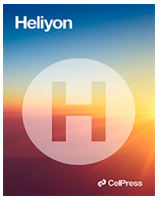
Heliyon 10 (2024) e37507

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Association of hemoglobin with plasma neurofilament light and white matter hyperintensities in Alzheimer's disease continuum

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ARTICLE INFO

Keywords:
Hemoglobin
Alzheimer's disease
White matter hyperintensities
Neurofilament light
APOE

ABSTRACT

Objective: This study aimed to investigate the association of hemoglobin (Hb) with axonal injury marker plasma neurofilament light (PNFL) and brain structure measurements in the Alzheimer's disease (AD) continuum.

Methods: The data used in this study were collected from the Alzheimer's Disease Neuroimaging Initiative database. Participants with cognitively normal, mild cognitive impairment, and mild dementia were included in the data analyses. All participants had available data on blood tests, PNFL levels, neuropsychological assessments, brain structure measurements (including volumes of white matter hyperintensities [WMH], hippocampus, gray matter, and total brain), and Aβ positron emission tomography standardized uptake value ratio (SUVR) at baseline. Aβ-positive was defined as SUVR threshold value > 1.11. Linear regression, restricted cubic spline, and causal mediation analyses were conducted to investigate the association of Hb concentration with PNFL levels and brain structure measurements. Stratified analyses were also employed to evaluate the association between Hb concentration and PNFL levels across different APOE genotypes and sex.

Results: In the Aβ-positive group, Hb concentration was associated with PNFL levels ($\beta = -0.022$, $p = 0.002$). Stratified analyses suggested an association between Hb concentration and PNFL in APOE ε4 carriers ($\beta = -0.031$, $p < 0.001$) and males ($\beta = -0.030$, $p < 0.001$) but not in non-carriers and females ($p > 0.05$). Hb concentration was also associated with WMH volume ($\beta = -0.04$, $p = 0.028$), especially in APOE ε4 carriers, with mediation analysis revealing that PNFL mediated the association between Hb concentration and WMH volume. The association of Hb concentration with other brain structure measurements was minimal.

Conclusion: In the AD continuum, Hb was associated with axonal injury marker PNFL and WMH volume, particularly in APOE ε4 carriers.

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[1] Data used in this study were collected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the ADNI investigators contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators is available at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

https://doi.org/10.1016/j.heliyon.2024.e37507
Received 29 April 2024; Received in revised form 3 September 2024; Accepted 4 September 2024
Available online 5 September 2024

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia among the elderly [1]. In addition to the accumulation of Amyloid-β (Aβ) senile plaques and hyperphosphorylated tau tangles, it also involves vascular pathologies concerning its mechanisms [2,3]. Increasing evidence suggests that AD patients often present with imaging markers of cerebral small vessel disease (CSVD), such as white matter hyperintensities (WMH) [4], which makes its treatment more challenging. However, the mechanism of comorbidity between AD and CSVD has not been fully elucidated. Investigating the comorbidity of AD and CSVD, particularly AD comorbid with WMH, may help to better understand the mechanism underlying the comorbidity of the two conditions.

WMH in CSVD was implied to be caused by both hypoperfusion and brain blood barrier impairment, and its pathological changes included demyelination and axonal injury [5]. Chronic hypoxia can lead to axonal injury, as central myelinated axons critically dependent on a continuous oxygen supply [6]. Previous literature indicates that axonal injury is an early event in AD [7], with structural brain damage occurring later in the progression of the disease [8]. Aβ pathology can also promote the development of axonal injury [9,10]. In light of this evidence, we hypothesize that risk factors leading to cerebral hypoxia or insufficient perfusion may induce axonal injury, interact with Aβ pathology, and ultimately cause macroscopic white matter damage (eg., WMH) when the lesions accumulate to a certain extent. Plasma neurofilament light (PNFL) has been widely used as a biomarker of axonal injury due to its convenient detection and low cost [11], showing good consistency with neurofilament light (NFL) in cerebrospinal fluid in reflecting the severity of AD and WMH [12–14]. Further investigation of the association between risk factors, axonal injury marker PNFL, and WMH in AD may contribute to understanding the comorbidity mechanisms of AD and CSVD, so as to implement targeted intervention.

