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Association of lower hemoglobin level and neuropathology in community-dwelling older persons

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Abstract

Lower hemoglobin levels have been associated with cognitive decline in older persons. The objective of this study was to investigate the relationship between lower hemoglobin levels and common, age-related neuropathologies associated with cognitive decline. Hemoglobin and neuropathology measures were available in 113 deceased, community-dwelling, older adults participating in the Rush Memory and Aging Project, a prospective, observational, clinical pathology study of aging. The mean hemoglobin level was 13.0 g/dL (SD=1.4) and was measured 3.2 (SD=1.3) years prior to death. Thirty-five participants had at least one chronic macroscopic infarction and twenty-nine had at least one chronic microscopic infarction. Eleven participants had Lewy Bodies. The mean Alzheimer's disease pathology score based on a summary measure of neuritic plaques, diffuse plaques, and neurofibrillary tangles was 0.56 unit (SD=0.56; range=0, 2.34). Using logistic regression models adjusted for age at death, gender, and education, each g/dL lower hemoglobin level increased the odds for having a chronic macroscopic infarction by 37% (95% CI=1.01, 1.86) but not for having a chronic microscopic infarction (OR=1.11; 95% CI=0.82, 1.52) or Lewy Bodies (OR=1.07; 95% CI=0.68, 1.68). In an adjusted multiple regression model, hemoglobin level was not associated with the global AD pathology measure (parameter estimate=−0.02, SE=0.03, p=0.6). In secondary analyses, lower hemoglobin levels were associated with higher odds of having a chronic macroscopic infarction in a subcortical region but not with higher total subcortical chronic macroscopic infarction volume. In conclusion, lower hemoglobin levels appear to be associated with chronic macroscopic infarctions but not other common age-related neuropathologies.

Keywords

hemoglobin; neuropathology; macroscopic infarction; microscopic infarction; neuritic plaque; diffuse plaque; neurofibrillary tangle; Lewy Body

INTRODUCTION

In community-dwelling men and women, the rate of cognitive decline tends to be greater in late-life compared to mid-life. [1] Cognitive decline in late-life has been associated with

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increased mortality, increased disability, and increased health resource use. [2, 3] Neuropathologic studies in community-dwelling cohorts of older persons point to cognitive decline being associated with common neuropathologies such as chronic infarctions and Lewy Bodies along with amyloid plaques and neurofibrillary tangles. [4–6] Over the last decade, there has been significant interest in identifying biologic markers that are associated with cognitive decline in late life. Features of an ideal prognostic biologic marker include being inexpensive to measure with no need for complex analytic techniques and being measurable in an easily accessible tissue. [7] Hemoglobin level has been identified as a potential biologic marker for cognitive decline. Lower hemoglobin levels are common in older adults [8, 9] and frequently are measured in clinical practice. In community-dwelling, older adults, lower hemoglobin levels have been associated with cognitive function in cross-sectional studies [10–12] and with cognitive decline in longitudinal studies. [13–16] Lower hemoglobin levels have been associated with development of stroke in middle-aged persons with impaired renal function [17], of Parkinson's disease [18], and of Alzheimer's disease [13, 19 but not 20]. However, the association of hemoglobin with the neuropathologies that underlie cognitive decline in late-life is uncertain.

In this study, we examined the relationship of hemoglobin level to common, age-related neuropathologies utilizing data from more than 100 community-dwelling participants in the Rush Memory and Aging Project with hemoglobin assessment and brain pathology measures for chronic macroscopic infarctions, chronic microscopic infarctions, Lewy Bodies, and Alzheimer's disease pathology. [21] In secondary analyses, we also examined the association of hemoglobin level to the location of chronic macroscopic infarctions as well as the volume of chronic macroscopic infarcts by location.

