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Anemia and Erythrocyte Indices are Associated with Neurocognitive Performance Across Multiple Ability Domains in Adults with HIV

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Abstract

Background: Anemia is linked to neurocognitive impairment (NCI) in people with HIV (PWH), but its impact within specific ability domains, and in diverse populations with HIV, is uncertain.

Methods: Participants included 1,339 PWH enrolled in observational HIV cohort studies with a mean of 3 comprehensive neurocognitive assessments over 30 months. Global and domain-specific neurocognitive function were assessed by the Global and Domain Deficit Score (GDS and DDS, respectively), or as GDS- or DDS-defined NCI (GDS≥0.5, DDS>0.5). Time-dependent associations of anemia or red-cell indices with neurocognitive function were evaluated by multivariable regression.

Results: Mean age at entry was 43.6 years (85% male, 23.9% Hispanic, 16.7% African-ancestry by self-report, 69.8% virally suppressed). Anemia occurred at entry in 297 (22.2%) and developed subsequently in another 129 (9.6%). Anemia (present in 26.8% of cognitively impaired PWH at entry) and lower hemoglobin were associated with higher (worse) GDS values; the association for anemia persisted after multivariable adjustment and in virally suppressed persons (p<0.0001). Anemia was also associated with reduced processing speed, motor function, learning, delayed recall, working memory (all p<0.01), executive function (p=0.021), and verbal fluency (p=0.035), and these findings persisted in longitudinal analyses (adjusted p<0.01 for all domains, except

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verbal fluency). Higher mean corpuscular volume and mean corpuscular hemoglobin were associated with less impairment in learning and recall (all $p<0.05$).

Conclusions: Anemia in diverse and virally suppressed PWH associates with reduced neurocognitive performance in multiple domains, cross-sectionally and over time. The impact of identifying and treating anemia to prevent or slow neurocognitive decline in PWH should be prospectively evaluated.

Keywords

anemia; neurocognitive function; ability domains; HIV

INTRODUCTION

People with HIV (PWH) on effective antiretroviral therapy (ART) experience a near-normal life expectancy but develop aging-related diseases such as neurocognitive (NC) impairment (NCI) at higher age-adjusted rates than the general population.[1,2] NCI occurs in 30–50% of PWH,[3] often with deficits in multiple ability domains. HIV infection of perivascular macrophages and glia, leading to virus latency and chronic inflammation within the central nervous system (CNS), contributes to, but does not fully explain, the continued high prevalence of NCI in treated PWH.[4]

Anemia is a significant risk factor for morbidity and mortality in PWH.[5,6] Anemia is often classified according to whether erythrocyte indices (*e.g.,* mean corpuscular volume, or MCV) are high, normal, or low: macrocytic (high-MCV) anemias may be due to micronutrient deficiencies or associated with the use of nucleoside reverse-transcriptase inhibitors like zidovudine;[7] microcytic (low-MCV) anemias may result from iron-deficient erythropoiesis, other iron-related disorders, thalassemias, or efavirenz use.[8] Systemic effects of anemia depend upon its duration and severity: reduced oxygen transport to the CNS can depress synaptic activity and promote neuronal-cell death in regions such as the hippocampus, basal ganglia, neocortex and thalamus.[9] Pre-ART-era and largely cross-sectional studies identified associations between anemia and worse NC performance in PWH but included participants who were ART-naïve or not receiving contemporary ART. We had reported the association of anemia and erythrocyte indices with worse NC performance in an observational cohort of PWH, the majority of whom were on ART.[10] Another study also found that lower hematocrit values predicted future NC decline.[6] The purpose of this study was to confirm and expand upon prior findings in an independent, largely virally suppressed (VS), HIV cohort of diverse race/ethnicity on ART. We hypothesized that participants with anemia and altered erythrocyte indices would have worse domain-specific as well as global NC function at entry and follow-up.

METHODS

Study Design

We analyzed data from 1,339 adults with HIV and a total of 4,266 visits across several observational cohort studies of the HIV Neurobehavioral Research Program (HNRP) at the University of California San Diego (UCSD). Participants were assessed between July

1999 and June 2017, reported receiving ART at all visits, and all underwent comprehensive neuromedical and NC evaluations and laboratory testing at each visit. PWH who had current or prior CNS opportunistic infections, or specific conditions which prevent attribution of NCI to HIV (*e.g.,* head injury with prolonged loss of consciousness, learning disability or developmental delay, untreated seizure disorder or psychosis, active substance use), were excluded.[11] Of evaluated participants, 784 (58.6%) had two or more NC assessments.

This study was approved by the UCSD Human Research Protections Program. All participants provided written informed consent for use of their data in research. The majority of PWH were enrolled in studies of the National Institute of Mental Health (NIMH)-funded HIV Neurobehavioral Research Center (HNRC, n=765) or the National Institute on Drug Abuse (NIDA)-funded Translational Methamphetamine AIDS Research Center (TMARC, n=190).