Current evidence suggests that in addition to vascular narrowing and decreased cerebral perfusion [15], a decrease in hemoglobin (Hb) concentration is also an important factor contributing to cerebral hypoxia [16]. Anemia or decreased Hb concentrations are not only associated with an increased risk of death and poor prognosis [17] but also with cognitive impairment diseases, such as AD [18]. Previous studies have reported associations of Hb concentration with AD biomarkers (e.g., cortical thickness and brain glucose metabolism) [19], vascular pathology (eg., WMH/cerebral microbleeds) [20,21], and cognitive function [22]. Nevertheless, the underlying mechanism linking Hb and AD remains unknown, and the relationship between Hb and axonal injury, particularly in AD, has not been investigated.

In this study, we investigated the association of Hb with axonal injury marker PNFL, brain structure measurements relevant to AD (including WMH, hippocampus, gray matter, white matter, and total brain), and cognitive function in the AD continuum. Given the genetic overlap between vascular pathology and AD, primarily driven by the APOE ε4 [23], and observed differences in Hb concentration between sexes [17], we conducted stratified analyses based on APOE genotypes and sex. Additionally, we assessed whether PNFL plays a mediating role in the relationship between Hb and brain structure measurements.

2. Materials and methods

2.1. Data source

The data utilized in this study were collected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (accessed on January 16, 2024), which was established in 2003 as a public-private partnership under the leadership of Principal Investigator Michael W. Weiner, MD. For the latest information, please refer to www.adni-info.org.

2.2. Participants

The detailed enrollment procedure and inclusion criteria for different diagnostic categories in the ADNI study have been described previously [24]. In this study, participants with baseline diagnoses of cognitively normal (CN), mild cognitive impairment (MCI), and mild dementia with clinical dementia rating (CDR) ranging from 0 to 1 underwent eligibility screening.

The inclusion criteria for this study were as follows: (1) aged 60 years or older; (2) availability of demographic information, PNFL data, blood tests data, brain magnetic resonance imaging (MRI), neuropsychological assessments, and Aβ (florbetapir) positron emission tomography (PET) at baseline. The following exclusion criteria were applied: (1) the usage of any psychoactive medications; (2) leukemia and hemolytic anemia; (3) acute inflammatory diseases (such as sepsis, systemic inflammatory response syndrome, and so on); (4) uremia.

According to the 2018 National Institute on Aging and Alzheimer's Association criteria, Aβ-positivity can be defined as the AD continuum [25]. In the present study, Aβ positive was defined as Aβ PET standardized uptake value ratio (SUVR) threshold value $>$ 1.11 [26].

2.3. Neuropsychological assessments

The neuropsychological assessments were performed by certified raters using standardized ADNI protocols (www.adni-info.org). Multiple scales were employed to assess cognitive performance, including the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), CDR, ADNI memory, executive, language, and visuospatial composite function scores. Meanwhile, scores for different subdomains of the MoCA test were collected. Cognitive impairment was defined as a global CDR score $\geq$0.5. The neuropsychological assessment data were collected from the files ("MMSE.csv", "MOCA.csv", "CDR.csv", "UWNPSYCHSUM.csv").

2.4. Neuroimaging data

MRI examinations were performed according to the ADNI MRI scanning protocol. Four brain tissue segmentation methods have been previously described, with detailed explanations available in the ADNI reference documentation "Four Tissue Segmentation in ADNI II." The volumes of WMH, hippocampus, gray matter, white matter, and total brain were collected from the file ("ADNI_UCD_WMH.csv").

The detailed protocols for Aβ PET image acquisition have been described in previous studies [27]. The average SUVR of Aβ PET was extracted from the ADNI file ("UCBERKELEY_AMY_6 MM.csv").

2.5. PNFL data and blood test data

PNFL was analyzed by the single molecule array (Simoa) technique, at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal Campus, Mölndal, Sweden. The PNFL data were collected from the ADNI file ("BLENNOWPLASMANFLLONG.csv"). Blood routine and renal function data were acquired from the file ("LABDATA.csv"). According to the World Health Organization standards, anemia was defined as Hb concentrations below 12 g/dL for females and below 13 g/dL for males [28]. Considering the impact of renal function on PNFL [29], we adjusted for renal function when analyzing the association between Hb and PNFL. The calculation of the estimated glomerular filtration rate (eGFR) was based on the Chronic Kidney Disease Epidemiology Collaboration equation [30].

