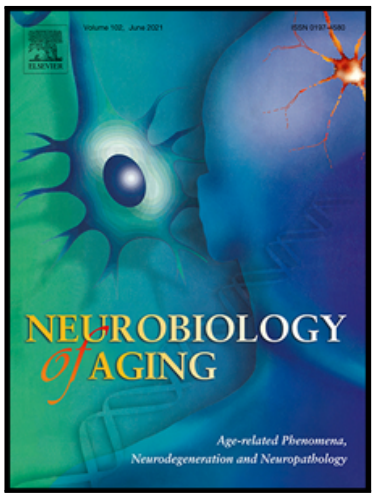
This work was written as part of one of the author's official duties as an Employee of the United States Government and is therefore a work of the United States Government. In accordance with 17 U.S.C. 105, no copyright protection is available for such works under U.S. Law. Access to this work was provided by the University of Maryland, Baltimore County (UMBC) ScholarWorks@UMBC digital repository on the Maryland Shared Open Access (MD-SOAR) platform.

Please provide feedback

Please support the ScholarWorks@UMBC repository by emailing scholarworks-group@umbc.edu and telling us what having access to this work means to you and why it's important to you. Thank you.

Journal Pre-proof



Red cell distribution width, anemia and their associations with white matter integrity among middle-aged urban adults

May A. Beydoun , Danielle Shaked , Sharmin Hossain , Jordan Weiss , Hind A. Beydoun , Ana I. Maldonado , Leslie I. Katzel , Christos Davatzikos , Rao P. Gullapalli , Stephen L. Seliger , Guray Erus , Michele K. Evans , Alan B. Zonderman , Shari R. Waldstein

PII: S0197-4580(21)00158-5
DOI: https://doi.org/10.1016/j.neurobiolaging.2021.05.004
Reference: NBA 11151

To appear in: *Neurobiology of Aging*

Received date: 21 December 2020
Revised date: 3 May 2021
Accepted date: 4 May 2021

Please cite this article as: May A. Beydoun , Danielle Shaked , Sharmin Hossain , Jordan Weiss , Hind A. Beydoun , Ana I. Maldonado , Leslie I. Katzel , Christos Davatzikos , Rao P. Gullapalli , Stephen L. Seliger , Guray Erus , Michele K. Evans , Alan B. Zonderman , Shari R. Waldstein , Red cell distribution width, anemia and their associations with white matter integrity among middle-aged urban adults, *Neurobiology of Aging* (2021), doi: https://doi.org/10.1016/j.neurobiolaging.2021.05.004

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Published by Elsevier Inc.

Journal Pre-proof

Highlights

- Greater RDW_{v1} was associated with poorer WMI, among males only, particularly in terms of lower mean global fractional anisotropy (FA).
- No such associations were found for anemia and δRDW (overall or sex-specific), or for RDW exposures among females and the non-anemic group.

Journal Pre-proof

Journal Pre-proof

Red cell distribution width, anemia and their associations with white matter integrity among middle-aged urban adults

May A. Beydoun[1,*]; Danielle Shaked[2]; Sharmin Hossain[1] ; Jordan Weiss[3]; Hind A. Beydoun[4]; Ana I. Maldonado[1,5]; Leslie I. Katzel[6,7] , Christos Davatzikos[8]; Rao P. Gullapalli[9]; Stephen L. Seliger[10]; Guray Erus[8]; Michele K. Evans[1]; Alan B. Zonderman[1]; Shari R. Waldstein[5,6,7]

[1]Laboratory of Epidemiology and Population Sciences, NIA/NIH/IRP, Baltimore, MD

[2] Department of Psychology, VA Boston Healthcare System, Boston, MA

[3] Department of Demography, University of California, Berkeley, Berkeley, CA, USA.

[4]Department of Research Programs, Fort Belvoir Community Hospital, Fort Belvoir, VA

[5] Department of Psychology, University of Maryland Baltimore County, Catonsville, MD

[6]Geriatric Research Education and Clinical Center, Baltimore VA Medical Center, Baltimore, MD

[7]Division of Gerontology & Geriatric Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

[8] Artificial Intelligence in Biomedical Imaging Lab, Center for Biomedical Image Computing and Analytics (CBICA), Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA

[9]Department of Diagnostic Radiology, University of Maryland School of Medicine, Baltimore, MD

[10]Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

*Corresponding author:

May A. Beydoun, PhD

Journal Pre-proof

Journal Pre-proof

NIH Biomedical Research Center

National Institute on Aging, IRP

251 Bayview Blvd.

Suite 100

Room #: 04B118

Baltimore, MD 21224, USA

E-mail: baydounm@mail.nih.gov

Fax: 410-558-8236

† MAB had full access to the data used in this manuscript and completed all the statistical analyses.

Type of manuscript: Regular Article

Abbreviated title: Red cell distribution width, anemia and white matter integrity

Journal Pre-proof

Journal Pre-proof

ABBREVIATIONS

Above Poverty (AP)

Below Poverty (BP)

Core for Translational Research in Imaging @ Maryland (C-TRIM)

Coefficient of Variation (CV)

Diffusion Tensor Magnetic Resonance Imaging (dMRI)

Dual X-ray absorptiometry (DXA)

Fractional Anisotropy (FA)

False Discovery Rate (FDR)

Fluid-Attenuated Inversion Recovery (FLAIR)

Field of View (FOV)

Gray Matter (GM)

Hemoglobin (Hb)

Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study

Healthy Eating Index 2010 release (HEI-2010)

Homocysteine (Hcy)

Journal Pre-proof

Journal Pre-proof

High School (HS)

Least absolute shrinkage and selection operator (LASSO)

Mean Cell Hemoglobin (MCH)

Mean Cell Volume (MCV)

Mean Diffusivity (MD)

Magnetization prepared rapid gradient echo (MP-RAGE)

Magnetic Resonance Imaging (MRI)

Medical Research Vehicle (MRV)

Multiplicative intrinsic component optimization (MICO)

Multi-atlas region Segmentation utilizing Ensembles (MUSE)

Red Blood Cells (RBC)

Red Cell Distribution Width (RDW)

Regions of Interest (ROI)

Sensitivity Analysis (SA)

Standard Deviation (SD)

Standard Error (SE)

Journal Pre-proof

Journal Pre-proof

Structural MRI (sMRI)

United States (US)

Wide Range Achievement Test, version 3 (WRAT-3)

White Matter (WM)

White Matter Integrity (WMI)

White Matter Lesion (WML)

White Matter Lesion Volume (WMLV)

World Health Organization (WHO)

Journal Pre-proof

Journal Pre-proof

ABSTRACT

Anemia (blood hemoglobin (Hb) <13 g/dL among males; <12 g/dL among females) and elevated red cell distribution width (RDW) are potential risk factors for reduced brain white matter integrity (WMI), reflected by lower fractional anisotropy or increased mean diffusivity. Cross-sectional data with exposure-outcome lag time was used, whereby hematological exposures (RDW and Hb) and covariates were compiled from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study with available visit 1 (v_1) (2004-2009) and/or v_2 (2009-2013) data; while diffusion tensor magnetic resonance imaging (dMRI) outcome data were collected at HANDLS SCAN visit (v_{scan}: 2011-2015, n=214, mean follow-up from v_1 ±SD: 5.6±1.8y). Multivariable-adjusted linear regression analyses were conducted, overall, stratifying by sex, and further restricting to the non-anemic for RDW exposures in part of the analyses. Among males, $RDW_{(v1)}$ was linked with lower global mean fractional anisotropy (standardized effect size b=-0.30, P=0.003, q<0.05; basic model), an association only slightly attenuated with further covariate adjustment. Anemia was not a risk factor for poor WMI, independently of RDW . Ultimately, pending further longitudinal evidence, initial RDW appears to be associated with poorer WMI among males.

Journal Pre-proof

Journal Pre-proof

Key words: Red cell distribution width, anemia, white matter integrity, aging.

1. Introduction

A consequence of a progressively aging population is an increase in the number of older adults living with chronic conditions like dementia, many of which have no cure and require a large unmet medical need (Winchester et al., 2018). Consequently, researchers have investigated multiple pathways through which progression to dementia can be slowed by delaying cognitive impairment. In addition to several structural brain neuroimaging markers [e.g., reduced brain volumes and increased white matter lesion volume (WMLV)], reduced white matter integrity (WMI) is associated with decrements in various cognitive abilities and may be used as an early marker for dementia risk (Cavedo et al., 2012; Glymour et al., 2012; Hsu et al., 2018; Louapre et al., 2016; Muller et al., 2020; Walter et al., 2019).

In recent years, anemia—defined by the World Health Organization (WHO) as reduced blood hemoglobin (Hb) <13 g/dL among males and <12 g/dL among females (World Health Organization, 1972)—has been identified as an important and understudied direct, mechanistic pathway to age-related cognitive decline (Chung et al., 2014; Hong et al., 2020; Park et al., 2019; Schneider et al., 2016; Sousa et al., 2018; Taniguchi et al., 2019; Trevisan et al., 2016; Weuve et al., 2014; Winchester et al., 2018; Wolters et al., 2019). It is also linked to numerous other markers of poor health and

Journal Pre-proof

physical function and potentially triggered by other nutritional insufficiencies (e.g. folate and vitamin B-12) (Dlugaj et al., 2016). Prior work examining anemia's relationship with cognitive decrements suggest several mechanisms including reduced blood oxygen-carrying capacity resulting in brain hypoperfusion, as well as heightened oxidative stress and inflammation, both of which can contribute to neurodegeneration (Faux et al., 2014; Zlokovic, 2011).

Relatedly, red cell distribution width (RDW) is a marker for variation in red blood cell sizes, known as anisocytosis. It has been associated with chronic disease morbidity and mortality (Hoffmann, 2012; Li et al., 2017; Patel et al., 2010; Perlstein et al., 2009; Tajuddin et al., 2017), and recent research demonstrated that RDW was related to reduced performance on tests of verbal memory, attention and higher prevalence of dementia (Beydoun et al., 2020; Ozturk et al., 2013; Wan et al., 2020; Weuve et al., 2014; Winchester et al., 2018). For example, a recent study observed independent associations of RDW and anemia with lower cognitive health (Winchester et al., 2018).