Clinical and NC Assessments

Information on demographic, neuromedical, and HIV-disease and treatment factors was collected. Not collected were dietary questionnaire data or specific information on iron intake. Common comorbidities that contribute to vascular causes of NC impairment, such as diabetes mellitus, hypertension, and hyperlipidemia, and hepatitis C virus (HCV) seropositivity, were included as covariates in our analyses.[12] Leukocyte and erythrocyte numbers, hemoglobin concentration, and erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)] were determined by Coulter counter. Anemia was defined as a hemoglobin <13.5 g/dL in males and <12 g/dL in females.[13] Erythrocyte indices were categorized as follows: a) MCV ≤80 fL (“low”) and MCV ≥100 fL (“high”); b) MCH ≤27 pg/cell (“low”), and c) MCHC ≤32 g/dL (“low”). (High MCH and MCHC categories were not included, because they do not provide any additional clinically useful information beyond what is provided by the MCV.) Other measures included: CD4+ T-cell count by flow cytometry, and plasma HIV RNA by reverse-transcriptase PCR (Roche Amplicor), with a lower limit of quantification of 50 copies/mL.

Assessments of NC Function

Participants underwent a standardized battery of NC tests to evaluate NC abilities in domains known to be affected by HIV infection: executive function, processing speed, learning, delayed recall, working memory/attention, verbal fluency, and motor function.[11] Test scores were transformed into demographically-adjusted T-scores after adjusting for age, education, sex, and self-reported race/ethnicity.[14] In addition, test scores from second or subsequent assessments were corrected for practice effects, as described in Cysique et al.[15] The Global Deficit Score (GDS) and Domain Deficit Score (DDS) were calculated as previously described, with higher scores (ranging from 0 to 5) indicating *worse* NC function, and NCI defined by a GDS ≥0.5 or a DDS >0.5.[14]

Statistical Analyses

Statistical analyses were performed using SPSS *version* 24 and JMP Statistical Software *version* 14.0, with two-sided α=0.05. Standard parametric (Student’s *t*-test) or non-

parametric (Fisher's *exact* or Pearson's chi-square) tests were used to compare variables between groups. Linear regression was used to determine associations of anemia, erythrocyte indices, and other variables with the GDS or DDS (as continuous variables) at baseline (entry) in univariate analyses. Other variables of interest included: age, sex, self-reported race/ethnicity, HIV-disease variables, ART, comorbidities (*e.g.,* HCV serostatus), and cohort membership (*e.g.,* HNRC, TMARC, or *other*). Variables with *p*-values ≤0.10 in univariate analyses were included as candidate covariates in multiple linear regression analyses at entry, and stepwise backward model selection using minimum Akaike Information Criteria (AIC), and *p*-values >0.05, were used to determine final models. Effect sizes were estimated using Cohen's d.

Regression analyses of longitudinal NC outcomes (*e.g.,* continuous GDS) were performed by generalized linear mixed-effects modeling, with participants as a random effects, adjusting for the duration (time) of observation. Variables with $p \leq 0.10$ in univariate analyses were included as candidate covariates in multivariable models. Again, backward model selection was performed to remove covariates with *p*-values >0.05, to determine final models.

Generalized estimating equations (GEE) were also applied as a more general approach, using repeated measures at different time-points to evaluate binary outcomes (GDS- or DDS-defined NCI) in longitudinal analyses. Analyses were repeated in virally suppressed (VS) participants with plasma HIV RNA values <200 copies/mL (n=1,034), to better reflect contemporary ambulatory HIV+ populations on treatment. Similar models were used to explore relationships of erythrocyte indices or anemia with global and domain-specific NCI at entry and over time.

RESULTS

Participant Characteristics

Among 1,339 PWH evaluated, the mean number of NC assessments was 3.2, and the mean duration of follow-up was 30.3 months. Table 1 summarizes participant demographic and clinical characteristics, stratified by anemia at the baseline (entry) visit. Anemia occurred at entry in 297 PWH (22.2%), and developed during follow-up in another 128 (9.6%). Anemia was not associated with age or biological sex, and up to two-thirds of anemic persons were VS. However, anemic PWH were significantly less likely be VS ($p<0.001$). Anemia was also more frequent in PWH of African ancestry, and anemia in females was more often moderately severe (hemoglobin 8.0–10.9 g/dL) *vs.* mild, compared to males (34.9% *vs.* 5.5%, respectively, $p<0.05$). Anemic PWH had a higher prevalence of AIDS, diabetes mellitus, zidovudine or lamivudine use and were more likely to have lower nadir and current CD4+ T-cell counts. At least one abnormal erythrocyte index was present in 206 anemic PWH (69.1%), compared with 508 non-anemic study participants (48.8%). Anemic PWH were also less likely to be receiving tenofovir- or emtricitabine-containing ART. Clinical characteristics of anemic PWH at baseline, stratified by self-reported race/ethnicity (excluding "Other" and Asian ethnicities due to low numbers), are shown in Table S1, Supplemental Digital Content. Hemoglobin values among anemic persons were similar across racial/ethnic groups, with over 50% in each group having *undetectable* HIV RNA in

