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Published in:
Journal of Gerontology: Medical sciences

DOI:
10.1093/gerona/glaa223

THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Medical Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2021, Vol. 76, No. 7, 1309–1315
doi:10.1093/gerona/glaa223
Advance Access publication September 4, 2020

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Research Article

White Blood Cell Count Mediates the Association Between Periodontal Inflammation and Cognitive Performance Measured by Digit Symbol Substitution Test Among Older U.S. Adults

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Received: March 26, 2020; Editorial Decision Date: August 24, 2020

Decision Editor: Jay Magaziner, PhD, MSHyg

Abstract

Background: Systemic effects of periodontal infection may increase the risk of central neuroinflammation, aggravating impaired cognition. This study aims to examine whether systemic inflammatory factors mediate the possible association between periodontal inflammation and cognitive function.

Methods: We conducted a cross-sectional analysis of 766 participants aged ≥ 60 years and who had completed periodontal and cognitive examinations in the National Health and Nutrition Examination Survey (NHANES) 2001–2002. We used multivariable linear regression to investigate the overall association between periodontal health and cognitive function as measured by the digit symbol substitution test (DSST). Bleeding on probing (BOP) and periodontal inflamed surface area (PISA) were used to assess the periodontal inflammatory activity and burden, respectively. Mediation analyses were used to test the indirect effects of the BOP/PISA on DSST via C-reactive protein, white blood cell (WBC) count, and fibrinogen.

Results: Participants with superior periodontal health obtained higher DSST scores than those with poorer periodontal health, adjusting for demographic factors and chronic conditions. Concerning the inflammatory activity, WBC count acted as a full mediator in the association between BOP and DSST (β = −0.091; 95% confidence interval [CI] = −0.174 to −0.008) and mediated 27.5% of the total association. Regarding the inflammatory burden, WBC count acted as a partial mediator in the association between PISA and DSST (β = −0.059; 95% CI = −0.087 to −0.031) and mediated 20.3% of the total association.

Conclusion: Our study indicated the potential role of systemic inflammatory factors as a mediator of associations between periodontal inflammation and cognitive function in the U.S. geriatric population.

Keywords: Cognitive function, Inflammation, Mediation analysis, Periodontal health, WBC count

Owing to the rapidly aging population, the number of individuals with dementia will more than triple by 2050, compared to 2010. Globally, the number of cases of dementia in people aged over 65 years is predicted to be 70 million (1,2). Identifying modifiable factors that can reduce the incidence of dementia should thus become a priority (3). Numerous studies have indicated the importance of systemic inflammation as a critical determinant of the cognitive impairment associated with dementia progression (4,5).

Periodontitis is an oral infection affecting tooth-supporting tissues that cause clinical attachment loss (CAL) and alveolar bone loss, ultimately leading to tooth loss (6). The prevalence of periodontitis increases with age (7). A systematic review indicated the association between oral health and cognitive function in old adults (8).

Periodontal health was initially defined as the absence of clinically detected inflammation by the 2018 World Workshop of the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) (9), that is, no deepening of the gingival sulcus and without the substantial extent of gingival bleeding. Previously, most studies defined a healthy periodontium as the opposite of case definitions of periodontitis (10,11). These case definitions are diagnosed with the accumulation of tissue defects, such as radiography findings as well as clinical measurements of CAL and alveolar bone loss. However, those have limitations for the detection of inflammatory activity, as these clinical signs are often indicators of chronic disease (12). The pathological progression of periodontitis is a slow continuous process of alveolar bone loss cyclically with periods of exacerbation and quiescence (6). Periods of exacerbation (activity) result in deeper periodontal pockets and exacerbate gingival bleeding. In contrast, the periods of quiescence (inactivity) are characterized by a reduced inflammatory response and less attachment loss. Thus, it is unclear whether differences exist about associations between cognitive function and different focuses of definitions of periodontal health (active inflammation vs accumulative deficiencies).

Bleeding on probing (BOP) is an essential parameter in the newly proposed EFP/AAP definition of periodontal health to assess the inflammatory activity of periodontium (9). Notably, the previous studies did not take account of BOP when evaluating the association between periodontal disease and cognitive function (13–16). On the other hand, periodontal inflamed surface area (PISA) value was recently proposed to reflect the area of bleeding pocket in mm^2 (17). It is believed to be an accurate method to quantify inflammatory burden posed by periodontal disease (18). PISA is calculated using conventional CAL, probing pocket depth (PPD), and BOP measurements (17). Periodontitis may contribute to neuroinflammation and may exacerbate Alzheimer's disease due to the release of inflammatory mediators into the bloodstream (19). The periodontal infection may increase the risk of neuroinflammation partly via exacerbating the systemic load of pro-inflammatory cytokines and inflammatory factors (20). Among the markers, C-reactive protein (CRP) (21), white blood cell (WBC) count (22), and fibrinogen (23) are all negatively associated with cognitive function. It is unknown whether these markers mediate possible associations of the periodontal inflammatory activity and inflammatory burden with cognitive function. In this study, we aim to analyze the relationship between periodontal inflammation (assessed by BOP and PISA) with cognitive function and to examine whether systemic inflammatory markers mediate these processes.

