50 and older) identified a relationship between platelet decline and reduced gray matter in adjusted analysis, supporting a possible relationship with neuronal injury in this subgroup.

The imaging substudy results are consistent with other findings implicating platelet activation and platelet-derived factors in HIV-associated neuronal injury. Platelet interactions with the HIV-1 viral protein, *tat*, induce activation and expression of CD154 (CD40L) and other platelet-derived immune modulators (Wang et al. 2011). Levels of sCD40L, from activated platelets, are higher in cognitively impaired HIV subjects (Sui et al. 2007). Some evidence indicates that sCD40L may synergize with *tat* to amplify monocyte/microglial activation and increase neurotoxicity (Sui et al. 2007).

Advances in platelet biology have uncovered physiologic significance extending beyond hemostasis to more direct involvement in immunomodulation. Activated platelets express immune receptors on their membranes and are directly involved in immune responses and in inflammation; for a review, see Brass (2010). Opposing roles have been shown for platelet-derived molecules, such as CD40L (CD154), which can promote immune response, yet also activate CD4+ T cells, dendritic cells, and macrophages, thereby enhancing viral replication (Kornbluth 2000; Martin et al. 2007). Activated platelets also express or induce cytokines, chemokines, matrix metalloproteinases, and other factors (Boehlen and Clemetson 2001; Gawaz et al. 2000, 1998; Klinger and Jelkmann 2002; Lin et al. 2006; Nagata et al. 1993; Price et al. 2007; Schonbeck and Libby 2001a, b; Weyrich et al. 1996; Weyrich and Zimmerman 2004). Many of these factors, such as MCP-1 (CCL2) and matrix metalloproteinases, have been implicated in cognitive impairment and brain injury in HIV infection (Conant et al. 1998, 1999; Ragin et al. 2011, 2010). Platelet activation may also be relevant to brain deposition of beta-amyloid in HIV-infected individuals (Achim et al. 2009; Green et al. 2005; Nebuloni et al. 2001). An activated platelet subset retaining amyloid precursor protein on the surface has been associated with cognitive decline in Alzheimer's disease (Prodan et al. 2011, 2008; Stellos et al. 2010). Platelet dynamics may reflect chronic immune activation and changes in the bone marrow, associated with viral infection (e.g., of marrow stromal cells) and with aging. Trafficking of activated and infected monocytes from the marrow, which is a viral reservoir, to the brain may play a critical role in viral entry and aberrant immune activation associated with CNS injury (Gartner 2000). Upregulation of cytokines, chemokines and some antiretrovirals also disturb hematopoiesis, thrombopoiesis, and circulating levels of erythropoietin and thrombopoietin. Thrombopoietin, which regulates platelet production, and erythropoietin, which is critical in hematopoiesis, have been found to have direct involvement in the brain, in neuroprotection and in neuronal apoptosis (Ehrenreich et al. 2002, 2005).

When interpreting these findings, it is important to appreciate that brain volumetric measurements were available for only a single timepoint, and therefore, it cannot be determined with certainty whether neuronal loss occurred in the time frame of infection or whether it was a pre-extant condition. This risk relationship should be investigated further in longitudinal imaging studies. It is also important to appreciate that hematological parameters are intrinsically related (e.g., due to common progenitors) and characterized by homeostatic compensatory mechanisms that may become exhausted across the course of infection. Co-morbidities, such as cardiovascular risk factors and chronic liver disease, may also be relevant in further studies. It is unclear why there was an effect of study site. This has been observed in other MACS studies; however, explanatory factors have not yet been determined. It may be relevant that UCLA was included in the consortium somewhat later and may have more AIDS participants and more drug use than other sites. The MACS is the longest study of HIV infection with up to 25 years of longitudinal data for some participants. The incidence and severity of dementia have declined in the HAART era and neurocognitive decline may be asymptomatic for longer periods. This may complicate interpretation of cognitive outcome measures. The possibility of survivor bias, as well as differences in pre-HAART and post-HAART treatment era distribution over this extensive follow-up, may contribute to differences for sites and between cohorts.

In summary, platelet decline was not an independent predictor of neurocognitive outcome in the MACS cohort. A relationship with reduced brain gray matter was observed in an imaging substudy of older participants. More comprehensive characterization of platelet dynamics and relation to neurological status may yield insights into the complex pathophysiology underlying HIV-associated brain injury and cognitive deterioration.

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Conflict of interest The authors declare that they have no conflict of interest.

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