plasma. Anemic Black PWH were significantly more likely to have microcytic anemia, and anemic whites were more likely to have macrocytic anemia, compared with other groups (both *p*-values<0.01). Anemic Hispanic PWH tended more often to be female (21.4% *vs.* 12.3%, *p*=0.059) than other anemic PWH, whereas anemic White PWH were much less likely to be female (8.9% *vs.* 19.9%, *p*=0.007) and more likely to be older (mean age 47.1 *vs.* 41.8 years, *p*=0.002). With regard to antiretroviral regimens, anemic White individuals were less likely to be on tenofovir (38.4% *vs.* 52.6%, *p*=0.002) and much more likely to be on zidovudine (34.3% *vs.* 16.1%, *p*<0.001).

Associations of Anemia with NCI and Global and Domain Deficit Scores at Entry

NCI was significantly more common in anemic than non-anemic PWH at baseline (49.2% *vs.* 38.3%, respectively, *p*<0.001, Table 1). Mean hemoglobin values in cognitively impaired PWH in this sample (mean hemoglobin 14.1 ± 1.5 g/dL) were not significantly different at baseline from hemoglobin values in unimpaired PWH, and mean MCV was 97.4 ± 9.8 femtoliters in the impaired group. However, the distribution of hemoglobin values differed significantly by cognitive impairment status: the proportion of cognitively impaired PWH with anemia was 26.8%, compared with 19.0% of cognitively normal individuals (*p*=0.001). This difference was actually most pronounced among cognitively impaired, VS PWH, 23.1% of whom were were anemic *vs.* 14.9% of cognitively normal, VS PWH (*p*=001, Supplemental Table 2). Worse GDS values were also associated with older age, self-reported non-African ancestry, longer duration of HIV infection, use of first ART regimen, ritonavir use, and certain comorbidities (all *p*-values<0.05). Lower hemoglobin (*p*=0.006) and anemia (*p*<0.0001) were each associated with higher (worse) GDS values at baseline in univariate analyses (Table 2). In multivariable-adjusted analyses, worse GDS values were associated with anemia (*p*<0.0001), older age (*p*=<0.0001), non-African ethnicity (*p*<0.0001), use of the first ART regimen (*p*=0.010), and ritonavir use (*p*=0.004). Similar associations were observed in VS participants (*p*<0.0001 for anemia; model R^2 = 0.06, *p*=0.0003; Table S3, Supplemental Digital Content).

We then evaluated domain-specific NC performance at baseline to identify specific NC domains which might be compromised by anemia. Figure 1 summarizes anemia associations with GDS as well as DDS values at baseline in all study participants. In addition to its association with worse global NC performance (*higher* GDS, *p*<0.0001, Cohen's d=0.32), anemia was also associated with significantly worse performance in every NC ability domain, including processing speed (*p*<0.0001, d=0.36), motor function (*p*<0.0001, d=0.25), learning (*p*=0.001, d=0.23), delayed recall (*p*=0.001, d=0.23), working memory (*p*=0.001, d=0.21), executive function (*p*=0.021, d=0.15), and verbal fluency (*p*=0.035, d=0.14). These effects are small-to-medium in magnitude.

Longitudinal Associations of Anemia with NC Performance: Global and Domain Deficit Scores

Results of univariate and multivariable mixed-model analyses of the NC performance over time are summarized in Table 3. Anemia (*p*<0.0001), as well as older age, non-African ancestry, diabetes, hypertension, higher plasma HIV RNA, use of first ART regimen, and ritonavir use (all *p*-values≤0.05) were each associated with worse longitudinal GDS

values in multivariable analyses. Similarly, in VS PWH, associations observed in the entire cohort were not significantly altered ($p<0.0001$ for anemia, Table S4, Supplemental Digital Content).

Furthermore, lower hemoglobin was associated with more DDS-defined NCI over time in executive function ($p=0.008$), processing speed ($p<0.0001$), learning ($p=0.040$), attention/working memory ($p=0.046$) and motor ($p<0.0001$) domains, but not verbal and recall ability (Table S5, Supplemental Digital Content). Anemia showed similar associations, including a nearly-significant association with worse recall. Erythrocyte indices were generally not associated with GDS-defined NCI over time. However, hypochromia (lower MCH or MCHC) was associated with more impairment in learning, recall, and/or working memory ($p<0.01$ for association of "low MCH" with impaired learning and delayed recall and p-values<0.01 for associations of lower MCHC values or "low MCHC" with impaired working memory). Higher MCV values tended to associate with less impairment in learning and recall domains (both p-values<0.05).