To date, few mechanistic studies in humans have examined associations of anemia or RDW with brain imaging markers (Dagistan and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). The few studies that have been conducted indicate an association between anemia and smaller total and gray matter (GM) brain volumes, as well as a positive association between RDW and white matter lesion volumes (WMLV)(Dagistan

Journal Pre-proof

and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). This limited research to date has not investigated sex differences in those associations. This is an important consideration as prior work has reported that females face higher prevalence of anemia compared with males (Beydoun et al., 2020). Moreover, researchers have observed a correlation between RDW and anemia (Beydoun et al., 2020). Sex-based differences in brain MRI outcomes are well established (Cosgrove et al., 2007), and are thought to often be independent of other socio-demographic characteristics. Furthermore, elevated RDW has been linked to poorer cognitive performance, particularly within the attention domain, in a sex-specific manner (Beydoun et al., 2020). Taken together, the literature suggests a relationship between RDW/anemia and worse neurocognitive outcomes, which may vary across population strata, including sex. Given that RDW and anemia have been shown to have adverse effects on other neurobiological endpoints (e.g., GM, WMLV), perhaps they also negatively impact WMI, captured by reduced global and regional fractional anisotropy (FA) and increased mean diffusivity (MD), and in a sex-specific manner.

We build on the extant literature by examining associations of anemia and RDW status—and change therein—with global and regional cortical WMI. We use a unique sample of urban adults that varies in balanced manner with respect to sex, socioeconomic position, and race. Our primary hypothesis is that baseline anemia and

Journal Pre-proof

RDW, and change in RDW over time, would be associated with reduced WMI and may vary with respect to sex.

2. Methods

2.1 Database

We used data from the Healthy Aging of Neighborhoods of Diversity across the Life Span (HANDLS) study, an ongoing prospective cohort study among a socio-demographically diverse sample of middle-aged White and African American urban adults aged 30-65 y at baseline in 2004-2009. Interviews were completed among participants who were identified by random sampling of addresses within every census tract. Participants were eligible and invited when meeting the following eligibility criteria: (1) age range: 30–64 y; (2) not pregnant; (3) no active cancer treatment within 6 months; (4) without an AIDS diagnosis; (5) capable of written informed consent, and (6) capable of producing valid government-issued identification and verifiable address. Details on sampling strategy and inclusion criteria are described elsewhere(Evans et al., 2010).

In visit 1, HANDLS researchers collected information on study participants in two phases. In Phase 1, in-home interviews were conducted during which researchers collected information on demographic characteristics, psychosocial health, and completed a dietary interview. Phase 2 was conducted on Medical Research Vehicles

Journal Pre-proof

(MRVs), which allowed researchers to collect a wide range of physical and mental health metrics as well as anthropometrics and biological samples. These included, for example, Dual X-ray Absorptiometry (DXA) for bone mineral density and body composition, an electrocardiogram, intima-media thickness by ultrasound, physical examination by a physician, neuropsychological tests, and inventories to assess psychiatric symptoms(Evans et al., 2010). Comparable information was collected on MRVs during a second visit (v_2: 2009-2013). Diffusion tensor magnetic resonance imaging (dMRI) measures were collected at a follow-up visit, as part of the HANLS SCAN ancillary study(Waldstein et al., 2017). Both v_1 and v_2 data were collected prior to the HANDLS SCAN visit (v_{scan}: 2011-2015) for all participants. The current study utilized hematological data (anemia and RDW) from visit 1 (v_1: 2004-2009) and change in RDW ascertained from v_1 and v_2 (2009-2013) among a sub-sample of N_{max}=240 participants within the HANDLS SCAN sub-study (v_{scan}: 2011-2015)(Evans et al., 2010; Waldstein et al., 2017).

Study protocols for HANDLS and HANDLS SCAN were approved by the National Institute on Environmental Health Sciences Institutional Review Board (IRB) of the National Institutes of Health. Moreover, HANDLS SCAN was also approved by IRBs of the University of Maryland School of Medicine and the University of Maryland, Baltimore County. Written informed consent was obtained for all participants.

Journal Pre-proof

Journal Pre-proof

2.2. Study participants

The baseline HANDLS sample was comprised of 3,720 participants (30-65 y, African Americans and Whites, Phase 1, v_1). Of the initial 3,720 participants, we retained individuals with RDW data at either v_1 or v_2 (N=3,017), among whom v_1 Hb was complete for 2,744 participants. We further restricted our sample to those who participated in the HANDLS SCAN sub-study (the largest available HANDLS SCAN sample was n=240). This yielded an analytic sample of 214 participants with complete dMRI parameters of interest, RDW at either visit, and v_1 Hb. Comparing the final sample (N=214) with the remaining excluded participants from the initial sample (n=3,720), the final sample had higher proportions of Whites (59% vs. 40%, $P<0.05$) and individuals living above poverty (67% vs. 58%, $P<0.05$). Sample selectivity for the non-anemic group at v_1 (n=190) was similar with respect to race ($p<0.05$), but not with respect to poverty status ($p\geq0.05$), while no socio-demographic differences were detected for the non-anemic group at either visit (i.e., v_1 and/or v_2: n=182) vs. those excluded from the initial sample (n=3,720). In the main final analytic sample (n=214), the length of follow-up time between v_1 and v_{scan} had a mean±SD of 5.6±1.8 y (range: 2.3-10.0y).

Journal Pre-proof

Journal Pre-proof

2.3. Brain dMRI

dMRI was assessed using multi-band spin echo EPI sequence with a multi-band acceleration factor of three (**supplemental method 1)**. FA and trace (TR, also known as mean diffusivity, MD) images were evaluated from tensor images, with higher FA values indicating healthier WMI. Summation of eigenvalues for diffusion tensor yields MD, with higher values suggesting poorer WMI (Jones, 2008). Computed FA and MD images were aligned to a common template space using deformable registration with a standard dMRI template (i.e., EVE (Wakana et al., 2004)). We calculated the average of right and left FA and MD values for each ROI corresponding to WM regions (See **supplemental Table 1** for list of ROIs). Global FA and MD were computed as the average across all WM ROIs. Selection of cortical WM sub-regions that comprised the bilateral (Left/Right) larger brain regions (Frontal, Temporal, Parietal, Occipital) was similar to previous studies (see Roalf et al., 2015; Shaked et al., 2019).

2.4. RDW at v_1 and δRDW

RDW was determined by an automated Coulter DXH 800 hematology analyzer as part of peripheral complete blood count (Beckman Coulter, Brea, CA). The main exposure of interest was RDW-CV (coefficient of variation, expressed in %), computed using RDW-Standard Deviation (SD, unit: fL) as a percent of mean cell volume (MCV), whereby RDW-CV=RDW-SD×100/MCV. In addition to RDW(v_1),

Journal Pre-proof

annual rate of change in RDW between v_1 and v_2 (aka δRDW) was also of interest (see **supplemental method 2**), to assess the relationships between longitudinal change in this exposure and follow-up outcome measures.

2.5. Hb and anemia

Hb was determined from 1 ml of blood sampled from participants after an overnight fast and refrigerated for ≤ 6 days (Quest diagnostics). We defined anemia based on the World Health Organization as low blood Hb levels (<13 g/dL in males and <12 g/dL in females (World Health Organization, 1972). Non-anemic participants were selected out for a secondary analysis for RDW exposures. Specifically, for RDW(v_1), absence of anemia was defined only for v_1, whereas in the case of δRDW, non-anemic reflected v_1, v_2 or both. V_1 anemia was the primary exposure of interest, while v_1 Hb was considered a secondary exposure.

2.6. Covariates

Covariates were selected for their potential association with the exposures and/or outcome. We included age (y) at baseline, sex (male, female), self-reported race (African American, White), poverty status determined by self-reported household income either <125% or ≥125% of the 2004 Health and Human Services poverty guidelines (Department of Health and Human Services, 2004) and time (days) between v_1 MRV

Journal Pre-proof

visit and v_{scan}. We adjusted for additional covariates after identifying them as associated with anemia and/or RDW exposures with machine learning techniques [Least absolute shrinkage and selection operator (LASSO) models], followed by backward elimination. Additional information is provided in **online supplemental method 3**. It is worth noting that even though MCV is a major hematological measure linked to RDW, it was dropped from covariate selection, given its high correlation with other measures such as mean cell hemoglobin (MCH), (Pearson's r>0.90). While sex was the main effect modifier in our analyses, other secondary stratifying variables included poverty status (defined as such by design at first-visit), race (self-identified), and age group categorized to distinguish younger middle-aged with older middle-aged adults (30-49 at v_1 vs. 50-66 at v_1). In the final selected sample (*n*=214), the breakdown by socio-demographic factors was as follows: sex (males: *n*=97; females: *n*=117); age group (<50 years: *n*=127; ≥ 50 years: *n*=87); race (Whites: *n*=127; African-Americans: *n*=87); poverty status (Below poverty: *n*=70; Above poverty: *n*=144).

2.7. Statistical analysis

We used Student's *t* and chi-square tests as appropriate to compare sample characteristics by sex. We further tested for sex differences in sample characteristics after covariate adjustment (age, race, and poverty status) using multivariable-adjusted models. We additionally examined sample characteristics across anemia and RDW_{v1}. To

Journal Pre-proof

Journal Pre-proof

test our main hypotheses, we fit a series of covariate-adjusted ordinary least square (OLS) regression models, overall and stratified by sex, using dMRI as the outcome. These models produced coefficient estimates indicating the association between each of the three primary exposures of interest (i.e. v_1 anemia, RDW and δRDW) to dMRI outcomes. We obtained standardized coefficient estimates which were interpreted as the fraction of 1 SD change in dMRI outcome per 1 SD change in a continuous exposure (i.e., RDW and δRDW). Estimates > 0.20 were considered moderate-to-strong; estimates between 0.10 and 0.20 were considered weak-to-moderate.

We conducted our analysis in two stages. *Analysis A* included global mean FA and mean MD. In addition to stratifying by sex, an additional analysis was carried out (*Analysis A'*), which considered age group (<50 years vs. ≥50 years), race and poverty status as alternate stratifying variables. *Analysis B* included measures of FA and MD for the frontal, temporal, parietal, and occipital cortical WM regions (left and right) yielding a total of 16 models. *Analysis B* was conducted on a case-by-case basis if at least one exposure-outcome (global mean FA/mean MD) association was statistically significant from *Analysis A* ($P_{uncorr}<0.05$), considering each main exposure separately (i.e. anemia at v_1, RDW at v_1 and δRDW), within each stratum (i.e. overall or within males and females) and each adjusted model.