Method

Study Design and Population

The data used in this study were retrieved from the National Health and Nutrition Examination Survey (NHANES) 2001–2002 database. NHANES is a national cross-sectional study administered by the National Center for Health Statistics (NCHS) to assess the health and nutritional status of the U.S. population-based on a variety of health-related data. The NCHS Ethics Review Board approved all NHANES protocols. Because this study analyzed public-use data, an additional research ethics review of our analysis was not necessary. The NCHS collected data from a stratified, multistage, clustered probability sample of noninstitutionalized civilians. The data were obtained from various sources: the questionnaire, including demographic information, lifestyle, and disease history; physical examination; and laboratory assessments. Details of the NHANES can be obtained at https://www.cdc.gov/nchs/nhanes/index.htm. Our study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting cross-sectional studies. For this study, individuals who were below 60 years, edentulous, and/or had no data available on DSST or periodontal measurements were excluded from our data set. In total, we included 776 respondents in the present study (Figure 1).

Sociodemographic and Health-Related Variables

Sociodemographic variables included age, gender, ethnicity, educational level, and annual household income. Body mass index was calculated using height and weight. Lifestyle variables included smoking status, alcohol intake, and the time since the last dental visit. Systemic biomarkers of inflammation (CRP, WBC count, and fibrinogen) were measured using blood samples taken from the participants. Hyperlipidemia was measured by total cholesterol and high-density lipoprotein cholesterol. A doctor-diagnosed of diabetes or glycohemoglobin of 6.5% or higher was used to define diabetes mellitus. The presence of hypertension was identified as a self-report of a doctor's diagnosis, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. Physician-diagnosed myocardial infarction and coronary heart disease were defined as heart disease. Other doctor-diagnosed conditions included arthritis and stroke. All of these variables were identified as potential confounders that might influence both periodontal inflammation and cognitive function (24).

Periodontal Variables

Trained and calibrated dentists from the NCHS examined the teeth of each participant. Periodontal status was evaluated by examining two randomly chosen quadrants in one person: one maxillary and one mandibular (random half-mouth protocol [RHMP]). The

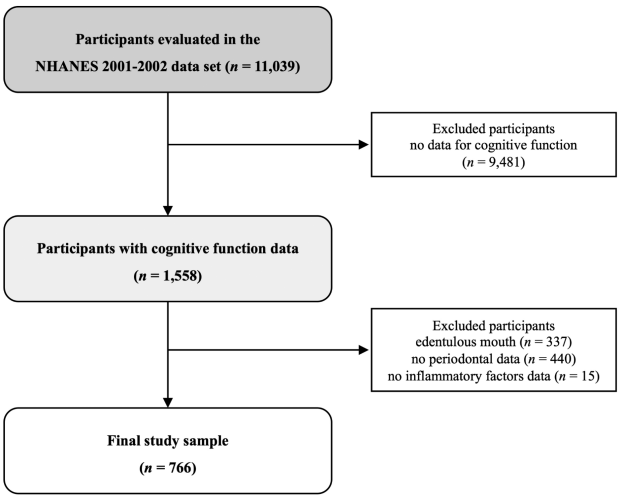

Figure 1. Flow chart indicating the subset of participants included for the analysis from the NHANES 2001–2002 data set.

examination included probing assessments for CAL, PPD, and BOP at three sites per tooth (mesiobuccal, midbuccal, and distobuccal). As third molars were excluded, a maximum of 14 teeth and 42 sites per individual could be examined to assess periodontal health status. Measurements were on the tooth level and after that classified according to the traditional and the newly proposed definitions of periodontal health: (i) the case definition developed by the Centers for Disease Control and Prevention (CDC) in partnership with the AAP (the "CDC/AAP definition") (25,26); (ii) the definition proposed by the world workshop of the EFP and the AAP on the classification of periodontal and peri-implant disease and condition (the "EFP/AAP definition") (9). For details concerning the criteria of these classifications, see Supplementary Table S1. In addition, the PISA value reflects the surface area of bleeding pocket epithelium and was used to quantify the total periodontal inflammatory burden in the patient (17).

Cognitive Function Variables

The DSST is a component of the Wechsler Adult Intelligence Test (27). This test assesses sustained attention, processing speed, and working memory, which is regarded as a cognitive phenotype that reveals the hidden progression of an individual's dementia or cognitive impairment. The DSST measures cognitive function by asking participants to pair digits with their corresponding symbols within 2 minutes. Following the standard scoring method, a score is the total number of correct matches. A higher score represents a superior cognitive function. The measurement has been widely used in large-scale epidemiological studies (28,29).