DISCUSSION

In this multi-cohort study, 22% of PWH met criteria for anemia at baseline or during follow-up. Anemia was associated with self-reported African ancestry, but surprisingly not with biological sex, AIDS status, lower CD4+ T-cell count, or higher plasma viral load. Use of zidovudine and lamivudine and, conversely, the absence of tenofovir- and emtricitabine-containing ART, were also associated with anemia. Importantly, anemia was associated with worse global and domain-specific NC performance in both cross-sectional and longitudinal analyses (except for verbal and possibly, learning and recall domains in longitudinal analyses).

Results from this study confirm prior associations of the GDS with anemia in the CHARTER Study.[6,10] We are unaware of other longitudinal analyses evaluating associations of erythrocyte characteristics and anemia with performance across NC ability domains. While the use of ART has dramatically reduced morbidity and mortality in PWH, some antiretroviral drugs have been implicated in anemia. In the past, zidovudine was one of the most common causes of macrocytic anemia, due to its dose-dependent inhibition of hemoglobin synthesis, globin-gene transcription and erythroid progenitor-cell differentiation and proliferation.[16] Consistent with these effects, nearly a fifth of our study participants had a history of zidovudine use. Lamivudine use was also associated with anemia, possibly due to its common co-formulation with zidovudine.

The frequency of anemia defined by sex-specific norms was higher in PWH of self-reported African ancestry. In some previous studies, including the CHARTER Study, African-ancestry PWH had better NC performance than other subgroups; our findings in this multi-cohort analysis were similar.[10] Nevertheless, in all PWH, including persons of African ancestry, anemia was associated with significantly worse NC performance. This may be partly explained by the fact that non-African-ancestry PWH in our sample had a significantly higher prevalence of comorbidities such as diabetes and hyperlipidemia (contributors to vascular causes of NCI) than PWH of other ancestries. Genetic differences

might also confer greater ability to compensate for anemia in African-ancestry PWH. Finally, it is possible that differences in anemia prevalence by race are artefactual, owing to newly recognized differences in the normal ranges for hemoglobin (and cut-offs for anemia) by race, which have yet to be translated into different reference intervals for these subpopulations in routine patient care.[17]

As noted earlier, anemia was equally prevalent in females and males with HIV at entry. Biological sex was not associated with the GDS at baseline or longitudinally in this study, but other investigators have reported sex differences in NC performance and vulnerability to NCI.[18] For example, a cross-sectional study found higher rates of NCI in males, while other studies have suggested that females may have greater cognitive vulnerability.[19,20] Only 15% of participants in this study were females, however, limiting power to assess the impact of sex on associations of anemia and erythrocyte indices with NC outcomes.

The effect of anemia on global NC performance in PWH has been previously studied, but not its influence on individual ability domains: this is a unique element of the current analysis. Anemia was associated with worse NC performance in all domains at baseline and four of seven domains longitudinally; associations with two other domains showed near-statistical significance. Systemic diseases commonly associated with anemia, like the metabolic syndrome, have been previously associated with worse learning, executive function, and motor skills.[21] Low-grade inflammation persists in PWH on ART, particularly in the presence of comorbidities that are strongly linked to NCI in the published literature. Inflammation due to comorbidities causes iron re-distribution and iron-deficient erythropoiesis, which are often reflected by reduced MCV and MCHC. Our findings are consistent with the concept that different underlying conditions affect different NC domains and their underlying neuronal circuits. Factors such as the duration of HIV disease, changes in ART, and the timing of NC assessments could also have influenced our results,[22] although we adjusted for potential confounders in our analyses, and the median number of visits did not differ between anemic and non-anemic persons (median of 2 visits in both groups, IQR 1–4).

Chronic anemia, even if mild, may significantly impact NC function, despite tightly regulated adaptations such as the upregulation of hypoxia-inducible factors (HIFs), vascular endothelial growth factors (VEGFs), erythropoietin and erythropoietin receptors, and nitric oxide production to mitigate the effects of reduced oxygenation on brain function. [23–25] Erythropoietin, a hormone produced mainly in the kidneys, induces erythropoiesis and may also be neuroprotective by preventing axonal degeneration and neuronal death.[26,27] Lower erythropoietin levels in PWH have been reported in some studies, and others have advocated the use of erythropoietin to reduce anemia[28] and possibly, neuronal injury.[29,30]