Secondary outcomes were bilateral means of FA/MD at each independent ROI (**Supplemental Table 1,** 51 WM-related ROIs highlighted in red). Volcano plots were

Journal Pre-proof

Journal Pre-proof

used to visualize select findings from these models (R Foundation for Statistical Computing, 2013). These plots display Log_{10} (p-values) for each set of models against standardized beta coefficients (*b*) on the X-axis, highlighting findings with larger *b*. Only ROIs with uncorrected p-value <0.05 are presented. Visualization of ROI-specific *b* with standard brain images was carried out using FSLeyes software (Jenkinson et al., 2002; Jenkinson and Smith, 2001) applied to these dMRI results (URL: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes).

We corrected for multiple testing using the false discovery rate (FDR, q-value) while treating the overall and sex-stratified analyses as separate hypotheses. Our correction for multiple testing was only applied to the minimally adjusted models (i.e., Model 1, explained below) for each of *Analysis A* and *Analysis B*. In Model 1, we adjusted for age, sex, race, poverty status, and follow-up time between v_1 and v_{scan}; FDR q-values were only presented for this model when $P_{uncorr}<0.05$ for exposure-outcome associations. Statistical significance in Model 1 was determined when FDR q-value<0.05. We considered a q-value<0.10 indicative of a trend. The results for *Analysis A'* were shown only for the minimally adjusted model without correction for multiple testing, though additional adjustment for other hematological factors was explored.

In sensitivity analyses, we used a data set where only the additional covariates were imputed (k=5 imputations, 10 iterations, chained equations) to obtain the largest possible sample size with complete exposure and outcome data. This sensitivity

Journal Pre-proof

analysis adjusted for those additional covariates which were selected using a multi-step process as described in **supplemental method 3**. The initial pool of selected and imputed covariates were associated with hematological measures and/or cognitive outcomes as identified in prior work (Beydoun et al., 2014).

Overall, our modeling approach consisted of: a model with limited covariate adjustment fit on the unimputed data (Model 1) with a sensitivity analysis on the imputed data; Model 2 built on Model 1 by adding hematological measures that are considered to be the most important potential confounders in the main associations, aside from socio-demographic factors; Model 3 built on Model 2 by adding nutritional/dietary characteristics (i.e., Healthy Eating Index-2010 total score, serum B-12 and serum folate), considered as potential confounders, after other hematological factors are explained away; Model 4 built on Model 2 through the addition of an alternate set of potential confounders, namely inflammatory conditions (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 built on Model 2 with measures of adiposity and metabolic disturbance factors, as a third alternate set of confounders (waist circumference, cholesterol, cholesterol:HDL ratio, triglycerides, creatinine); and Model 6 built on Model 2 with additional covariate adjustment, which may act as additional confounders to the main socio-demographic and hematological factors (e.g., education, Wide Range Achievement Test [WRAT], smoking). In sensitivity analyses for these models, we tested for effect modification by including 2-way

Journal Pre-proof

interaction terms between the exposure and sex with a type I error of 0.10 used for 2-way interaction terms due to reduced statistical power (Selvin, 2004).

We replicated all analyses in the subsample of participants who were non-anemic at v_1 for the $RDW_{(v1)}$ and the non-anemic at v_1 and v_2 for δRDW (See **Figure 1**). We also conducted a secondary analysis for visit 1 Hb (as a continuous exposure), overall and stratified by sex. We did not account for multiple testing in these analyses nor in models 2-6. All analyses were conducted using Stata version 16.0 (STATA, 2019) or R version 3.6.1.

3. Results

3.1. Sample characteristics

Sample characteristics included in this study, overall and stratified by sex, are presented in **Table 1**. The selected sample consisted of 97 males and 117 females, with v_1 ages of mean±SD 47.7±9.1y, 40.7% of whom were African American and 67.3% living above poverty. Males had a higher proportion than females with household incomes above 125% of the federal poverty line (aka "above poverty"), lower mean RDW_{v1} (13.7 vs. 14.2, p=0.007; overall SD=1.55) and had a reduced $anemia_{(v1)}$ prevalence (6.1% vs. 14.0%, p=0.034). Mean follow-up times did not differ significantly by sex (p=0.75, *t*-test), nor did they differ significantly by anemia status at v_1 or v_1/v_2 (p>0.05, data not shown). Nevertheless, follow-up times were longer on average by 0.8y among African

Journal Pre-proof

Journal Pre-proof

Americans vs. Whites, and by 1.3y among individuals living below poverty vs. those above poverty at v_1 (data not shown). While mean FA did not differ by sex, mean MD was higher among males, suggesting reduced WMI compared to females (P<0.010). However, within cortical regions, both FA and MD regional measures showed reduced WMI among males (i.e., lower FA and higher MD). Other imputed covariates that exhibited sex differences that survived adjustment for age, race and poverty status included C-reactive protein (Males(M)<Females(F)), albumin (M>F), cholesterol:HDL-C ratio (M>F), triglycerides (M>F), creatinine (M>F), mean cell hemoglobin (M>F), serum iron (M>F), and ESR (M<F). Importantly, v_1 Hb was significantly more elevated among males compared with females (P<0.001).

Supplemental Table 2 presents those same study characteristics across anemia and $RDW_{(v1)}$ tertile groups, in the total sample, as well as stratified by sex. Generally, African Americans were more represented in the anemic group (vs. non-anemic) and in the uppermost RDW tertile vs. the lower two tertiles exhibiting a dose-response relationship. RDW was similarly more elevated in the $anemia_{(v1)}^{+}$ vs. $anemia_{(v1)}^{-}$ groups. Overall and among females, anemia and RDW tertiles were generally not associated with FA or MD global or cortical regional measures, with few exceptions. However, among males, there was a linear dose-response relationship between $RDW_{(v1)}$ tertiles and mean global FA as well as FA in specific regions, mainly frontal, parietal and occipital, reflecting poorer WMI (lower FA), with higher $RDW_{(v1)}$. Similar patterns were

Journal Pre-proof

observed among males with respect to the association of FA measures with anemia$_{(v1)}$ groups, suggesting that anemia is linked to poorer WMI (lower FA) among males. Due to reduced statistical power (only n=3 were anemic), with few exceptions, no significant associations were found among males across anemia status at v_1/v_2, with respect to means of FA and MD global or cortical regional measures.

3.2. Anemia, Hb and RDW's associations with global and cortical WMI: sex-specific findings

Tables 2-3 and **supplemental Tables 3-4** test our main hypotheses, namely the associations of anemia and/or RDW exposures with WMI markers, in covariate-adjusted linear regression models and accounting for multiple testing. All results are represented in the total sample, among males and females separately and in the non-anemic group for RDW exposures. Anemia$_{(v1)}$ was not associated with WMI measures (FA or MD) as shown in **Table 2**. In a sensitivity analysis, Hb$_{(v1)}$ was directly associated with global FA among males in the minimally adjusted model, reflecting a potential beneficial effect on WMI, particularly in frontal and occipital regions. This association, was, however, markedly attenuated in Model 2, upon adjustment for other hematological measures, including RDW$_{(v1)}$.

However, based on **Table 3** findings, RDW$_{(v1)}$ was linked to lower global WMI in males, particularly in terms of reduced mean FA (**Table 3**, Model 1, *Analysis A*,

Journal Pre-proof

standardized effect size b=-0.30, P=0.003, q<0.05). In region-specific analyses, (*Analysis B)*, associations of greater RDW with lower WMI was observed specifically in frontal and parietal FAs and occipital FAs and MDs. These associations among males remained statistically significant upon further adjustment for hematological measures (**Table 3**, Model 2), and our further analyses indicated statistically significant sex differences in those associations (**Table 3**, Model 2, sex×$RDW_{(v1)}$ P<0.05 in non-stratified models). When further adjustment was made for additional covariates, these relationships remained largely unaltered (**supplemental Table 3**). One exception was Model 6 for $RDW_{(v1)}$ vs. mean FA among males, suggesting potential confounding effects by education, literacy and smoking status, factors known for their associations with various neuro-cognitive outcomes (Beydoun et al., 2014). Another exception was Model 5 for $RDW_{(v1)}$ vs. mean MD among males, suggesting potential confounding by adiposity and metabolic disturbance factors(Beydoun et al., 2008). There were no significant associations of FA/MD measures with $RDW_{(v1)}$ among females or in the non-anemic group (Models 1 and 2, **Table 3**); as well as between the δRDW exposure and the main outcomes of interest (**Supplemental Table 4**, Models 1-2).

3.3. RDW's association with ROI-specific WMI among males

Figure 2 displays the findings from the minimally adjusted models among males with respect to the association of $RDW_{(v1)}$ with regional FAs and MDs. As shown in the volcano plots, all associations were in the expected direction whereby a higher $RDW_{(v1)}$

Journal Pre-proof

was associated with lower regional FAs and higher regional MDs. Among significant associations, some of the strongest effects sizes (b<-0.30) for $RDW_{(v1)}$ vs. FA were concentrated in the parietal region (e.g. supramarginal WM, pre-cuneus WM, precentral WM, and angular WM), while others were found in occipital (e.g lingual WM), frontal (e.g. middle fronto-orbital WM) and temporal cortical regions (e.g. inferior temporal WM). Nevertheless, the strongest association was found in the cingulate WM among males with respect to FA (b=-0.35), and other associations with b<-0.30 included $RDW_{(v1)}$ vs. FA in the splenium of the corpus callosum, cerebellar and middle cerebellar peduncle. However, with respect to MD, only three ROIs had effect sizes b>+0.30, namely lingual WM (occipital), medial lemniscus and the fornix. Thus, poor lingual WM integrity was associated with higher $RDW_{(v1)}$ among males, consistently in terms of lower FA and higher MD, with a strong effect size.

3.4. Anemia and RDW's associations with global and cortical WMI: age-, race- and poverty status-specific findings

Figure 3 presents the findings from the minimally adjusted models with all exposures and global FA/MD as outcomes, stratifying by age group, race and poverty status (*Analysis A'*). The results indicate that anemia was associated with lower mean FA in the older group only (≥50: β±SE: -0.0152±0.00070, p=0.033), as well as among individuals living above poverty (AP: β±SE: -0.00886±0.00445, p=0.048). These findings were, however, markedly attenuated and did not retain statistical significance (p>0.05) with

Journal Pre-proof

Journal Pre-proof

further adjustment for other hematological factors, including $RDW_{(v1)}$, (i.e., Model 2, data not shown). In contrast, $RDW_{(v1)}$ was linked with lower FA among individuals living above poverty in the minimally adjusted model (AP: β±SE: -0.00209±0.00089, p=0.020), an association that remained statistically significant in Model 2, with adjustment for other hematological factors including blood Hb (AP: β±SE: -0.00391±0.00130, p=0.004). No racial differences were detected in the main associations between anemia/RDW exposures and global FA/MD outcomes. No stratum-specific associations with global FA/MD outcomes were found for the δRDW exposure.