MATERIALS AND METHODS

Participants

All participants were older, community-dwelling persons without known dementia who agreed to participate in the Rush Memory and Aging Project. The Rush Memory and Aging Project was approved by the Rush University Medical Center Institutional Review Board and the study was conducted in accord with the ethical standards of the Helsinki Declaration of 1975. Participants provided written informed consent to annual clinical evaluations and brain donation at the time of death. [21] Rolling enrollment for the Rush Memory and Aging Project began in 1997 and continues. At the time of these analyses, more than 1350 participants had completed an initial evaluation. They came from more than 40 groups in the Chicago, Illinois, vicinity (see "Acknowledgments"). Through March 10, 2010, approximately 85% of deceased participants had undergone brain autopsy resulting in 315 participants with neuropathology data. Blood collection for complete blood counts began in February 2003. For these analyses, the annual evaluation with the initial hemoglobin level measurement was defined as the baseline. Inclusion in these analyses required: a) complete neuropathology data; b) at least one hemoglobin measure; and, c) absence of dementia at the visit associated with the first hemoglobin measurement.

Neuropathology Assessment

Utilizing a previously described standard autopsy protocol [22, 23], brains were removed and weighed. One cerebral hemisphere was designated for freezing and the other hemisphere for fixation for 3–21 days in 4% paraformaldehyde. Both hemispheres were cut into 1-cm coronal slabs in a Plexiglas jib; and all slabs were photographed and assessed for macroscopic infarctions by a neuropathologist or trained technician blinded to all clinical data. The age, location, and size of each infarction were recorded. Using gross and histological features, an infarction was defined as chronic if it was assessed as occurring at

least three months prior to death. Only chronic infarctions were included in these analyses. Chronic macroscopic infarction was summarized as being present or absent. [24] The location of the macroscopic infarction was determined as being cortical only, subcortical only, or both cortical and subcortical. Volume was estimated by multiplying the recorded width, length, and depth for each infarction. Using the location designation and size measurements for each infarction, the total volume of chronic macroscopic infarcts in subcortical regions and in cortical regions was determined. Routine tissue blocks were dissected from all visualized pathologies including infarcts and from the midfrontal, middle temporal, inferior parietal cortex, entorhinal cortices, hippocampus, anterior basal ganglia, anterior thalamus, and midbrain with substantia nigra. Tissue blocks were embedded in paraffin, cut into 6- μ m sections, and mounted on glass slides. Hematoxylin and eosin stained sections from all blocks were examined for chronic microscopic infarctions, as previously described [25] Lewy bodies were detected using α -synuclein immunohistochemistry (Zymed Laboratories, South San Francisco, CA; 1:100) as previously described. [24] Lewy bodies were classified as nigral, limbic, or neocortical. [26] In order to determine a composite measure of global Alzheimer's disease pathology, 0.5 cm thick blocks of tissue from five regions (CA1/subiculum of the hippocampus, entorhinal cortex, inferior parietal gyrus, middle temporal gyrus, and midfrontal gyrus) were embedded in paraffin wax, cut into 6mm sections, and stained with modified Bielschowsky silver. Neuritic plaques, diffuse plaques, and neurofibrillar tangles in each brain region were counted in a 1-mm² area under 610 magnification in the site judged to have the most of a given type of pathology. The raw count of neuritic plaques in each region was divided by the standard deviation of all counts of neuritic plaques in the region to yield a standard score. A similar procedure was followed to derive the standard scores for diffuse plaques and neurofibrillary tangles. Then, the standard scores were averaged to produce a composite measure of global Alzheimer's disease pathology.

Hemoglobin Level Measurement

Phlebotomists and nurses skilled in venipuncture using sterile technique collected blood in a 2-mL EDTA tube. A Beckman/Coulter LH750 automated processor (Quest Laboratories, Wood Dale, IL) was used to perform a complete blood count analysis. [12] As hemoglobin levels can be lower proximate to death, we chose to use the first available hemoglobin level measure as our main predictor. We also calculated the average hemoglobin level from all annual hemoglobin values available at least one year prior to death.