Lower MCH and MCV indices were associated with more global and domain-specific NCI over time, differing from prior findings confined to the CHARTER Study.[10] An important difference, however, is that all participants in this study were receiving ART by design, compared with 66% of CHARTER Study participants, and the reasons for hypochromia and/or altered MCV (*e.g.,* inflammation, mitochondrial dysfunction, alcohol use) may have differed.[10] Prior studies have reported associations of higher MCV

with ART-induced mitochondrial dysfunction and blood lactate levels.[31–32] Our study population had a lower prevalence of current/prior zidovudine use and possibly, less heavy alcohol use (causes of macrocytic anemia) and a higher prevalence of iron deficiency than prior studies. Erythrocyte size depends on both hemoglobin content and membrane lipid biosynthesis, but hypochromia typically indicates defective hemoglobin production in, and/or incorporation into, developing erythrocytes. Iron homeostasis is essential for hemoglobin synthesis and diverse cellular metabolic functions.[33] Thus, iron dysregulation disrupts a multitude of processes, leading to anemia and ultimately organ dysfunction. Studies in children, adolescents, and women without HIV have associated altered iron status with NC function,[34] including reduced working memory[35] and attention.[36] Dysregulated iron transport is commonly observed in chronic inflammatory diseases like HIV, due to increased synthesis of the master iron-regulatory hormone, hepcidin. Hepcidin promotes iron sequestration within the monocyte-macrophage compartment and reduces iron bioavailability for erythropoiesis.[37] Although a majority of the PWH we studied are likely to have had anemia of chronic inflammation, we cannot be certain of the cause of the anemia in these individuals based on the available data, and this constitutes a limitation of the present study. Higher hepcidin levels are reported in PWH, even during ART, compared with HIV-negative persons.[38] While we did not measure circulating iron or hepcidin levels in this study, it is well-established that low hemoglobin levels generally reflect reduced iron levels and/or bioavailability.[39]

Another potential limitation of this study is that data were combined from multiple cohorts; despite similar assessments and adjustment for cohort membership in our analyses, cohort heterogeneity may have influenced our results. Secondly, the models did not explain a large amount of variance in NC outcomes, despite their strong statistical significance. In 306 (22.9%) of the assessed visits, study participants also had detectable plasma virus. To determine whether viral suppression, which is associated with higher levels of inflammation, impacted our findings, we performed analyses limited to VS PWH, and the anemia (and hemoglobin) associations with NC performance and NCI persisted. As we did not have reliable information on substance (including alcohol) use disorders, we could not adjust for them in analyses, and they remain a possible source of confounding. While residual confounding is possible due to differences in comorbidity across cohorts, however, the persistence of observed associations in all analyses after adjusting for major comorbidities linked to inflammation lends validity to our findings. Estimated effect sizes for associations of hemoglobin and anemia with NC outcomes were modest but similar to the effect sizes reported for known risk factors for NCI in PWH, such as age and the nadir CD4+ T-cell count.[40]

In conclusion, this study confirms prior associations of anemia and erythrocyte indices with global NC performance and shows that anemia remains very common even among VS PWH. Importantly, these findings also extend our knowledge of the impact of anemia on NC function by demonstrating that anemia is independently associated with worse performance across multiple NC ability domains, cross-sectionally and over time, in diverse PWH. Future investigations of iron metabolism (*e.g.,* with measurement of hepcidin, and/or specific iron-transport proteins) may provide new insights into the observed associations. Hemoglobin and erythrocyte indices are inexpensive and routinely available measures and may be useful