4. Discussion

This study is among a few to examine the association between anemia and WMI and to our knowledge, the first to link RDW with WMI, particularly in a racially and socio-economically diverse sample of middle-aged urban adults. Among key findings, greater RDW_{v1} was consistently associated with poorer WMI among males. Most notably, in males, $RDW_{(v1)}$ was linked with lower global mean FA (standardized effect size b=-0.30, P=0.003, q<0.05) in the basic model adjusted for socio-demographic factors, an association that remained statistically significant upon adjustment for other hematological measures and other potential confounders and detected in all cortical regions except the temporal lobe. No such associations were found for anemia, and δRDW (overall or sex-specific), or for RDW exposures among females and the non-anemic group.

Journal Pre-proof

Journal Pre-proof

Before our study, there was no evidence for relationships between anemia or RDW and WMI in humans. Most prior studies examined relations between Hb or anemia and other brain MRI outcomes (e.g., volumetric markers) and only a few tested associations of those various brain MRI outcomes with RDW exposures (Dagistan and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). For instance, one study reported an association between lower Hb and smaller GM and intracranial volumes, with a trend observed towards reduced WM volume (Jonassaint et al., 2014). Given the link between anemia, RDW and cardiovascular disease (Mozos, 2015), it has been opined that among brain outcomes, these variables may be especially related to cerebrovascular markers (Wolters et al., 2019). Indeed, studies have found relations between Hb, RDW, and anemia with key markers of cerebrovascular health, including vascular brain disease, global cerebral perfusion (Wolters et al., 2019), and WM lesion burden (Dagistan and Cosgun, 2019; Lee et al., 2016). This is consistent with our findings given that WMI is also a measure of cerebrovascular health (Burgmans et al., 2010; Falvey et al., 2013; Kennedy and Raz, 2009).

Our findings further the literature by demonstrating the deleterious effect of RDW on WMI among males, above and beyond the contribution of Hb and other hematological factors. Our results on the positive relation between Hb and FA, particularly among males, held only in reduced models that did not adjust for RDW and other variables. It is our understanding that our sex-specific findings are novel as

Journal Pre-proof

this is the first study to examine these differential relations of dMRI outcomes across sex groups. These findings are consistent with results of our previous study indicating that RDW was associated with poorer baseline performance on the Brief Test of Attention in the total population (γ = -0.123 ± 0.039; P = 0.001), a relationship detected mostly among men (γ = -0.221 ± 0.068; P = 0.001)(Beydoun et al., 2020). Given the potential clinical relevance of these findings (e.g., males may be uniquely susceptible to poorer neurological outcomes as a function of these hematologic variables), it would be prudent for other studies to attempt to replicate our finding, which would provide clarity on the validity and generalizability of these results.

Elevated RDW may reflect iron deficiency early before MCV changes and when multiple nutritional deficiencies are present that would lead to opposing directional changes in MCV. Although the exact pathophysiological mechanism linking elevated RDW and severity of white matter hyperintensity (leukoaraiosis) is unclear, Jonassaint and colleagues suggested that RDW reflects an underlying inflammation due to its positive correlation with various inflammatory markers (Lee et al., 2016). Inflammation induces ineffective erythropoiesis and enables immature RBCs to enter the circulation, which leads to anisocytosis. Recent studies have suggested that elevated RDW might also reflect impairment of microcirculation (Patel et al., 2013). Over their life, RBCs gradually lose cell membrane deformability and eventually rupture and are eliminated in the spleen. Elevated RDW is strongly associated with reduced RBC deformability

Journal Pre-proof

(Patel et al., 2013). As chronic ischemic disease and inadequate blood supply in the cerebral microcirculation are important mechanisms for the development and progression of leukoaraiosis, it is plausible that reduced RBC deformability impairs the cerebral microcirculation and subsequently leads to the development of leukoaraiosis in people with elevated RDW level. This mechanism might be extended to the effect of RDW on WMI, given the inverse association between white matter lesion volume and WMI in our cohort (Absolute value of Pearson's correlation, |r| between 0.29 and 0.33 for both mean FA and mean MD), although further studies are needed. The mechanisms behind sex differences in the effects of RDW on WMI, particularly the stronger effect on FA among males are unknown, despite the well-known fact that anemia and elevated RDW are both more common among females, triggered mainly by menstruation during the pre-menopausal period. In contrast, among males, gastro-intestinal bleeding and cancer are some of the main causes of anemia. Pending replication of our findings, animal and other mechanistic studies are needed to uncover pathways behind those sex differences. It is also possible that the main causes for elevated RDW among men underlies the link with WMI, rather than RDW itself. Thus, triggers for gastro-intestinal bleeding should be studied in relation to WMI and their association with WMI measures compared between men and women. Such triggers may include but are not limited to certain infections that may be conducive to anemia of inflammation and elevated RDW (e.g. *H. pylori* infection)(Haile and Timerga, 2021). In

Journal Pre-proof

Journal Pre-proof

fact, *H. pylori* infection is more prevalent and can lead to atrophic gastritis to a greater extent among men than among women (Ferro et al., 2019) and was shown recently to be associated with incident AD mostly among men (Beydoun et al., 2018). Nevertheless, more studies are needed to examine those complex relationships.

Our findings also indicate that RDW may affect mostly WMI in the occipital and parietal regions of the brain, with a strong effect consistently found with respect to FA and MD in the lingual WM (|b|>0.30). The latter small region located in the occipital lobe is known to be involved in visual processing pathways including color perception, visual word processing and analyzing complex features of visual forms (Szeszko et al., 2005).

Our novel examination of the associations between anemia-related biomarkers with brain structural dMRI measures—reflecting global and regional white matter integrity, potentially underlying various neuropathologies—are among our study's key strengths. Despite our cross-sectional design, our study provided 5-6 years of latency between exposure (RDW_{v1} and anemia) and outcome (brain MRI measures) while accounting for longitudinal changes in RDW as an additional exposure of interest. Our study sample provided sufficient power (power>0.80 for an effect size of 0.30; power>0.68 for an effect size of 0.25 for both male and female strata) to estimate sex-stratified models, an important consideration given the reported associations between sex, anemia, and cognitive impairment. Other stratified models were also well-powered, with the

Journal Pre-proof

Journal Pre-proof

possible exception of the "below poverty" group. Our analytic approach and data sample adds to the strength of the current study. We were able to test our hypotheses in our overall sample, and separately for males and females while correcting for multiple testing and adjusting for a wide range of potential confounders, including socio-demographic, lifestyle and health-related characteristics including hematological measures, and additional nutritional biomarkers. Those potential confounders were selected using machine learning (i.e. LASSO) and backward elimination to reduce bias without that being at the expense of statistical efficiency.

Despite the strengths of our study, it is not without limitations. Due to the cross-sectional design, our findings are only speculative in nature despite the 5-6 year latency period. Further, despite our inclusion of several potential confounders previously identified for their association with the exposure and/or outcome, we are not able to rule out residual confounding. Finally, our final sample differed significantly with the initially recruited HANDLS sample, particularly with respect to distribution according to poverty status, affecting generalizability to Baltimore city or other similar urban settings across the United States. However, the differences were minor when comparing the initial largest available HANDLS SCAN sample (n=240) with the final sample selected based on exposure variable availability.

In conclusion, initial RDW was consistently associated with poorer WMI among males. Further longitudinal studies should be directed at elucidating the mechanisms

Journal Pre-proof

that mediate or link higher RDW with poor white matter integrity, particularly among males.

ACKNOWLEDGEMENT

The authors would like to thank participants, staff and investigators of HANDLS and HANDLS SCAN and the internal reviewers of the manuscript at NIA/NIH/IRP. We wish to acknowledge the Core for Translational Research in Imaging @ Maryland (C-TRIM) which is part of the Center for Innovative Biomedical Resources at the University of Maryland School of Medicine.

Author contributions:

MAB contributed to study concept, planned analysis, conducted data management and statistical analysis, conducted literature review, wrote and revised the manuscript.

DS planned analysis, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript.

SH planned analysis, conducted data management, conducted literature review, wrote and revised parts of the manuscript.

JW planned analysis, conducted literature search and review, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript.

Journal Pre-proof

Journal Pre-proof

HAB planned the analysis, conducted literature review, wrote parts of the manuscript, revised the manuscript.

AM planned analysis, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript.

LIK acquired data, wrote and revised parts of the manuscript.

CD acquired data, wrote and revised parts of the manuscript.

RPG acquired data, wrote and revised parts of the manuscript.

SLS acquired data, wrote and revised parts of the manuscript.

GE acquired data, planned analysis, assisted in data management and statistical analysis,

wrote and revised parts of the manuscript.

MKE acquired data, wrote and revised parts of the manuscript.

ABZ acquired data, planned analysis, wrote and revised parts of the manuscript.

SW acquired data, planned analysis, wrote and revised parts of the manuscript.

All authors read and approved the final version of the paper.

Data availability statement:

Journal Pre-proof

Journal Pre-proof

Data are available upon request to researchers with valid proposals who agree to the confidentiality agreement as required by our Institutional Review Board. We publicize our policies on our website https://handls.nih.gov, which contains the code book for the parent study, HANDLS. Requests for data access may be sent to the PIs or the study manager, Jennifer Norbeck at norbeckje@mail.nih.gov. These data are owned by the National Institute on Aging at the National Institutes of Health. The Principal Investigators, have restricted public access to these data because (1) the study collects medical, psychological, cognitive, and psychosocial information on racial and poverty differences that could be misconstrued or willfully manipulated to promote racial discrimination; and (2) although the sample is fairly large, there are sufficient identifiers that the PIs cannot guarantee absolute confidentiality for every participant as we have stated in acquiring our confidentiality certificate. Analytic scripts and code book specific to HANDLS-SCAN can be obtained from the corresponding author upon request.

Financial disclosure: This work was supported in part by the Intramural research Program of the NIH, National institute on Aging. This work was also supported by the National Institutes of Health, R01-AG034161 to S.R.W, ZIA–AG000513 to M.K.E. and A.B.Z., and The University of Maryland Claude D. Pepper Older Americans Independence Center (NIH grant P30 AG028747).

Journal Pre-proof

Journal Pre-proof

Conflict of interest disclosure: The authors declare no conflict of interest.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government. Reference to any commercial products within this publication does not create or imply any endorsement by Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government.