2.6. Other assessments and data collection

The following data were collected from the clinical evaluation files ("ADNIMERGE.csv", "APOERES.csv", "VITALS.csv", "RECMHIST.csv"): age, sex, education years, APOE genotypes, body mass index, blood pressure, usage of anti-dementia, anti-hypertensive, and antiplatelet aggregation medications, and vascular risk factors (including hypertension and diabetes mellitus). Participants with at least one copy of the APOE ε4 allele were considered as APOE ε4 carriers.

Table 1
Demographic and clinical characteristics of participants.

Index	All (N = 686)	Aβ-positive (N = 377)	Aβ-negative (N = 309)	p value
Age (years)	73.2 (6.74)	74.4 (6.58)	71.8 (6.66)	<0.001
Female (%)	313 (45.6 %)	173 (45.9 %)	140 (45.3 %)	0.94
Education years (years)	16.2 (2.63)	16.0 (2.74)	16.5 (2.47)	0.024
Body mass index (kg/㎡)	27.4 (5.01)	26.9 (5.08)	28.0 (4.87)	0.003
Systolic blood pressure (mmHg)	136 (15.8)	137 (15.4)	135 (16.3)	0.14
Diastolic blood pressure (mmHg)	75.4 (9.36)	75.1 (9.33)	75.7 (9.40)	0.475
Hypertension (%)	320 (46.6 %)	181 (48.0 %)	139 (45.0 %)	0.475
Diabetes mellitus (%)	81 (11.8 %)	47 (12.5 %)	34 (11.0 %)	0.637
Anaemia (%)	66 (9.62 %)	41 (10.9 %)	25 (8.09 %)	0.271
APOE ε4 carrier (%)	314 (45.8 %)	249 (66.0 %)	65 (21.0 %)	<0.001
Red blood cell (10^12)	4.56 (0.41)	4.55 (0.42)	4.56 (0.39)	0.602
Hemoglobin (g/dL)	14.0 (1.25)	14.0 (1.22)	14.0 (1.29)	0.988
Mean corpuscular volume (fL)	90.5 (8.59)	91.0 (6.66)	90.0 (10.5)	0.141
White blood cell (10^9)	6.44 (1.61)	6.46 (1.64)	6.41 (1.58)	0.647
eGFR (ml/min/1.73㎡)	69.2 (13.5)	68.7 (13.4)	69.7 (13.7)	0.333
Plasma neurofilament (pg/mL)	35.3 [26.9, 47.3]	39.9 [30.5, 51.0]	30.5 [23.8, 41.3]	<0.001
Hippocampal volume (mL)	6.31 (0.91)	6.12 (0.88)	6.54 (0.90)	<0.001
Gray matter volume (mL)	586 (54.6)	581 (55.7)	591 (52.9)	0.02
White matter volume (mL)	474 (59.7)	468 (59.4)	482 (59.1)	0.002
White matter hyperintensities (mL)	4.27 [1.85, 8.61]	5.33 [2.46,11.3]	2.79 [1.48, 6.31]	<0.001
Total brain volume (mL)	1401 (138)	1401 (141)	1400 (134)	0.976
Aβ PET SUVR	1.23 (0.26)	1.42 (0.19)	1.00 (0.06)	<0.001
MMSE (0–30 points)	28 [26,29]	27 [25,29]	29 [28,30]	<0.001
MOCA (0–30 points)	23 [20,26]	22 [19,25]	25 [23,27]	<0.001
Cognitive function type:				<0.001
cognitively normal	179 (26.1 %)	55 (14.6 %)	124 (40.1 %)	
mild cognitive impairment	443 (64.6 %)	265 (70.3 %)	178 (57.6 %)	
mild dementia	64 (9.33 %)	57 (15.1 %)	7 (2.3 %)	
Usage of medications:				
anti-dementia (%)	270 (39.4 %)	216 (57.3 %)	54 (17.5 %)	<0.001
antiplatelet aggregation (%)	407 (59.3 %)	221 (58.6 %)	186 (60.2 %)	0.734
anti-hypertension (%)	276 (40.2 %)	181 (48.0 %)	139 (45.0 %)	0.475

Abbreviation: eGFR, estimated glomerular filtration rate; SUVR, standard uptake value ratio.

2.7. Statistical analysis

Statistical significance thresholds were set at a two-tailed p-value of <0.05. All statistical analyses were performed using R programming (version 4.2.2).