for screening and risk stratification of PWH. Further studies are now indicated, aimed at understanding the causes and reversibility of anemia in PWH on contemporary ART, and on determining the value of early, aggressive treatment of anemia in PWH to reduce NC decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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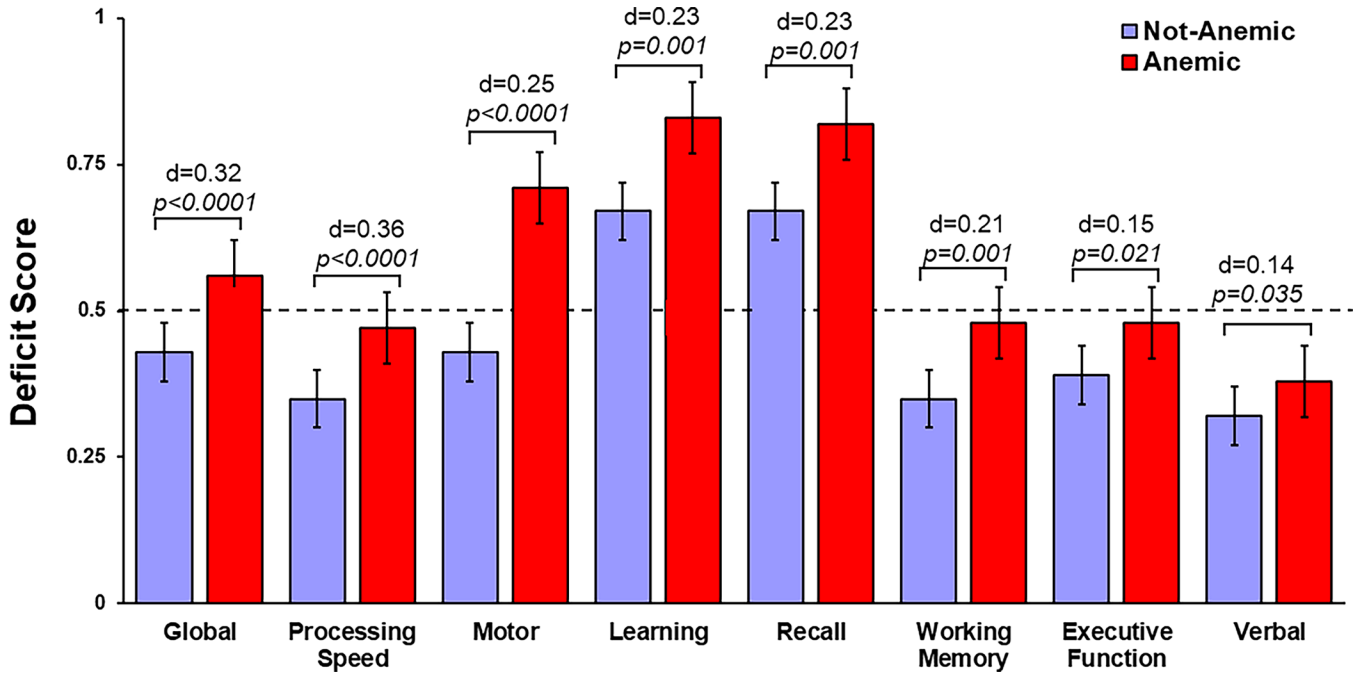

Figure 1.

Associations of Global or Domain-Specific Deficit Scores and Anemia Status at Baseline in 1,339 People with HIV. Bars indicate the mean global deficit score and error bars are the standard error of the mean. Effect sizes, as indicated by Cohen's d (d), are in the small-to-medium range. Values above the dotted line are in the impaired range.

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Table 1:

Demographic and Clinical Characteristics of Study Participants Stratified by Anemia Status at Baseline (Entry).

Variable	All Participants (N=1,339)	Anemic (N= 297)	Not Anemic (N= 1,042)	*p*-value
Sex [1]				0.78
Female	201 (15.0%)	43 (14.5%)	158 (15.2%)	
Male	1138 (85.0%)	254 (85.5%)	884 (84.9%)	
Age (Years)[2]	43.6 (10.2)	44.4 (10.6)	43.4 (10.0)	0.14
Self-Reported Race/Ethnicity [1]				**<0.0001**
Asian	16 (1.2%)	2 (0.67%)	14 (1.3%)	
African-ancestry	224 (16.7%)	75 (25.3%)	149 (14.3%)	
Hispanic	320 (23.9%)	70 (23.6%)	250 (24.0%)	
European	737 (55.0%)	146 (49.2%)	591 (56.7%)	
Other	43 (3.2%)	4 (1.4%)	39 (3.7%)	
HIV Disease Characteristic				
AIDS[1]	779 (58.2%)	207 (69.9%)	572 (54.9%)	**<0.0001**
Duration of HIV (Years)[2]	10.3 (7.5)	10.0 (7.5)	10.4 (7.5)	0.42
Nadir CD4+ T-Cell Count (cells/μL)[3]	199 (69, 319)	148 (40, 277.0)	206 (81.5, 333.0)	**<0.0001**
CD4+ T-Cell Count (cells/μL)	472 (292, 669)	333 (183, 554)	499 (336, 689)	**<0.0001**
CD8+ T-Cell Count (cells/μL)[3]	871 (632, 1184)	898 (610, 1210)	866 (638, 1178)	0.81
HIV RNA (log_{10} cp/mL)[3]	2.6 (1.6, 2.6)	2.6 (1.7, 2.8)	2.4 (1.6, 2.6)	**<0.0001**
HIV RNA ≤ 50 cp/mL[1]	934 (69.7%)	171 (57.6%)	763 (73.2%)	**<0.0001**
HIV RNA ≤ 200 cp/mL[1]	1,034 (77.2%)	198 (66.7%)	836 (80.2%)	**<0.0001**
Antiretroviral Therapy				
First ART Regimen[1]	555 (41.4%)	134 (45.1%)	421 (40.4%)	0.08
Duration of ART (Months)[3]	44.1 (13.3, 93.1)	38.1 (10.3, 86.3)	46.5 (14.1, 94.3)	0.063
Tenofovir Use[1]	680 (50.7%)	127 (42.8%)	553 (53.1%)	**0.002**
Lamivudine Use[1]	568 (42.4%)	152 (51.2%)	416 (39.9%)	**<0.0001**