Journal Pre-proof

Journal Pre-proof

REFERENCES

Beydoun, M.A., Beydoun, H.A., Elbejjani, M., Dore, G.A., Zonderman, A.B., 2018. Helicobacter pylori seropositivity and its association with incident all-cause and Alzheimer's disease dementia in large national surveys. Alzheimers Dement 14(9), 1148-1158.

Beydoun, M.A., Beydoun, H.A., Gamaldo, A.A., Teel, A., Zonderman, A.B., Wang, Y., 2014. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. BMC Public Health 14, 643.

Beydoun, M.A., Beydoun, H.A., Wang, Y., 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obes Rev 9(3), 204-218.

Beydoun, M.A., Hossain, S., Beydoun, H.A., Shaked, D., Weiss, J., Evans, M.K., Zonderman, A.B., 2020. Red Cell Distribution Width Is Directly Associated with Poor Cognitive Performance among Nonanemic, Middle-Aged, Urban Adults. J Nutr 150(1), 128-139.

Burgmans, S., van Boxtel, M.P., Gronenschild, E.H., Vuurman, E.F., Hofman, P., Uylings, H.B., Jolles, J., Raz, N., 2010. Multiple indicators of age-related differences in cerebral white matter and the modifying effects of hypertension. Neuroimage 49(3), 2083-2093.

Cavedo, E., Galluzzi, S., Pievani, M., Boccardi, M., Frisoni, G.B., 2012. Norms for imaging markers of brain reserve. J Alzheimers Dis 31(3), 623-633.

Chung, S.D., Sheu, J.J., Kao, L.T., Lin, H.C., Kang, J.H., 2014. Dementia is associated with iron-deficiency anemia in females: a population-based study. J Neurol Sci 346(1-2), 90-93.

Cosgrove, K.P., Mazure, C.M., Staley, J.K., 2007. Evolving knowledge of sex differences in brain structure, function, and chemistry. Biol Psychiatry 62(8), 847-855.

Dagistan, E., Cosgun, Z., 2019. Could hemogram parameters be predictors of dementia in elderly patients? The aging male : the official journal of the International Society for the Study of the Aging Male 22(3), 192-197.

Journal Pre-proof

Journal Pre-proof

Department of Health and Human Services, O.o.t.A.S.f.P.a.E., 2004. The 2004 HHS Poverty Guidelines. https://aspe.hhs.gov/2004-hhs-poverty-guidelines. (Accessed September 1st 2019).

Dlugaj, M., Winkler, A., Weimar, C., Durig, J., Broecker-Preuss, M., Dragano, N., Moebus, S., Jockel, K.H., Erbel, R., Eisele, L., Heinz Nixdorf Recall Study Investigative, G., 2016. Anemia and Mild Cognitive Impairment in the German General Population. J Alzheimers Dis 49(4), 1031-1042.

Evans, M.K., Lepkowski, J.M., Powe, N.R., LaVeist, T., Kuczmarski, M.F., Zonderman, A.B., 2010. Healthy aging in neighborhoods of diversity across the life span (HANDLS): overcoming barriers to implementing a longitudinal, epidemiologic, urban study of health, race, and socioeconomic status. Ethn Dis 20(3), 267-275.

Falvey, C.M., Rosano, C., Simonsick, E.M., Harris, T., Strotmeyer, E.S., Satterfield, S., Yaffe, K., Health, A.B.C.S., 2013. Macro- and microstructural magnetic resonance imaging indices associated with diabetes among community-dwelling older adults. Diabetes Care 36(3), 677-682.

Faux, N.G., Rembach, A., Wiley, J., Ellis, K.A., Ames, D., Fowler, C.J., Martins, R.N., Pertile, K.K., Rumble, R.L., Trounson, B., Masters, C.L., Group, A.R., Bush, A.I., 2014. An anemia of Alzheimer's disease. Mol Psychiatry 19(11), 1227-1234.

Ferro, A., Morais, S., Pelucchi, C., Dierssen-Sotos, T., Martin, V., Lopez-Carrillo, L., Malekzadeh, R., Tsugane, S., Hamada, G.S., Hidaka, A., Hernandez-Ramirez, R.U., Lopez-Cervantes, M., Zaridze, D., Maximovitch, D., Pourfarzi, F., Zhang, Z.F., Yu, G.P., Pakseresht, M., Ye, W., Plymoth, A., Leja, M., Gasenko, E., Derakhshan, M.H., Negri, E., La Vecchia, C., Peleteiro, B., Lunet, N., 2019. Sex differences in the prevalence of Helicobacter pylori infection: an individual participant data pooled analysis (StoP Project). Eur J Gastroenterol Hepatol 31(5), 593-598.

Glymour, M.M., Chene, G., Tzourio, C., Dufouil, C., 2012. Brain MRI markers and dropout in a longitudinal study of cognitive aging: the Three-City Dijon Study. Neurology 79(13), 1340-1348.

Haile, K., Timerga, A., 2021. Evaluation of Hematological Parameters of Helicobacter pylori-Infected Adult Patients at Southern Ethiopia: A Comparative Cross-Sectional Study. J Blood Med 12, 77-84.

Journal Pre-proof

Journal Pre-proof

Hoffmann, J.J., 2012. Red cell distribution width and mortality risk. Clin Chim Acta 413(7-8), 824-825.

Hong, C.T., Hsieh, Y.C., Liu, H.Y., Chiou, H.Y., Chien, L.N., 2020. Association between Anemia and Dementia-A Nationwide, Population-Based Cohort Study in Taiwan. Current Alzheimer research.

Hsu, F.C., Sink, K.M., Hugenschmidt, C.E., Williamson, J.D., Hughes, T.M., Palmer, N.D., Xu, J., Smith, S.C., Wagner, B.C., Whitlow, C.T., Bowden, D.W., Maldjian, J.A., Divers, J., Freedman, B.I., 2018. Cerebral Structure and Cognitive Performance in African Americans and European Americans With Type 2 Diabetes. J Gerontol A Biol Sci Med Sci 73(3), 407-414.

Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17(2), 825-841.

Jenkinson, M., Smith, S., 2001. A global optimisation method for robust affine registration of brain images. Med Image Anal 5(2), 143-156.

Jonassaint, C.R., Varma, V.R., Chuang, Y.F., Harris, G.C., Yasar, S., Polinder-Bos, H., Carlson, M.C., 2014. Lower hemoglobin is associated with poorer cognitive performance and smaller brain volume in older adults. J Am Geriatr Soc 62(5), 972-973.

Jones, D.K., 2008. Studying connections in the living human brain with diffusion MRI. Cortex 44(8), 936-952.

Kennedy, K.M., Raz, N., 2009. Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk. Brain Res 1297, 41-56.

Lee, H.B., Kim, J., Oh, S.H., Kim, S.H., Kim, H.S., Kim, W.C., Kim, S., Kim, O.J., 2016. Red Blood Cell Distribution Width Is Associated with Severity of Leukoaraiosis. PLoS One 11(2), e0150308.

Li, N., Zhou, H., Tang, Q., 2017. Red Blood Cell Distribution Width: A Novel Predictive Indicator for Cardiovascular and Cerebrovascular Diseases. Dis Markers 2017, 7089493.

Louapre, C., Govindarajan, S.T., Gianni, C., Madigan, N., Nielsen, A.S., Sloane, J.A., Kinkel, R.P., Mainero, C., 2016. The association between intra- and juxta-cortical

Journal Pre-proof

pathology and cognitive impairment in multiple sclerosis by quantitative T2* mapping at 7 T MRI. Neuroimage Clin 12, 879-886.

Mozos, I., 2015. Mechanisms linking red blood cell disorders and cardiovascular diseases. Biomed Res Int 2015, 682054.

Muller, T., Payton, N.M., Kalpouzos, G., Jessen, F., Grande, G., Backman, L., Laukka, E.J., 2020. Cognitive, Genetic, Brain Volume, and Diffusion Tensor Imaging Markers as Early Indicators of Dementia. J Alzheimers Dis 77(4), 1443-1453.

Ozturk, Z.A., Unal, A., Yigiter, R., Yesil, Y., Kuyumcu, M.E., Neyal, M., Kepekci, Y., 2013. Is increased red cell distribution width (RDW) indicating the inflammation in Alzheimer's disease (AD)? Arch Gerontol Geriatr 56(1), 50-54.

Park, S., Kim, D.K., Myung, W., Yoo, J.H., Shin, S.J., Na, D.L., Kim, S.Y., Lee, J.H., Kim, S.Y., Han, S.H., Choi, S.H., Shin, J., 2019. Risk Factors of Behavioral and Psychological Symptoms in Patients with Alzheimer Disease: The Clinical Research of Dementia of South Korea Study. Korean J Fam Med 40(1), 16-21.

Patel, K.V., Mohanty, J.G., Kanapuru, B., Hesdorffer, C., Ershler, W.B., Rifkind, J.M., 2013. Association of the red cell distribution width with red blood cell deformability. Adv Exp Med Biol 765, 211-216.

Patel, K.V., Semba, R.D., Ferrucci, L., Newman, A.B., Fried, L.P., Wallace, R.B., Bandinelli, S., Phillips, C.S., Yu, B., Connelly, S., Shlipak, M.G., Chaves, P.H., Launer, L.J., Ershler, W.B., Harris, T.B., Longo, D.L., Guralnik, J.M., 2010. Red cell distribution width and mortality in older adults: a meta-analysis. J Gerontol A Biol Sci Med Sci 65(3), 258-265.

Perlstein, T.S., Weuve, J., Pfeffer, M.A., Beckman, J.A., 2009. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med 169(6), 588-594.

R Foundation for Statistical Computing, 2013. R: A Language and Environment for Statistical Computing

http://www.R-project.org/.

Journal Pre-proof

Journal Pre-proof

Roalf, D.R., Gur, R.E., Verma, R., Parker, W.A., Quarmley, M., Ruparel, K., Gur, R.C., 2015. White matter microstructure in schizophrenia: associations to neurocognition and clinical symptomatology. Schizophr Res 161(1), 42-49.

Schneider, A.L., Jonassaint, C., Sharrett, A.R., Mosley, T.H., Astor, B.C., Selvin, E., Coresh, J., Gottesman, R.F., 2016. Hemoglobin, Anemia, and Cognitive Function: The Atherosclerosis Risk in Communities Study. J Gerontol A Biol Sci Med Sci 71(6), 772-779.

Selvin, S., 2004. Statistical analysis of epidemiologic data, 3rd ed. ed. Oxford University Press.

Shaked, D., Leibel, D.K., Katzel, L.I., Davatzikos, C., Gullapalli, R.P., Seliger, S.L., Erus, G., Evans, M.K., Zonderman, A.B., Waldstein, S.R., 2019. Disparities in Diffuse Cortical White Matter Integrity Between Socioeconomic Groups. Front Hum Neurosci 13, 198.