Demographic Covariates and Clinical Diagnoses

Participants were asked for demographic information including date of birth, gender, and highest number of years of education completed. Date of death also was collected. At study baseline and follow-up evaluations, each participant underwent a uniform structured clinical evaluation that included a medical history, neurologic examination, and cognitive performance testing. The presence of dementia was determined using a multi-step process, as previously described. [27] Data from a battery of 21 cognitive function tests were reviewed by an experienced neuropsychologist who determined if cognitive impairment was present. Then, participants were evaluated in person by an experienced clinician who used all available cognitive and clinical testing results from the current year's evaluation to diagnose dementia and Alzheimer's disease using the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. [28] Self-report along with clinician assessment was utilized to diagnose Parkinson's disease, and stroke, as previously described. [21]

Statistical Analysis

Analyses were programmed in SAS[®], Version 9.2 (SAS Institute Inc., Cary, NC). First, we determined the distribution for the baseline hemoglobin levels and other demographic characteristics for the study cohort. We compared the demographic characteristics of participants included in these analyses to participants in the Rush Memory and Aging Project who did not have a hemoglobin measure prior to neuropathology assessment. Then, for participants included in these analyses, we determined the frequencies of macroscopic infarctions, microscopic infarctions, and Lewy Bodies along with the distribution of the global Alzheimer's disease pathology measure.

In order to determine the odds ratio for having a chronic macroscopic infarction on neuropathological assessment (as compared to not having a chronic macroscopic infarction) for each g/dL lower level of hemoglobin, we constructed a logistic regression model adjusted for age at death, gender, and education level. We conducted an additional sensitivity analysis by repeating the original model after excluding participants with clinical stroke or Parkinson's disease proximate to the hemoglobin level measurement. In secondary analyses, we examined the association between hemoglobin level and location and total volume of macroscopic infarctions. We used a 4-category polychotomous logistic regression model to determine the odds ratio for each g/dL lower level of hemoglobin and the likelihood of having a chronic macroscopic infarction in a subcortical region only, a cortical region only, or both a cortical and subcortical region. The odds ratio for each location was compared to having no chronic macroscopic infarction in any location. Then, in participants with the presence of a chronic macroscopic infarction, we repeated a linear regression model adjusted for demographics two times to determine the association of hemoglobin level with subcortical chronic macroscopic infarction volume and cortical macroscopic infarction volume as the respective outcomes.

In order to examine the association of hemoglobin level with other neuropathologies, we repeated the logistic regression model adjusted for demographics twice by replacing the presence of chronic macroscopic infarction with the presence of chronic microscopic infarction and the presence of Lewy Bodies. We also used a linear regression model adjusted for demographic variables to determine the association between hemoglobin level and global Alzheimer's disease pathology. As the distribution of the measure of global Alzheimer's disease pathology was skewed, a square root transformation was used in analyses.

Finally, we repeated our neuropathology outcome models adjusted for age at death, gender, and education level by replacing the level of hemoglobin based on the first available measure with the average hemoglobin level from all available annual readings collected greater than one year prior to death.

RESULTS

Cohort Characteristics

At the time of these analyses, 113 deceased and consecutively autopsied Rush Memory and Aging Project participants without baseline dementia had available neuropathology measures along with at least one measure of hemoglobin. Compared to the group of deceased participants without hemoglobin measures ($n=202$), the group of participants with available hemoglobin and neuropathology measures did not have a significantly different age of death (88.2 years vs. 88.5 years, $t_{313}=0.33$, $p=0.7$), education level (14.2 years vs. 14.6 years, $t_{312}=1.08$, $p=0.3$), or the percentage of females (32.7% vs. 40.1%, $\chi^2_1=1.67$, $p=0.2$). The mean level of the first available hemoglobin level was 13.0 g/dL (SD=1.4). First available hemoglobin was collected on average 3.2 years prior to death (SD=1.3). Other

cohort characteristics are summarized in Table 1 along with the burden of common, age-related neuropathologies.