The Shapiro-Wilk test was used to assess data distribution types. Data were reported as mean (standard deviation [SD]), frequency (%), and median (interquartile range). The Student's t-test was utilized to compare normally distributed data between two groups. The chi-square test was employed for comparing categorical variables, while the Wilcoxon Rank-Sum test was used for comparing non-normally distributed continuous variables. Variables that did not adhere to a normal distribution were transformed before conducting the regression analysis. MMSE scores were z-transformed, and WMH volume and PNFL levels were log-transformed.

Linear regression was performed to examine the association of Hb concentration with PNFL levels, brain structure measurements, and cognitive performance, adjusting for age, sex, APOE genotypes, hypertension status, and eGFR. Additionally, stratified analyses were conducted based on APOE genotypes and sex to explore the relationship between Hb and PNFL levels/WMH volume in the Aβ-positive and Aβ-negative groups. Linear regression analysis was also performed to investigate the relationship between PNFL levels and brain structure measurements. If the variance inflation factor exceeded 5, it was considered a significant indication of multicollinearity. None of the linear regression models exhibited significant collinearity. Restricted cubic spline (RCS) is a statistical analysis method used to assess the nonlinear relationship between continuous variables and binary outcome variables. RCS analysis was conducted to assess the non-linear relationship between Hb and PNFL, WMH volume, hippocampal volume (consecutive variables were grouped into two categories using the median), and cognitive impairment, adjusting for the aforementioned confounding variables using the "rms" package. Based on the values of the Akaike Information Criterion, the number of knots that best approximates the model was selected (knots = 4). Finally, causal mediation analysis [31] was employed to assess the potential mediating effects of PNFL on the association between Hb and brain structure measurements. The extent of the indirect effect was estimated, and the significance was determined using 1000 bootstrapped iterations with the "mediate" package.

3. Results

3.1. Participants' characteristics

Fig. S1 illustrated the screening process for the participants. A total of 686 participants were included in the data analyses, comprising 377 cases of Aβ-positive and 309 cases of Aβ-negative individuals. The demographic information of the excluded participants can be found in Table S1.

The average age of the included participants was 73.2 years (SD 6.74), with 45.6 % being female, 45.8 % being APOE ε4 carrier, and an average Hb concentration was 14.0 g/dL (SD 1.25). Compared to the Aβ-negative group, the Aβ-positive group was elderly (74.4 ± 6.58 years vs. 71.8 ± 6.66 years), had higher PNFL levels, and larger WMH volume. The prevalence of anemia and the difference in average Hb concentration between the two groups were insignificant. For more information, please refer to Table 1.

3.2. Association between Hb and PNFL

In the overall population, Hb concentration was associated with PNFL levels ($\beta = -0.017$, $p = 0.002$). Subgroup analyses suggested an association between Hb and PNFL in Aβ-positive individuals ($\beta = -0.022$, $p = 0.002$), APOE ε4 carriers ($\beta = -0.029$, $p < 0.001$), and males ($\beta = -0.021$, $p = 0.002$). However, no significant association was observed between Hb concentration and PNFL levels in Aβ-negative individuals, APOE ε4 non-carriers, and females (refer to Table 2).

Table 2
Association between hemoglobin concentration and plasma neurofilament light in the total sample and different subgroups.

	Beta (Standard error)	p value
Total sample (N = 686)		
Hemoglobin	−0.017 (0.005)	**0.002**
Aβ-positive (N = 377)		
Hemoglobin	−0.022 (0.007)	**0.002**
Aβ-negative (N = 309)		
Hemoglobin	−0.014 (0.008)	0.085
ε4 carriers (N = 314)		
Hemoglobin	−0.029 (0.008)	**<0.001**
Non-carriers (N = 372)		
Hemoglobin	−0.008 (0.007)	0.289
Female (N = 313)		
Hemoglobin	−0.012 (0.009)	0.167
Male (N = 373)		
Hemoglobin	−0.021 (0.007)	**0.002**

Note: All models were adjusted for age, hypertension status, and estimated glomerular filtration rate.

In the Aβ-positive group, stratified analyses indicated an association between Hb and PNFL in APOE ε4 carriers (β = −0.031, p < 0.001) and males (β = −0.030, p < 0.001). Additionally, a significant non-linear association was found between Hb concentration and PNFL in the Aβ-positive group (p for overall 0.006, p for non-linear 0.039; refer to Fig. 1).