Sousa, N.D.S., Menezes, T.N., Silva, N.A., Eulalio, M.D.C., Paiva, A.A., 2018. [Prevalence of anemia and correlation between the concentration of hemoglobin and cognitive factors among the elderly]. Cien Saude Colet 23(3), 935-944.

STATA, 2019. Statistics/Data Analysis: Release 16.0. Stata Corporation, Texas.

Szeszko, P.R., Ardekani, B.A., Ashtari, M., Malhotra, A.K., Robinson, D.G., Bilder, R.M., Lim, K.O., 2005. White matter abnormalities in obsessive-compulsive disorder: a diffusion tensor imaging study. Arch Gen Psychiatry 62(7), 782-790.

Tajuddin, S.M., Nalls, M.A., Zonderman, A.B., Evans, M.K., 2017. Association of red cell distribution width with all-cause and cardiovascular-specific mortality in African American and white adults: a prospective cohort study. J Transl Med 15(1), 208.

Taniguchi, Y., Kitamura, A., Kaito, S., Yokoyama, Y., Yokota, I., Shinozaki, T., Seino, S., Murayama, H., Matsuyama, Y., Ikeuchi, T., Fujiwara, Y., Shinkai, S., 2019. Albumin and Hemoglobin Trajectories and Incident Disabling Dementia in Community-Dwelling Older Japanese. Dement Geriatr Cogn Disord 47(4-6), 233-242.

Trevisan, C., Veronese, N., Bolzetta, F., De Rui, M., Maggi, S., Zambon, S., Musacchio, E., Sartori, L., Perissinotto, E., Crepaldi, G., Manzato, E., Sergi, G., 2016. Low Hemoglobin Levels and the Onset of Cognitive Impairment in Older People: The PRO.V.A. Study. Rejuvenation Res 19(6), 447-455.

Journal Pre-proof

Journal Pre-proof

Wakana, S., Jiang, H., Nagae-Poetscher, L.M., van Zijl, P.C., Mori, S., 2004. Fiber tract-based atlas of human white matter anatomy. Radiology 230(1), 77-87.

Waldstein, S.R., Dore, G.A., Davatzikos, C., Katzel, L.I., Gullapalli, R., Seliger, S.L., Kouo, T., Rosenberger, W.F., Erus, G., Evans, M.K., Zonderman, A.B., 2017. Differential Associations of Socioeconomic Status With Global Brain Volumes and White Matter Lesions in African American and White Adults: the HANDLS SCAN Study. Psychosom Med 79(3), 327-335.

Walter, S., Dufouil, C., Gross, A.L., Jones, R.N., Mungas, D., Filshtein, T.J., Manly, J.J., Arpawong, T.E., Glymour, M.M., 2019. Neuropsychological Test Performance and MRI Markers of Dementia Risk: Reducing Education Bias. Alzheimer Dis Assoc Disord 33(3), 179-185.

Wan, J., Luo, P., Du, X., Yan, H., 2020. Preoperative red cell distribution width predicts postoperative cognitive dysfunction after coronary artery bypass grafting. Bioscience reports 40(4).

Weuve, J., Mendes de Leon, C.F., Bennett, D.A., Dong, X., Evans, D.A., 2014. The red cell distribution width and anemia in association with prevalent dementia. Alzheimer Dis Assoc Disord 28(2), 99-105.

Winchester, L.M., Powell, J., Lovestone, S., Nevado-Holgado, A.J., 2018. Red blood cell indices and anaemia as causative factors for cognitive function deficits and for Alzheimer's disease. Genome Med 10(1), 51.

Wolters, F.J., Zonneveld, H.I., Licher, S., Cremers, L.G.M., Heart Brain Connection Collaborative Research, G., Ikram, M.K., Koudstaal, P.J., Vernooij, M.W., Ikram, M.A., 2019. Hemoglobin and anemia in relation to dementia risk and accompanying changes on brain MRI. Neurology 93(9), e917-e926.
World Health Organization, 1972. Nutritional Anaemias,, Technical Report Series 503. WHO, Geneva.

Zlokovic, B.V., 2011. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12(12), 723-738.

Journal Pre-proof

Journal Pre-proof

Table 1. Descriptive analyses by sex: Study sample characteristics of eligible study sample by sex; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	Total (N=214)	*Females* (N=117)	*Males* (N=97)	P_{sex}
Demographic factors				
Sex, % males	45.3	—	—	
Age_{v1}	47.7±9.1	47.6±0.9	47.8±0.9	0.85
Race, % African American	40.7	41.9	39.2	0.69
% above poverty	67.3	60.7	75.3	0.024
Follow-up time (v_1 through v_{scan}), years	5.6±1.8	5.6±0.2	5.5±0.2	0.75
Imputed covariates, % or Mean±SE				
Education, years				
<High School	7.1	7.4	6.8	0.88
High School	54.4	54.2	54.8	—
> High School	38.4	38.5	38.4	0.96
WRAT-3 score	43.7±0.50	43.7±0.6	43.9±0.8	0.83
Current smoker, % yes	45.3	48.2	42.0	0.36
HEI-2010 total score	42.3±0.8	43.6±1.2	40.8±1.1	0.10
Serum vitamin B-12, pg/mL	517.4±16.8	534.3±25.9	497.1±20.0	0.27
Serum folate, ng/mL	15.2±0.4	15.0±0.6	15.3±0.6	0.88
C-reactive protein, mg/L	4.3±0.6	5.7±1.0	2.7±0.5	0.013
Albumin, g/dL	4.34±0.02	4.29±0.03	4.42±0.03	<0.001
White blood cell,	6.7±0.2	6.9±0.2	6.4±0.2	0.088

Journal Pre-proof

Journal Pre-proof

count*10^9/L				
Waist size, cm	99.0±1.1	99.1±1.6	99.1±1.5	0.98
Total cholesterol, mg/dL	190.7±3.1	192.4±4.4	188.7±4.4	0.55
Cholestrol:HDL-Cholesterol ratio	3.9±0.1	3.6±0.1	4.1±0.2	0.023
Triglycerides, mg/dL	124.6±5.0	113.9±5.0	137.5±8.9	0.018
Creatinine, mg/dL	0.89±0.03	0.79±0.04	1.02±0.03	<0.001
Other hematological measures at v_1				
Imputed covariates, Mean±SE				
Mean Cell Hemoglobin, pg	30.3±0.2	30.0±0.3	30.7±0.2	0.028
Serum iron, μg/dL	87.1±2.6	78.7±3.4	97.3±3.7	<0.001
Erythrocyte Sedimentation Rate, mm/hr	13.7±0.8	17.2±1.2	9.5±0.9	<0.001
RDW (v_1)				
CV (%), Mean±SD/SE	13.98±1.55	14.2±0.2	13.7±0.1	0.0065
Median	13.6	13.8	13.5	
IQR	13.1;14.3	13.1;14.6	13.1;14.1	
RDW (v_2-v_1, annual), δRDW				
CV (%)	+0.054±0.068	+0.057±0.007	+0.052±0.005	0.62
Hemoglobin (v_1), g/dL, *Mean±SD/SE*	13.93±1.46	13.19±0.13	14.82±0.10	<0.001

Journal Pre-proof

Journal Pre-proof

Anemia (v_1)				
Yes, %	11.21	15.4	6.2	0.034
Anemia (v_1 and v_2)	*(N=196)*	*(N=107)*	*(N=89)*	
Yes, %	7.14	10.3	3.4	0.061
dMRI measures, *Mean±SD/SE*	*(N=214)*	*(N=117)*	*(N=97)*	
Global WMI measures	***mean±SD***	***mean±SE***	***mean±SE***	
Mean FA	+0.299±0.017	+0.300±0.0014	+0.298±0.002	0.25
Mean MD, mm^2/sec	+0.00254±0.0016	+0.00252±0.00002	+0.00258±0.00002	0.0018
Regional cortical WMI measures	***mean±SD***	***mean±SE***	***mean±SE***	
Left Brain				
Frontal FA	+0.234±0.002	+0.236±0.002	+0.232±0.002	0.054
Frontal MD, mm^2/sec	+0.00239±0.00014	+0.00236±0.00001	+0.00244±0.00002	<0.0001
Temporal FA	+0.240±0.016	+0.243±0.001	+0.237±0.002	0.012
Temporal MD, mm^2/sec	+0.00243±0.00014	+0.00240±0.00001	+0.00247±0.00001	0.0026
Parietal FA	+0.230±0.016	+0.232±0.001	+0.228±0.0017	0.043
Parietal MD, mm^2/sec	+0.00266±0.00020	+0.00260±0.00002	+0.00273±0.00002	<0.0001
Occipital FA	+0.203±0.015	+0.204±0.001	+0.200±0.001	0.039
Occipital MD, mm^2/sec	+0.00248±0.00016	+0.00251±0.00001	+0.00251±0.00002	0.0058
Right Brain				
Frontal FA	+0.234±0.015	+0.236±0.001	+0.231±0.002	0.017

Journal Pre-proof

Journal Pre-proof

Frontal MD, mm^2/sec	+0.00237±0.00014	+0.00233±0.000012	+0.00240±0.00002	0.0001
Temporal FA	+0.248±0.016	+0.251±0.001	+0.246±0.002	0.020
Temporal MD, mm^2/sec	+0.00234±0.00014	+0.00232±0.00001	+0.00237±0.00001	0.0196
Parietal FA	+0.230±0.017	+0.229±0.001	+0.223±0.002	0.005
Parietal MD, mm^2/sec	+0.00271±0.00022	+0.00264±0.00002	+0.00280±0.00002	<0.0001
Occipital FA	+0.204±0.015	+0.205±0.001	+0.202±0.002	0.073
Occipital MD, mm^2/sec	+0.00256±0.00018	+0.00252±0.00002	+0.00259±0.00002	0.0058

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); CV=Coefficient of Variation; IQR=Interquartile Range; dMRI=Diffusion Magnetic Resonance Imaging; δRDW=Red Cell Distribution Width annualized change between visits 1 and 2; FA=Fractional Anisotropy; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; HDL=High Density Lipoprotein; HDL=High Density Lipoprotein; HEI-2020=Healthy Eating Index, 2010 release; IQR=Interquartile range (25^{th}-75^{th} percentile); MCH=Mean Cell Hemoglobin; MD=Mean Diffusivity; RDW=Red Cell Distribution Width; v_1=visit 1 of HANDLS (2004-2009); v_2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015); WMI=White Matter Integrity; WRAT-3=Wide Range Achievement Test, 3^{rd} version.