Hemoglobin Level and Chronic Macroscopic Infarctions

Chronic macroscopic infarcts were present in 35 of the 113 participants (31%). In order to determine if hemoglobin level was associated with chronic macroscopic infarctions, we constructed a logistic regression model with the presence of chronic macroscopic infarctions as the outcome and hemoglobin level as the predictor of interest. We adjusted the model with terms for age at death, gender, and years of education. As shown in Table 2, Model A, each 1 g/dL lower level of hemoglobin was associated with 37% higher odds for having macroscopic infarction (95% CI=1.01, 1.86). As lower hemoglobin levels have been associated with clinical stroke and Parkinson's disease and these diseases also may be associated with neuropathology, we explored whether hemoglobin level was not just a marker for the presence of clinical stroke or Parkinson's disease at baseline. After removing participants with a clinical stroke or Parkinson's disease proximate to hemoglobin level measurement, the odds ratio between lower hemoglobin level and chronic macroscopic infarction remained significant (Table 2, Model B). In secondary analyses, we examined the association of hemoglobin level with the distribution pattern for chronic macroscopic infarctions. As shown in Table 3, a lower initial hemoglobin level was associated with an increased odds for having a chronic macroscopic infarction in a subcortical region but not in a cortical region or a combined subcortical and cortical region. We also examined whether a lower hemoglobin level was associated with volume of chronic macroscopic infarction. For the subset of participants with a chronic macroscopic infarction, lower hemoglobin level was not associated with either total volume of subcortical infarction ($p=0.2$) or total volume of cortical infarction ($p=0.7$) in a linear regression model adjusted for demographics.

Hemoglobin Level and Microscopic Neuropathology

Using a logistic regression model adjusted for demographics, lower hemoglobin level was not associated with an increased odds ratio for the presence of at least one microscopic infarction ($p=0.5$). Lower hemoglobin level also was not associated with an increased odds ratio for the presence of Lewy Bodies ($p=0.8$). In an adjusted linear regression model, hemoglobin level was not associated with a greater level of the global AD pathology measure ($p=0.6$).

Average Hemoglobin Level and Neuropathology

As a single hemoglobin level measure may not accurately capture chronic hemoglobin level, we determined the average hemoglobin level based on available annual readings greater than one year prior to death (mean number of readings = 2.7, SD=1.4, range 1 to 6). The average hemoglobin level did not differ from the first available hemoglobin level (difference = -0.03 g/dL, SD=0.5, $t_{111}=-0.61$, $p=0.5$). When the first available hemoglobin measure was replaced by the average hemoglobin level in models adjusted for demographics with neuropathology as the outcome, the results were unchanged. Each g/dL lower average hemoglobin level was associated with an increased odds for the presence of chronic macroscopic infarctions (odd ratio=1.45, 95% CI=1.06, 1.97), but not for the presence of chronic microscopic infarctions ($p=0.6$), the presence of Lewy Bodies ($p=0.6$), or a greater level of the global AD pathology measure ($p=0.3$).

DISCUSSION

In more than 100 older persons initially without clinical dementia who subsequently died and had available neuropathology results, a lower initial hemoglobin level was associated with greater odds for having a chronic macroscopic infarct but not with other common

neuropathologies of old age. Each unit lower in hemoglobin level was associated with a 37% increased odds of having a chronic macroscopic infarct on neuropathology assessment. Lower hemoglobin levels were associated with an increased odds ratio for having a chronic macroscopic infarction in a subcortical region but not with the total volume of infarction.