In the Aβ-negative group, there was no significant association between Hb concentration and PNFL levels across different APOE genotypes and sex types (p > 0.05; refer to Table 3). The non-linear association between Hb concentration and PNFL was also insignificant (p > 0.05; refer to Fig. S2).

3.3. Association of Hb with brain structure measurements and cognitive function

In the overall population, there was no significant association between Hb concentration and volumes of WMH, hippocampus, gray matter, white matter, and total brain (p > 0.05). However, when Hb concentration was grouped into quartiles, the third quartile was associated with higher gray (β = 11.44, p = 0.024) and total brain (β = 30.13, p = 0.014) volumes relative to the first quartile, while the second (β = −0.12, p = 0.02) and upper (β = −0.14, p = 0.008) quartiles were associated with lower WMH volume (refer to Table 4). RCS analysis indicated that the non-linear associations between Hb concentration and brain structure measurements were insignificant (p > 0.05; refer to Fig. S3).

In the Aβ-positive group, Hb concentration was associated with WMH volume (β = −0.04, p = 0.028). When Hb concentration was divided into quartiles, the second (β = −0.14, p = 0.024) and upper (β = −0.19, p = 0.007; refer to Table 4) quartiles were associated with lower WMH volume relative to the first quartile. Stratified analyses indicated an association between Hb and PNFL in APOE ε4 carriers (β = −0.059, p = 0.02), but not in non-carriers, males, and females (refer to Table S2). No significant association was found between Hb concentration and other brain structure measurements. RCS analysis showed that the non-linear associations between Hb and brain structure measurements were insignificant (p > 0.05; refer to Fig. S3).

In the Aβ-negative group, the linear and non-linear associations between Hb concentration and brain structure measurements were insignificant (p > 0.05).

Additionally, there was no significant association between Hb and cognitive function in the total study population, Aβ-positive group, and Aβ-negative group (p > 0.05). RCS analysis also indicated that the non-linear association between Hb concentration and cognitive impairment was insignificant (p > 0.05; refer to Fig. S4). However, in individuals with CN, Hb was associated with MoCA visuospatial/executive domain scores (β = 0.129, p = 0.026), while in individuals with MCI, Hb concentration was associated with language composite score (β = 0.067, p = 0.035; refer to Table S3).

3.4. Association between Hb and WMH volume was mediated by PNFL

In the Aβ-positive group, mediation analysis indicated that PNFL levels mediated the association between Hb concentration and WMH volume (refer to Fig. 2). Nevertheless, in the Aβ-negative group, the mediating effect of PNFL on the relationship between Hb and WMH volume was insignificant.

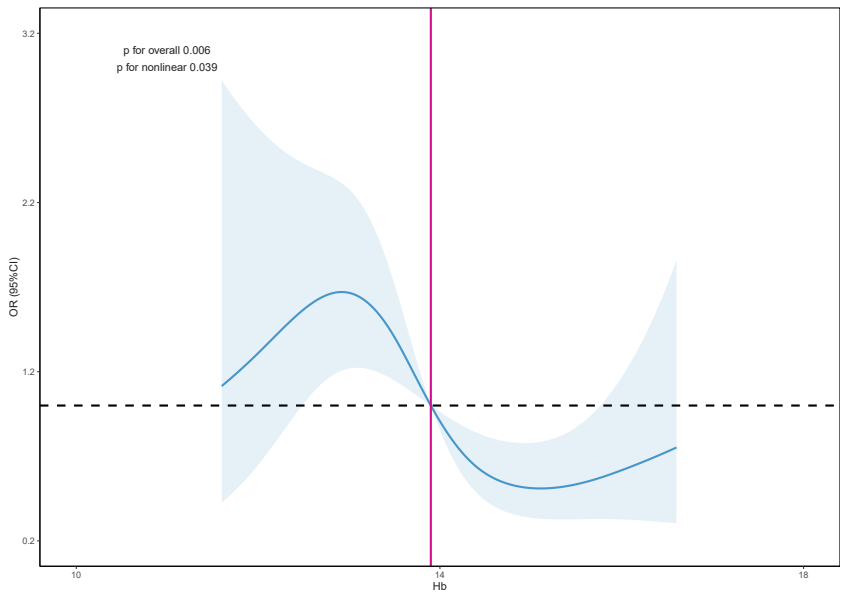

Fig. 1. The non-linear association between hemoglobin concentration and plasma neurofilament light levels in the Aβ-positive group. Abbreviation: OR, odds ratio; CI, confidence interval; Hb, hemoglobin.