[a] Values are Mean±SD for totals and Mean±SE for stratum-specific, or %. For continuous imputed covariates, values are Mean±SE for all 3 columns. For RDW, medians and inter-quartile ranges (IQR) were also provided. Psex was obtained from χ^2 and *t*-tests for the unimputed covariates and from multinomial logit and linear regression models for the imputed data. Additional models with sex, race, age and poverty status were conducted to test whether the sex differences were independent other socio-demographic factors. All statistically significant sex differences at type I error of 0.05 retained their statistical significance after further adjustment for age, race and poverty status.

Journal Pre-proof

Journal Pre-proof

Table 2. Anemia analyses: Minimally and hematological measure adjusted associations from analyses A (global FA/MD), B (Regional cortical FA/MD, Left/Right) vs. visit 1 Anemia (overall and stratified by sex) and continuous Hb level: ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*				***Model 2**: hematological measure-adjusted, sensitivity analysis (SA)[b]*			
Visit 1 Anemia								
Total sample (N=214)	*β*	*(SE)*	*P*	*q-value*	*β*	*(SE)*	*P*	*P for Interaction of Anemia$_{(v1)}$ by sex*
dMRI, Analysis A								
Mean FA	-0.0048	(0.004)	0.19	__	-0.0037	(0.004)	0.40	0.21
Mean MD	+0.0000297	(0.000032)	0.35	—	+0.0000202	(0.0000385)	0.60	0.30
Males (N=97)								
dMRI, Analysis A								
Mean FA	-0.0108	(0.00759)	0.16	__	-0.00418	0.0085242	0.63	__
Mean MD	+0.0000936	(0.0000654)	0.16	__	+0.0000445	(0.0000744)	0.55	__
Females (N=117)								
dMRI, Analysis A								
Mean FA	-0.002265	(0.003989)	0.57	__	-0.00307	(0.00498)	0.54	__
Mean MD	+7.78e-06	(0.0000362)	0.83	—	+0.000016	(0.000045)	0.72	—
Visit 1 Hb								
Total sample (N=214)	*β*	*(SE)*	*P*	*q-value*	*β*	*(SE)*	*P*	*P for Interaction By of Hb$_{(v1)}$ sex*

Journal Pre-proof

Journal Pre-proof

dMRI, Analysis A								
Mean FA	+0.0018	(0.0009)	0.060	SA	+0.0023	(0.0012)	0.070	0.15
Mean MD	-0.0000104	(2.28E-06)	0.21	SA	-0.0000122	(0.000011)	0.27	0.052
Males (N=97)								
dMRI, Analysis A								
Mean FA	+0.0040	(0.0018)	0.028	SA	+0.0034	(0.0020)	0.098	—
Mean MD	-0.000029	(0.000015)	0.061	SA	-0.000023	(0.000018)	0.20	—
dMRI, Analysis B								
Left Brain								
Frontal FA	+0.00278	(0.00165)	0.095	SA	—	—	—	—
Frontal MD	-0.000017	(0.000015)	0.26	SA	—	—	—	—
Temporal FA	+0.00264	(0.00166)	0.12	SA	—	—	—	—
Temporal MD	-0.000021	(0.000014)	0.14	SA	—	—	—	—
Parietal FA	+0.00251	(0.0016)	0.13	SA	—	—	—	—
Parietal MD	-1.51E-07	(0.000019)	0.99	SA	—	—	—	—
Occipital FA	+0.0032	(0.0015)	0.033	SA	—	—	—	—
Occipital MD	-0.000028	(0.000016)	0.075	SA	—	—	—	—
Right Brain								

Journal Pre-proof

Journal Pre-proof

Frontal FA			0.01					
	+0.0040	(0.0016)	3	SA	—	—	—	—
Frontal MD		(0.0000014	0.09					
	-0.000023	)	8	SA	—	—	—	—
Temporal FA			0.06					
	+0.0032	(0.0018)	2	SA	—	—	—	—
Temporal MD	-0.000010	(0.000013)	0.42	SA	—	—	—	—
Parietal FA	+0.0025	(0.0018)	0.18	SA	—	—	—	—
Parietal MD	5.24E-06	(0.00002)	0.80	SA	—	—	—	—
Occipital FA			0.05					
	+0.0034	(0.0017)	0	SA	—	—	—	—
Occipital MD			0.06					
	-0.000031	(0.000017)	6	SA	—	—	—	—
Females (N=117)								
dMRI, Analysis A								
Mean FA	+0.00076	(0.0011)	0.48	SA	+0.0019	(0.0016)	0.23	—
Mean MD	-2.85E-06	(9.64E-06)	0.77	SA	-8.92E-06	(0.000014)	0.53	—

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); CV=Coefficient of Variation; dMRI=Diffusion Magnetic Resonance Imaging; ESR=Erythrocyte Sedimentation Rate; FA=Frational anisotropy; FDR=False Discovery Rate; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; MCH=Mean Cell Hemoglobin; MD=Mean Diffusivity; RDW=Red Cell Distribution Width; SA=Sensitivity Analysis; SE=Standard Error; v_1=visit 1 of HANDLS (2004-2009); v_2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015).

Journal Pre-proof

Journal Pre-proof

a Values are adjusted linear regression coefficients β with associated SE, standardized beta, uncorrected p-values, corrected q-values (false discovery rate) and results of sensitivity analysis. (N) is the sample size in each analysis. Q-values presented only for uncorrected P-values<0.05 for model 1, __ otherwise. Model 1 was adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan}. MD is measured in mm^2/sec.

[b] Model 2 is a sensitivity analysis further adjusting Model 1 for selected hematological measures [i.e RDW + other hematological measures (MCH, Serum iron, ESR)] after screening using machine learning techniques (See Supplemental methods 3).

Table 3. RDW analyses: Minimally and hematological measure adjusted associations from analyses A (global FA/MD), B (Regional cortical FA/MD, Left/Right) vs. visit 1 RDW (overall and stratified by sex; and among non-anemic participants): ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*					***Model 2**: Hematological measure-adjusted, sensitivity analysis (SA)[b]*			
Total sample (N=214)	*β*	*(SE)*	*b*	*P*	*q-value*	*β*	*(SE)*	*P*	*P for Interaction of $RDW_{(v1)}$ by sex*
dMRI, Analysis A									
Mean FA	-0.00091	(0.00075)	-0.085	0.23	__	-0.00131	(0.00106)	0.22	__

Journal Pre-proof

Mean MD	+4.50e-06	(6.68e-06)	+0.043	0.50	__	+3.14e-06	(9.49e-06)	0.91	__
Males (N=97)									
dMRI, Analysis A									
Mean FA	-0.00593	(0.00196)	-0.30	0.003	0.038	-0.006473	(0.002433)	0.009	0.004
Mean MD	+4.28e-05	(1.71e-05)	+0.24	0.014	0.086	+0.000045	(0.000021)	0.038	0.029
dMRI, Analysis B									
Left Brain									
Frontal FA	-0.005704	(0.001796)	-0.31	0.002	0.037	-0.0066874	(0.002241)	0.004	0.011
Frontal MD	+0.0000316	(0.000017)	+0.18	0.066	__	+0.0000351	(0.0000213)	0.10	0.028
Temporal FA	-0.0046025	(0.001839)	-0.26	0.014	0.14	-0.0043301	(0.0023103)	0.064	0.022
Temporal MD	+0.0000257	(0.000016)	+0.17	0.11	__	+0.000021	(0.00002)	0.31	0.17
Parietal FA	-0.005925	(0.001778)	-0.33	0.001	0.030	-0.007343	(0.002212)	0.001	0.003
Parietal MD	+0.0000345	(0.0000209)	+0.16	0.10	__	+0.000061	(0.000026)	0.021	0.20
Occipital FA	-0.0051361	(0.001637)	-0.32	0.002	0.037	-0.0056694	(0.0020237)	0.006	0.003
Occipital MD	+0.0000507	(0.0000173)	+0.28	0.004	0.051	+0.0000619	(0.0000213)	0.005	0.020
Right Brain									
Frontal FA	-0.005948	(0.0017423)	-0.33	0.001	0.028	-0.006399	(0.002151)	0.004	0.001
Frontal MD	+0.0000286	(0.0000152)	+0.18	0.064	__	+0.000030	(0.000019)	0.12	0.036
Temporal FA	-0.004967	(0.001916)	-0.27	0.011	0.12	-0.005407	(0.002380)	0.026	0.011
Temporal MD	+0.000027	(0.0000139)	+0.19	0.058	__	+0.0000331	(0.0000175)	0.061	0.14
Parietal FA	-0.0067969	(0.001986)	-0.34	0.001	0.030	-0.0085593	(0.002467)	0.001	0.001
Parietal MD	+0.0000388	(0.0000232)	+0.16	0.097	__	+0.0000702	(0.0000286)	0.016	0.18
Occipital FA	-0.0065511	(0.0018366)	-0.36	0.001	0.028	-0.0076675	(0.0022795)	0.001	0.001
Occipital MD	+0.0000534	(0.0000182)	+0.29	0.004	0.051	+0.0000628	(0.0000225)	0.006	0.040

Journal Pre-proof

Journal Pre-proof

Females (N=117)									
dMRI, Analysis A									
Mean FA	+0.0001416	(0.0007934)	+0.017	0.86	__	-0.0003437	(0.0012155)	0.78	__
Mean MD	-2.99e-06	(7.18e-06)	-0.037	0.68	__	-7.01e-06	(0.0000111)	0.53	__
Non-Anemic (N=190)									
dMRI, Analysis A									
Mean FA	-0.0005986	(0.0011114)	-0.041	0.59	SA	-0.0008299	(0.0012592)	0.51	__
Mean MD	-4.42e-06	(0.0000104)	-0.030	0.67	SA	-6.08e-06	(0.0000118)	0.61	__

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); CV=Coefficient of Variation; dMRI=Diffusion Magnetic Resonance Imaging; ESR=Erythrocyte Sedimentation Rate; FA=Fractional Anisotropy; FDR=False Discovery Rate; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; MCH=Mean Cell Hemoglobin; MD=Mean Diffusivity; RDW=Red Cell Distribution Width; SA=Sensitivity Analysis; SE=Standard Error; v_1=visit 1 of HANDLS (2004-2009); v_2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015).

a Values are adjusted linear regression coefficients β with associated SE, standardized beta, uncorrected p-values, corrected q-values (false discovery rate) and results of sensitivity analysis. (N) is the sample size in each analysis. Standardized betas for RDW are computed as SD in outcome per SD in visit 1 RDW. Q-values presented only for uncorrected P-values<0.05 for model 1, __ otherwise. Model 1 was adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan}. MD is measured in mm^2/sec.