Variable	All Participants (N=1,339)	Anemic (N= 297)	Not Anemic (N= 1,042)	*p*-value
Emtricitabine Use[1]	553 (41.2%)	95 (32.0%)	458 (43.9%)	**<0.0001**
Ritonavir Use[1]	534 (39.8%)	123 (41.4%)	411 (39.4%)	0.525
Efavirenz Use[1]	321 (24.0%)	59 (19.9%)	262 (25.1%)	0.062
Abacavir Use[1]	286 (21.3%)	74 (24.9%)	212 (20.3%)	0.087
Zidovudine Use[1]	252 (18.8%)	85 (28.6%)	167 (16.0%)	**<0.0001**
Lopinavir Use[1]	179 (13.4%)	47 (15.8%)	132 (12.6%)	0.155
Atazanavir Use[1]	169 (12.6%)	34 (11.5%)	135 (12.9%)	0.497
Darunavir Use[1]	119 (8.9%)	22 (7.4%)	97 (9.3%)	0.314
Raltegravir Use[1]	76 (5.7%)	15 (5.1%)	61 (5.8%)	0.602
Elvitegravir Use[1]	63 (4.7%)	14 (4.7%)	49 (4.7%)	1.00
Dolutegravir Use[1]	52 (3.9%)	12 (4.0%)	40 (3.8%)	0.869
Erythrocyte Indices				
MCV (fL)[3]	95.2 (90.5, 103)	95.3 (88.8, 104)	95.2 (90.9, 102.5)	0.255
MCV Low[1]	29 (2.2%)	19 (6.4%)	10 (1.0%)	**<0.0001**
MCV High[1]	413 (30.8%)	103 (34.7%)	310 (29.7%)	0.100
MCH (pg/cell)[3,4]	32.4 (30.6, 35.0)	32.2 (29.4, 35.0)	32.5 (30.8, 35)	**0.014**
MCH Low[1]	46 (3.4%)	30 (10.1%)	16 (1.5%)	**<0.0001**
MCH High[4]	427 (31.9%	98 (33.0%)	329 (31.6%)	0.650
MCHC (g/dL)[3,4]	34 (33.3, 34.6)	33.7 (32.8, 34.4)	34 (33.4, 34.7)	**<0.0001**
MCHC Low[1]	361 (27.0%)	120 (40.5%)	241 (23.1%)	**<0.0001**
At least 1 abnormal index	714 (53.3%)	205 (69.0%)	509 (48.8%)	**<0.0001**
Comorbidities (Current or Past)				
Diabetes Mellitus[1]	110 (8.2%)	33 (11.2%)	77 (7.4%)	**0.037**
Hypertension[1]	313 (23.7%)	77 (26.9%)	236 (23.0%)	0.241
Hyperlipidemia[1]	271 (20.5%)	48 (16.4%)	223 (21.7%)	**0.046**

Variable	All Participants (N=1,339)	Anemic (N= 297)	Not Anemic (N= 1,042)	*p*-value
HCV Seropositive[1]	253 (19.1%)	59 (20.14%)	194 (18.8%)	0.612
Neurocognitive Performance				
Global Deficit Score (GDS)[3]	0.35 (0.11,0.72)	0.47 (0.19,0.94)	0.33 (0.11,0.67)	**<0.0001**
Neurocognitive impairment[1]	546 (40.7%)	146 (49.2%)	400 (38.3%)	**0.001**

[1]Number (percent)

[2]Mean (standard deviation)

[3]Median (interquartile range)

Statistically significant associations ($p < 0.05$) are indicated by **bold** font.

Abbreviations: *ART*: Antiretroviral Therapy; *cp/mL*: copies per milliliter; *MCV*: Mean Corpuscular Volume; *MCH*: Mean Corpuscular Hemoglobin; *MCHC*: Mean Corpuscular Hemoglobin Concentration; *fl:* femtoliters; *g:* grams*; pg*: picograms; *dL*: deciliter; *HCV*: hepatitis C virus.

[4]"High" MCH and MCHC categories do not add to information provided by the MCV categories and are rarely used clinically. Proportions with "high" MCH values (>34 picograms) are similar to the proportions of "high" MCV, as expected.

Note: Higher (continuous) GDS values indicate worse neurocognitive performance. Anemia was defined by sex-specific criteria (hemoglobin <12 g/dL in females and <13.5 g/dL in males). Gender identity information was not available for the majority of participants.

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Table 2:

Univariate and Multivariable Associations with the GDS at Baseline (N=1,339).