Prior studies have linked hemoglobin to lower cognitive function and greater cognitive decline in the elderly with the specific cognitive domains that were affected fitting more of a vascular pattern. [11–14] In a prospective cohort including over 400 community-dwelling women between the ages of 70 to 80, a hemoglobin level less than 12 g/dL was associated with worse executive function at baseline and with a faster three year rate of decline in episodic memory. [14] Also, prior work in the Rush Memory and Aging Project in almost 900 community-dwelling women and men with a mean age of 80 years at time of initial hemoglobin measure and a mean follow-up time slightly over 3 years, each g/dL lower or higher than 13.7 g/dL was associated with a greater rate of cognitive decline on episodic memory, semantic memory, working memory, and perceptual speed but not visuospatial ability. [13] In limited, prospective cohort studies, lower hemoglobin levels also have been associated with clinical diagnosis of stroke, Parkinson's disease, and Alzheimer's disease. [13, 17–19] However, to the best of our knowledge, no prior study has reported the relationship between hemoglobin levels and neuropathology in older, community-dwelling persons. As mixed neuropathology is commonly found in older persons [29] and as hemoglobin may be related to each of the common neuropathologies, being able to model the association of hemoglobin level to chronic infarctions, Lewy Bodies, and Alzheimer's disease pathology is a novel feature of this analysis. Our work adds to the literature by showing that lower hemoglobin level seems to be linked to having a chronic macroscopic infarction on neuropathological assessment rather than chronic microscopic infarctions, Lewy Bodies, or Alzheimer's disease pathology. Given that lower hemoglobin levels have been linked to cognitive decline, chronic macroscopic infarcts have been linked to cognitive decline, [22] and lower hemoglobin levels have been linked to chronic macroscopic infarctions in subcortical regions in this study, a potential causal pathway between low hemoglobin levels, chronic macroscopic infarctions, and cognitive decline in older persons is beginning to emerge. Such a causal pathway increases confidence in lower hemoglobin levels being a biologic marker for cognitive decline in older persons. However, further epidemiologic evidence for lower hemoglobin levels as a risk factor for cognitive decline requires analyses that show the relationship of lower hemoglobin levels and cognitive decline in a large cohort of older persons is attenuated by subcortical macroscopic infarctions as measured by neuropathology or neuroimaging.

Why lower hemoglobin levels are linked to increased odds for having a chronic macroscopic infarction and not other neuropathology is uncertain. Before examining cause-effect mechanisms, it must be acknowledged that the association between hemoglobin level and chronic macroscopic infarction may be indirect. For instance, lower hemoglobin levels are associated with earlier mortality. [30, 31] If there is a temporal relationship with developing macroscopic infarctions earlier in the lifespan than microscopic infarcts, Lewy Bodies, or Alzheimer's disease pathology, then persons with lower hemoglobin levels may present to autopsy at an earlier age and may not have had sufficient time to develop microscopic infarcts, Lewy Bodies, and Alzheimer's disease pathology. Given that the average age of our cohort at time of death was 88 years and other studies have shown a significant presence of chronic microscopic infarctions, Lewy Bodies, and Alzheimer's disease pathology in the oldest old, [32, 33] the influence of time on differential development of neuropathology seems less likely. Also, lower hemoglobin levels may be an earlier manifestation of a common pathologic process that also influences the development of macroscopic infarctions. For instance, lower hemoglobin levels in older community-dwelling persons may represent a decline in erythropoietin production by the kidneys. [34] As erythropoietin

has shown neuroprotective effects in ischemic stroke models in animal models, [35] lower erythropoietin may increase the likelihood of macroscopic infarctions.

With the above caveats in mind, a causal link between hemoglobin levels and chronic macroscopic infarctions can be postulated. Our study points to lower hemoglobin levels being associated with subcortical rather than cortical infarcts. One risk factor for subcortical macroscopic infarctions is white matter hyperintensities on neuroimaging. [36–38] White matter hyperintensities commonly are associated with ischemic demyelination due to narrowing of the lumens of small penetrating arteries. [39, 40] Narrowing of the lumens of penetrating arteries may result in the decreased cerebral blood flow observed with white matter hyperintensities on neuroimaging. [41] In the background of an ischemic environment, hypoxia resulting from lower hemoglobin levels may worsen the development of white matter hyperintensities. As a result, hypoxia could accelerate the conversion of ischemic demyelization towards incomplete lacunar infarction and chronic macroscopic infarction. [42] A recent magnetic resonance imaging study in community-dwelling older persons has linked lower hemoglobin levels with worsening white matter hyperintensities, especially in the presence of hypertension. [43] Further studies combining neuroimaging of white matter hyperintensities and subsequent neuropathology are needed to determine the biologic basis for the association between hemoglobin and chronic macroscopic infarctions in older persons.