Table 3
Stratified analyses of the relationship between hemoglobin and plasma neurofilament light by APOE genotypes and sex types in the Aβ-positive and Aβ-negative groups.

Group	Aβ-positive (N = 377)		Aβ-negative (N = 309)	
	Beta (Standard error)	p value	Beta (Standard error)	p value
APOE genotypes				
ε4 carriers	−0.031 (0.008)	**< 0.001**	−0.021 (0.022)	0.329
non-carriers	−0.004 (0.012)	0.749	−0.010 (0.009)	0.261
Sex types				
female	−0.012 (0.012)	0.332	−0.018 (0.013)	0.167
male	−0.030 (0.008)	**< 0.001**	−0.011 (0.011)	0.313

Note: All models were adjusted for age, sex, hypertension status, APOE genotypes, and estimated glomerular filtration rate.

Table 4
Association of hemoglobin concentration and neurofilament light levels with brain structure measurements in all participants and Aβ-positive individuals.

	Hippocampus	Gray matter	White matter	WMH	Total volume
All (N = 686)	Beta (Standard error) p value				
Model 1					
Second quartile	0.02 (0.09) p = 0.711	−1.13 (4.91) p = 0.818	1.54 (5.20) p = 0.767	−0.12 (0.05) **p = 0.02**	−3.92 (11.86) p = 0.741
Third quartile	0.09 (0.09) p = 0.313	11.44 (5.06) **p = 0.024**	9.31 (5.36) p = 0.083	−0.08 (0.05) p = 0.1	30.13 (12.23) **p = 0.014**
Upper quartile	0.02 (0.10) p = 0.828	4.98 (5.38) p = 0.355	0.69 (5.69) p = 0.904	−0.14 (0.05) **p = 0.008**	8.14 (12.99) p = 0.531
Hb	−0.01 (0.03) p = 0.762	1.54 (1.54) p = 0.317	0.24 (1.63) p = 0.882	−0.02 (0.02) p = 0.115	2.74 (3.72) p = 0.462
Model 2					
PNFL	−0.54 (0.20) **p = 0.008**	−11.90 (11.14) p = 0.286	−7.33 (11.76) p = 0.533	0.31 (0.11) **p = 0.006**	24.88 (26.92) p = 0.356
Aβ-positive (N = 377)					
Model 1					
Second quartile	0.05 (0.12) p = 0.649	−2.58 (6.67) p = 0.699	−0.74 (7.15) p = 0.918	−0.14 (0.06) **p = 0.024**	−10.47 (16.06) p = 0.515
Third quartile	0.004 (0.12) p = 0.973	7.31 (6.80) p = 0.283	4.10 (7.29) p = 0.574	−0.08 (0.07) p = 0.215	24.08 (16.39) p = 0.143
Upper quartile	−0.12 (0.13) p = 0.341	3.29 (7.17) p = 0.647	4.22 (7.68) p = 0.583	−0.19 (0.07) **p = 0.007**	14.72 (17.26) p = 0.394
Hb	−0.03 (0.04) p = 0.467	1.03 (2.11) p = 0.626	1.27 (2.25) p = 0.573	−0.04 (0.02) **p = 0.028**	3.31 (5.09) p = 0.516
Model 2					
PNFL	−0.67 (0.027) p = **0.015**	−23.50 (15.44) p = 0.129	−17.95 (16.49) p = 0.277	−0.14 (0.06) **p = 0.005**	0.97 (37.52) p = 0.00.98

Abbreviation: WMH, white matter hyperintensities; Hb, hemoglobin; PNFL, plasma neurofilament light. Note: WMH volume and PNFL levels were log-transformed before regression analyses. Model 1 with hemoglobin concentration or quartiles as the predictor variable was adjusted for age, sex, hypertension status, APOE genotypes, and estimated glomerular filtration rate using the lowest quartile as reference. Model 2 with PNFL as the predictor variable was adjusted for age, sex, hypertension status, and APOE genotypes. The unit of volume is milliliter.