Variable	Univariable Analysis[1]		Multivariable analysis (N=1,339)	
	β (95% CI)	*p*-value	β (95% CI)[2]	*p*-value
Age (Years)	0.007 (0.004, 0.010)	**<0.0001**	0.007 (0.004, 0.009)	**<0.0001**
African ancestry	−0.134 (−0.211, −0.056)	**0.001**	−0.154 (−0.230, −0.078)	**<0.0001**
Duration of HIV (Years)	0.005 (0.0007, 0.01)	**0.02**		
Nadir CD4+ T-cell Count (cells/μL)	−0.0001 (0.0003, 1.1^{-5})	0.07		
HIV RNA (log_{10} cp/mL)	−0.031 (−0.061, −0.0004)	**0.05**		
First ART regimen	0.066 (0.007, 0.125)	**0.03**	0.076 (0.018, 0.134)	**0.01**
Ritonavir use	0.0863 (0.027, 0.145)	**<0.01**	0.085 (0.027, 0.143)	**<0.01**
Tenofovir use	0.0484 (−0.009, 0.106)	0.101		
Darunavir use	0.094 (−0.008, 0.196)	0.070		
Hemoglobin (g/dL)	−0.027 (−0.046, −0.008)	**<0.01**		
Anemia	0.173 (0.104, 0.243)	**<0.0001**	0.179 (0.11, 0.247)	**<0.0001**
Diabetes	0.132 (0.026, 0.237)	**0.01**		
Hypertension	0.090 (0.022, 0.159)	**0.01**		
Hyperlipidemia	0.071 (−0.001, 0.143)	0.06		

[1] All variables with $p \leq 0.10$ in the univariate analysis are shown (statistically significant *p*-values in **bold** font). Variables tested but not shown include: sex, AIDS, current CD4+ and CD8+ T-cell counts, HCV co-infection, duration of ART use, history of lamivudine, emtricitabine, efavirenz, abacavir, zidovudine, lopinavir, atazanavir, raltegravir, elvitegravir and dolutegravir use, as *p*-values were > 0.10. Erythrocyte indices – MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) - were not statistically significant, with *p*-values > 0.10 and therefore were not included.

[2] Multivariable analysis after adjusting for cohort.

Abbreviations: *CI* = Confidence Interval; g/dL = grams per deciliter

Note: Higher (continuous) GDS values (positive estimate) indicate *worse* neurocognitive performance.

Table 3:

Univariate and Multivariable Associations with the GDS Over Time in All Participants (N=1,339)

Variable	Univariable Analysis β (95% CI)	*p*-value	Multivariable Analysis β (95% CI)[2]	*p*-value
Age (Years)	0.008 (0.005, 0.011)	**<0.0001**	0.005 (0.002, 0.008)	**<0.0001**
African Ancestry	−0.14 (−0.214, −0.072)	**<0.0001**	−0.165 (−0.238, −0.091)	**<0.0001**
AIDS	0.054 (−0.002, 0.109)	0.06		
Duration of HIV (Years)	0.002 (−.0002978 .004)	0.09	0.003 (−2.988e-4, 0.005)	0.08
CD4+ T-Cell Count (cells/μL)	−5.8e-5 (−0.0001, −9.04e-06)	**0.02**		
HIV RNA (log_{10} cp/mL)	0.010 (−0.002, 0.023)	0.10	0.018 (0.003, 0.032)	0.02
First ART Regimen	0.047 (0.018, 0.076)	**<0.01**	0.062 (0.03, 0.095)	**<0.0001**
Ritonavir Use	0.040 (0.015, 0.065)	**<0.01**	0.041 (0.014, 0.069)	**<0.01**
Emtricitabine	−0.021 (−0.045, 0.002)	0.08		
Hemoglobin (g/dL)	−0.018 (−0.036, 0.0003)	0.05		
Anemia	0.145 (0.079, 0.211)	**<0.0001**	0.146 (0.077, 0.215)	<0.0001
HCV	0.084 (0.028, 0.140)	**<0.01**	0.033 (−0.04, 0.106)	0.37
Diabetes Mellitus	0.171 (0.071, 0.270)	**<0.01**	0.110 (3.05E-5, 0.221)	**0.05**
Hypertension	0.120 (0.056, 0.185)	**<0.0001**	0.072 (0.002, 0.143)	0.04

[1] All variables with $p \leq 0.10$ in the univariate analysis are shown.

Variables evaluated but not shown: sex, duration of HIV infection, current CD8+ T-cell count, duration of ART use, hyperlipidemia, and zidovudine, efavirenz, tenofovir and lamivudine use, since *p*-values were > 0.10. Erythrocyte indices - MCV, low and high MCV, MCH, MCHC, and low MCHC were not statistically significant (*p*-values > 0.10), so they were not included in the final model.

[2] Multivariable models were adjusted for variables with *p*-values < 0.10 in univariate analyses, as well as for time and cohort.

Note: Higher (continuous) GDS values (positive estimate) indicate *worse* neurocognitive performance.