The strengths of our study include the analysis of common, age-related neuropathologies associated with cognitive decline in older, community-dwelling persons initially without dementia. Second, the Rush Memory and Aging Project had a high autopsy rate resulting in limited loss of outcome data. Third, having all brains evaluated at the same center using an established protocol lessened the potential for rater differences in the neuropathology assessment. Finally, having multiple annual measures of hemoglobin level to calculate an average hemoglobin level in addition to a first available hemoglobin level strengthens the likelihood that lower hemoglobin levels are associated with chronic macroscopic infarctions but not other neuropathology. Our study has limitations. The Rush Memory and Aging Project is a volunteer cohort comprising of individuals agreeing to autopsy at time of consent. Also, as hemoglobin levels were added into the cohort after the study was already underway, the number of neuropathologic cases for analyses was reduced. Our findings require confirmation as more neuropathology data becomes available in Rush Memory and Aging Project participants. Also, replication of the association of hemoglobin to neuropathology in other community-based, clinical pathologic studies of aging is needed.

If the causal pathway of lower hemoglobin being associated with cognitive decline via increased presence of chronic macroscopic infarction is confirmed, then clinicians may have a readily available, low-cost biologic marker for cognitive decline in older persons without known dementia, stroke, or Parkinson's disease. A lower hemoglobin level may lead to increased clinical efforts to manage known risk factors associated with subcortical infarctions such as high blood pressure. It is unclear that raising hemoglobin levels with currently available interventions such as recombinant erythropoietin will result in less infarction burden [44] or reduced cognitive decline. However, with a greater understanding of the causes of lower hemoglobin levels in older persons, other interventions may potentially be developed.

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Table 1

Characteristics of Participants (n=113)

Characteristic	Value
Age at baseline, mean (SD), years	85.0 (6.1)
Education, mean (SD), years	14.2 (2.6)
Women, Number of Participants (%)	76 (67.3)
Hemoglobin, mean (SD), g/dL	13.0 (1.4)
Baseline to Death Interval, mean (SD), y	3.2 (1.3)
Macroscopic Infarct Present, Number of Participants (%)	35 (31.0)
Microscopic Infarction Present, Number of Participants (%)	29 (25.7)
Lewy Body Present, Number of Participants (%)	11 (9.7)
Global Alzheimer's Disease Pathology Summary Score, (SD)	0.56 (0.56)

Table 2

Odds ratios for chronic macroscopic infarction for persons with hemoglobin level 1 g/dL below reference persons

Model	Model Description and Exclusions	Numbers of participants		Odds Ratio for Chronic Macroscopic Infarction*
		Total	With Chronic Macroscopic Infarction	
A	Primary Model	113	35	1.37 (1.01, 1.86)
B	Removing Participants with Baseline Clinical Stroke or Parkinson's Disease	102	28	1.47 (1.05, 2.05)

* Odds ratios (95% CI) for each g/dL lower hemoglobin level were derived from logistic regression models adjusted for age at death, gender, and education.

Table 3

Odds ratios for chronic macroscopic infarction by location for each 1 g/dL lower level in hemoglobin

Location of Chronic Macroscopic Infarct	Odd Ratio for Chronic Macroscopic Infarction in Location* (95% CI)
Subcortical Only	1.33 (1.03, 1.69)
Cortical Only	1.11 (0.79, 1.56)
Subcortical and Cortical	1.08 (0.73, 1.59)

* Odds ratios (95% CI) for each g/dL lower level of hemoglobin is derived from a polychotomous logistic regression model adjusted for age at death, gender, and education where the reference group is participants with no chronic macroscopic infarction.