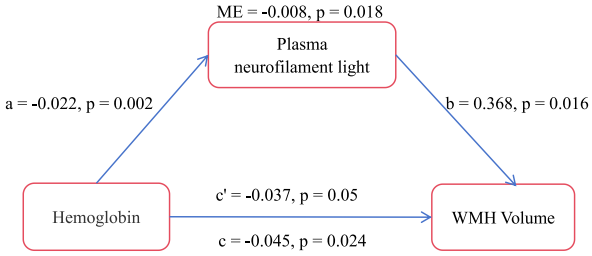

Fig. 2. Association between hemoglobin and white matter hyperintensities volume was mediated by PNFL in the Aβ-positive group.
Note: ME, mediation effect; a, the effect of hemoglobin on plasma neurofilament light; b, the effect of plasma neurofilament light on WMH volume; c', direct effect; c, total effect. WMH, white matter hyperintensities.

4. Discussion

In the present study, we investigated the association of Hb with PNFL and brain volume measurements. Our results revealed that: (1) Hb exhibited independent associations with PNFL levels only in the Aβ-positive group, not in the Aβ-negative group; (2) Stratified analyses suggested a significant association between Hb and PNFL in APOE ε4 carriers and males; (3) In the Aβ-positive group, Hb concentration was associated with WMH volume, with PNFL mediating the association between Hb and WMH volume. These findings strengthen the association between Hb and AD vascular pathology. To our knowledge, no studies have previously investigated the relationship between Hb and axonal injury marker PNFL.

Our study showed that Hb concentration was independently associated with PNFL in the Aβ-positive group. This result can be explained by the evidence that lower Hb concentration may exacerbate axonal injury in the presence of Aβ pathology [7,9]. We also observed a significant non-linear association (with the turning point at 13.9 g/dL) between Hb and PNFL in the Aβ-positive group, consistent with Wolters et al., who found a non-linear association between Hb levels and white matter connectivity reflected by diffusion tensor imaging [32]. Combining the study by Wolters et al. and our research, it may be beneficial to maintain a slightly higher Hb concentration within the range of 14–15 g/dL in AD.

To further clarify the relationship between Hb and PNFL, we conducted stratified analyses based on APOE genotypes and sex in the Aβ-positive group. Our findings revealed a significant association between Hb and PNFL in APOE ε4 carriers but not in non-carriers. Previous literature has shown that in AD, the presence of APOE ε4 is associated with a greater susceptibility to axonal injury compared to non-carriers [33]. This supports a large impact of Hb on PNFL levels in the presence of APOE ε4. Some studies have shown that the combination of APOE ε4 genotype, Hb, and folate can increase the sensitivity of predicting AD pathology [34], suggesting a synergistic relationship between APOE ε4 carrier and Hb with AD pathology, emphasizing the importance of monitoring and managing Hb in APOE ε4 carriers. Then, we evaluated the impact of sex on the relationship between Hb and PNFL levels. We found an association between Hb and PNFL levels in males, while the association was not significant in females. This result may be related to the slightly higher prevalence of anemia in males compared to females in this study (13.2 % vs. 8.09 %). Additionally, prior studies have shown that there was not much difference in brain energy metabolism rate between males and females. Males have a higher arterial Hb concentration than females, while females can increase cerebral blood flow through neurovascular regulatory mechanisms [35]. Therefore, changes in Hb concentration may have a greater impact on male brain oxygenation and metabolism. Furthermore, research has indicated that the axonal density in the temporal lobe cortex was higher in males than in females [36], potentially making males more sensitive to changes in Hb concentration.

In AD patients, the primary MRI signs of vascular pathology are characterized by WMH [4]. In this study, we found that Hb concentration was negatively associated with WMH volume in the Aβ-positive group, consistent with some previous studies [32]. This result can be explained by the fact that reduced Hb concentration may affect its oxygen-carrying function and brain oxygenation/metabolism, exacerbating pre-existing white matter lesions (axonal injury, age-related small artery sclerosis, or lumen narrowing) [37], leading to white matter damage manifested as WMH on MRI. Previous studies have indicated that genetically predicted vascular pathological changes in AD are mainly driven by APOE ε4, including white matter lesions [23]. By stratified analysis, we found that the association between Hb and WMH volume was significant in APOE ε4 carriers. This result indicated that in the presence of both Aβ pathology and APOE ε4 carriers, risk factors or internal environmental changes (Hb concentration) are more strongly associated with WMH. We previously hypothesized that axonal injury may act as an intermediary link between changes in Hb concentration and brain structural alterations. Through causal mediation analysis, we found that only in the Aβ-positive group, the axonal injury marker PNFL mediated the association between Hb concentration and WMH volume, suggesting that axonal injury may involve the pathological processes of Hb, and this process may rely on the existence of Aβ pathology. The above evidence indicated that Hb may be associated with axonal injury and subsequent development of WMH, with a tighter association in the presence of Aβ pathology and APOE ε4 carriers. That is to say, in AD patients with APOE ε4, risk factors are more likely to trigger vascular pathological changes (like WMH), emphasizing the need for early risk factor management in these patients.