[b] Model 2 is a sensitivity analysis further adjusting Model 1 for selected hematological measures [i.e Hb + other hematological measures (MCH, Serum iron, ESR)] after screening using machine learning techniques (See Supplemental methods 3).

Journal Pre-proof

FIGURE LEGENDS

FIGURE 1

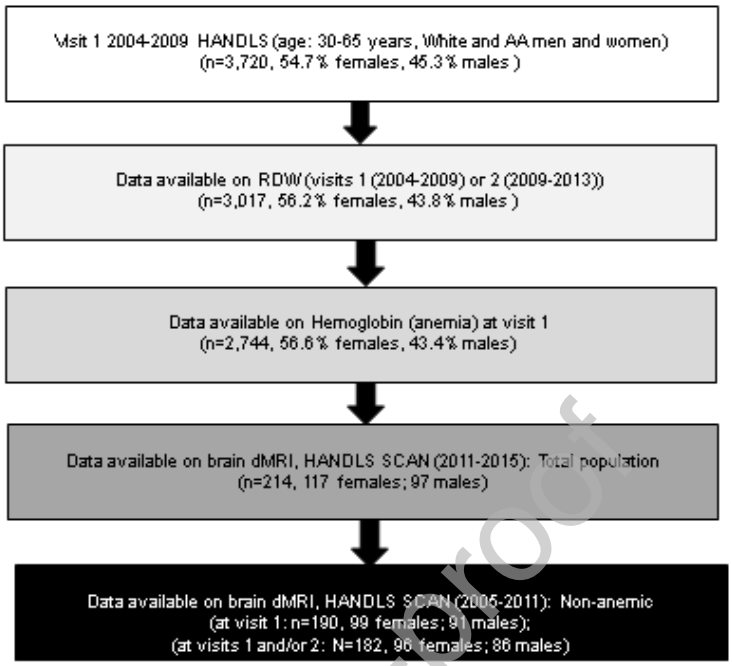


Figure 1. Study participant schematic: HANDLS 2004-2013 and HANDLS-SCAN 2011-2015[a]

Abbreviations: HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=HANDLS brain MRI ancillary study; MRI=Magnetic Resonance Imaging

[a]Visit 1 refers to HANDLS 2004-2009; Visit 2 refers to HANDLS 2009-2013; and HANDLS-SCAN visit (v_{scan}) was carried out between 2011 and 2015.

Journal Pre-proof

Journal Pre-proof

FIGURE 2

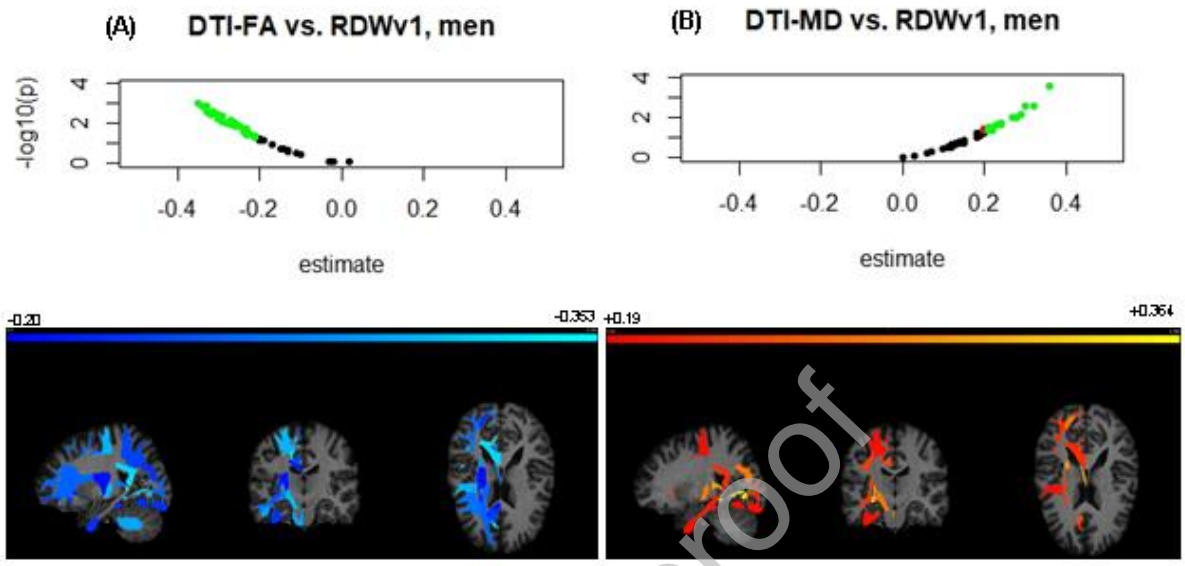


Figure 2. Volcano plots and brain images of FA and MD vs. RDW(v_1) among males: HANDLS 2004-2013 and HANDLS-SCAN 2011-2015[a,b,c]

Abbreviations: bayes1RDW= δRDW ; DTI=Diffusion Tensor Imaging; FA=Fractional Anisotropy; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=HANDLS brain MRI ancillary study; MD=Mean Diffusivity; MRI=Magnetic Resonance Imaging; TR=Tracts.

[a] In both the volcano plot and the brain images: Values are effect sizes from adjusted linear regression models restricted to males only with RDW(v_1) as the exposure and outcomes being alternately regional bilateral FA and regional bilateral MD. The multiple linear models were adjusted for age, race, poverty status and time of follow-up between visit 1 and v_{scan}.

[b] The volcano plot represents the results of the two models among men, with outcomes being regional FA and MD (A) RDW(v_1) vs. DTI-FA; (B) RDW(v_1) vs. DTI-MD. "Estimate" refers to the standardized beta coefficient or effect size; -Log10(p) is the associated negative Log base 10 of the p-value for each regional association. Red dots represent $p<0.05$ with $b<+0.20$ for positive associations or $b>-0.20$ for negative associations; Green dots represent $p<0.05$ with $b>+0.20$ for positive associations or $b<-0.20$ for negative associations.

Journal Pre-proof

Journal Pre-proof

c The brain images represent the same results of the two models among men, using FSLEYES software for visualizing effect sizes. Those effect sizes were selected for regions with p<0.05 and are presented at a threshold of 0.20 up to the maximum effect size, using a color gradient. Colder (blue) colors are for negative associations (expected for DTI-FA) and warmer colors (red through yellow) are for positive associations (expected for DTI-MD). Lighter colors indicate stronger effect sizes.

FIGURE 3

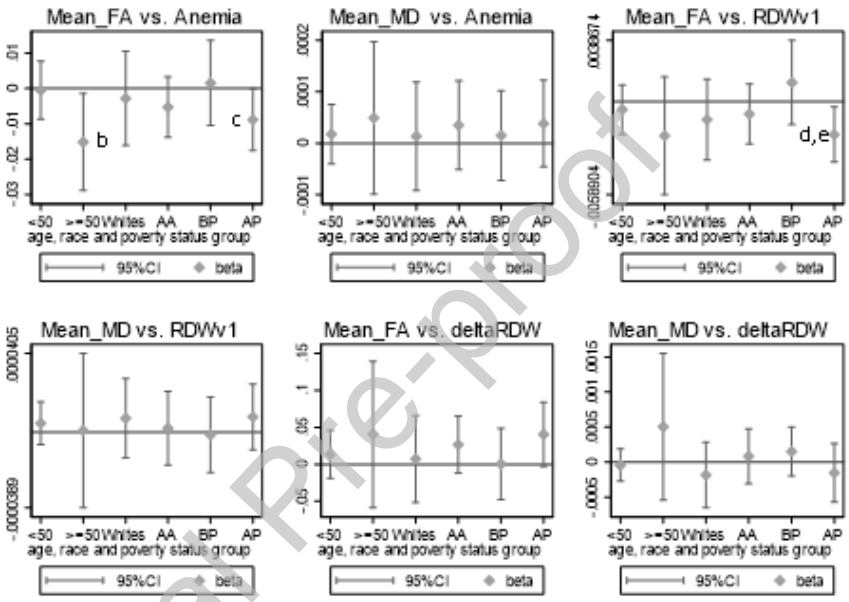


Figure 3. Associations of mean FA and MD with the 3 main exposures (Anemic(v_1), RDW(v_1) and δRDW) by age group, race and poverty status, minimally adjusted model (Model 1): HANDLS 2004-2013 and HANDLS-SCAN 2011-2015[a]

Abbreviations: AA=African Americans; AP=Above Poverty; BP=Below Poverty; ESR=Erythrocyte Sedimentation Rate; FA=Fractional Anisotropy; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=HANDLS brain MRI ancillary study; Hb=Hemoglobin; MCH=Mean Cell Hemoglobin; MD=Mean Diffusivity; MRI=Magnetic Resonance Imaging.

[a] Values are adjusted linear regression coefficients β with associated 95% CI. All stratified multiple linear models with outcomes being global Mean FA and global Mean MD and the 3 exposures entered alternately, were adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} (*Analysis A'*) The models

Journal Pre-proof

presented are stratified by age group (<50 years: group 1, *n*=127; ≥ 50 years: group 2, *n*=87); race (Whites: group 3, *n*=127; African-Americans: group 4, *n*=87); and poverty status (Below poverty: group 5, *n*=70; Above poverty: group 6, *n*=144). δRDW is the empirical bayes estimator from mixed-effects regression model using the full HANDLS cohort with two repeats, over time, representing annual rates of change at the individual-level in RDW. Anemia (using sex-specific WHO criteria) and RDW(v_1) are measured at visit 1. Model 2 was conducted on selected findings, further adjusting Model 1 by RDW (for anemia), Hb (for RDW), ESR, serum iron and MCH.

[b] Mean FA vs. anemia at v_1 among older adults (aged ≥50 years) in Model 1: β=-0.0152, SE=0.00070, p=0.033. P>0.05 in Model 2.

[c] Mean FA vs. anemia at v_1 among individuals living above poverty in Model 2: β=-0.0088, SE=0.0044, p=0.048. P>0.05 in Model 2.

[d] Mean FA vs. RDW at v_1 among individuals living above poverty in Model 1: β=-0.0021, SE=0.0009, p=0.020.

[e] Mean FA vs. RDW at v_1 among individuals living above poverty in Model 2: β±SE: -0.00391±0.00130, p=0.004.

Journal Pre-proof