However, there was a lack of association between Hb and other brain volume measurements in this study. Some previous studies have also reported that the connection between Hb and brain structural measurements was not notably strong [20,38,39]. Previously, we found that axonal injury (as represented by PNFL) may be involved in the pathological process of Hb. Since axons are mainly located in white matter and axonal injury predominantly affects white matter fiber integrity [40], this may explain the lack of association between Hb and other brain volume measurements, such as the hippocampus, gray matter, or total brain volume. Additionally, recent studies have revealed that Hb is expressed in dopaminergic neurons, and to some extent, in cortical and hippocampal astrocytes. Neuronal Hb exerts biochemical activities and biological functions similar to its roles in erythroid cells [41]. This implies that neuronal Hb may alleviate some of the negative effects caused by reduced Hb concentration inside the red blood cells under conditions of hypoxia. This evidence may help explain the insignificant association of Hb with the volumes of the hippocampus, gray matter, and total brain. The association between Hb and cognitive function was also limited. This is possibly due to the lack of significant association between Hb and brain volume measurements, with brain volume (hippocampus, gray matter volume) being an indicator reflecting the severity of cognitive function [42]. Considering the complicated association between Hb and cognitive function, and an inverted U-shaped relationship may exist [43]. Future research needs to further investigate the relationship between Hb and cognitive function, especially in AD.

In this study, some limitations need to be pointed out. Firstly, being a cross-sectional study, our results were insufficient to accurately elucidate the complex relationship between Hb and AD. Second, there may be some confounding factors associated with both Hb and AD that could potentially affect the results, such as vitamin D levels. Vitamin D may be associated with both blood parameters (like red blood cell count and mean corpuscular hemoglobin concentration) and AD [44,45]. However, due to the unavailability of vitamin D data, we were unable to assess the potential influence of vitamin D on the relationship between hemoglobin and AD. In the RCS analysis, categorizing a continuous dependent variable into two groups based on the median may not be very accurate, but it is a method commonly used in the majority of studies. Additionally, the causal mediation analysis in this study is based on traditional epidemiological observations, and the interpretation of the results should be cautious due to the continuous and heterogeneous nature of AD, with potential variations in pathology at different stages and types of AD. Future studies should explore this topic in different stages of AD.

5. Conclusion

In conclusion, our study identified an association between Hb and axonal injury marker PNFL in the AD continuum, with differences in their association observed under different APOE genotypes and sex types. In the AD continuum, Hb concentration was also associated with WMH volume, with PNFL mediating the relationship between Hb and WMH volume. These findings suggest that Hb is closely associated with vascular pathological changes (eg., WMH) in AD patients, particularly in APOE ε4 carriers, highlighting the importance of early risk factor management in these individuals. Considering the inverted U-shaped association between Hb and cognitive function demonstrated in previous studies, we suggest that maintaining a slightly higher Hb concentration (about 14–15 g/dL) may be beneficial for the elderly population, especially in AD patients with APOE ε4 carriers and males.

Data availability statement

The datasets generated and analyzed in the current study are available in the ADNI data repository: https://adni.loni.usc.edu/data-samples/adni-data/#AccessData.

Ethics statement

The ADNI study obtained approval from the institutional review boards of all participating institutions, and written informed consent was obtained from all participants or their authorized representatives in accordance with the principles outlined in the Declaration of Helsinki.

Ethical approval

Not required.

Consent for publication

Not applicable.

Funding

This present research was funded by the Chongqing Natural Science Fund Key Project No: cstc2019jcyj-zdxmX0029 and the Kuanren Talents Program of the Second Affiliated Hospital of Chongqing Medical University.

CRediT authorship contribution statement

Qin Li: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiehong Zhan:** Writing – review & editing, Validation, Methodology. **Zixuan Liao:** Writing – review & editing, Validation. **Jiayu Li:** Writing – review & editing, Validation. **Xiaofeng Li:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37507.

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