Statistical Analysis

Normality of distribution of continuous variables was tested by one-sample Kolmogorov–Smirnov test. Continuous variables with normal distribution were reported as mean (standard deviation [*SD*]); non-normal variables were presented as median (interquartile range). Categorical variables were described as number and frequency. Univariable linear regressions were used to evaluate the associations of the covariates with the DSST score. Variables with a significant association ($p < .10$) in the univariable analyses were incorporated into the multivariable analyses. Multivariable linear regressions were used to evaluate the overall association between periodontal health and DSST score adjusting for sociodemographics, health-related behaviors, and systemic diseases.

Multivariable linear regressions were used to investigate the potential mediating effect of systemic inflammatory factors (CRP, WBC count, and fibrinogen) on the association between BOP/PISA (exposure) and DSST (outcome) (30). Three pathways (a, b, and c) and the following criteria were used to establish mediation (Figure 2): Step1, regress "outcome" on "exposure" to test if the "exposure" is significantly associated with "outcome" (total effect). Step2, regress "mediator" on "exposure" to examine if "exposure" is a significant predictor of the "mediator" (path a). If not, then it is unlikely to mediate anything. Step3, regress "outcome" on both "exposure" and "mediator" to test if "mediator" is a significant predictor of "outcome," and to observe whether the association between "exposure" and "outcome" is attenuated when the "mediator" is included (path c, direct effect). The total effect is a sum of a direct effect and a mediation (indirect) effect. The proportion of the mediated effect was calculated using the following formula: $(\beta_{\text{total effect}} - \beta_{\text{direct effect}}) \times 100/\beta_{\text{total effect}}$ (31). Complete

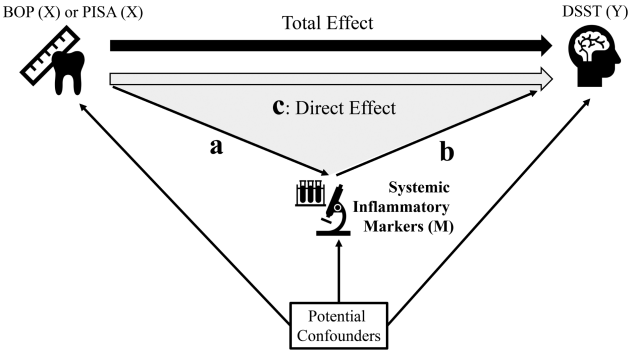

Figure 2. Graphic representation of the relationship between periodontal inflammatory activity/burden and cognitive function, and mediated effects (n = 766). Total effect: the relationship between BOP/PISA (exposure) and DSST score (outcome). Path a: the relationship between BOP/PISA and systemic inflammatory markers (mediator). Path b: the relationship between systemic inflammatory markers (mediator) and DSST score (outcome). Path c: systemic inflammatory markers mediation models of the relationship between BOP/PISA (exposure) and DSST score (outcome). BOP = bleeding on probing; DSST = digit symbol substitution test; PISA = periodontal inflamed surface area; X = exposure variable; Y = outcome variable; M = mediator.

case analysis was used for handling missing data. All statistical analyses were computed in SPSS version 25 (SPSS Inc., Chicago, IL). A value of $p < .05$ was considered significant.

Results

Characteristics of the Study Population

The characteristics of the samples are presented in Table 1 (n = 766). Participants were on average 70.3 (SD = 7.6) years old; 49% were male, and predominantly non-Hispanic White (61.1%). The mean DSST score was 45.2 (SD = 18.1). According to the CDC/AAP, EFP/AAP, and Hugoson's definitions, the prevalences of periodontal health were 56.1%, 59.3%, and 71.3%, respectively.

BOP As a Key Indicator of the Association

Univariable linear regression analyses showed that sociodemographics (age, gender, ethnicity, education, and annual household income), time since the last dental visit, the number of teeth, disease history (diabetes mellitus, stroke, hypertension, and heart disease), alcohol intake, total cholesterol, and high-density lipoprotein cholesterol were significantly associated with the DSST score (Supplementary Table S2). These potential confounders were used to adjust the multivariable regression models. Although the current smoking was not associated with DSST scores (β = –0.76, 95% confidence interval [CI]: –4.52 to 2.99), we still included it into the subsequent models to correct for smoking habit's possible confounding effect on periodontal inflammation.

Multivariable linear regressions showed the individuals with periodontal health defined by EFP/AAP performed higher DSST scores, compared to periodontal patients (β = 4.08, 95% CI: 1.87–6.29; Figure 3). In contrast, there was no association between periodontal health defined by CDC/AAP and DSST score (β = 1.75, 95% CI: –0.52 to 4.02). Nevertheless, CDC/AAP case definition was negatively associated with DSST score in the participants with moderate/severe periodontitis and BOP ≥10% (β = –6.05; 95% CI: –9.39 to –2.71; Supplementary Table S3).

Table 1. Characteristics of the Study Population (n = 766)

Variables	NHANES 2001–2002 (n = 766)
Continuous variables, mean (*SD*)/median (IQR)	
Age (year)	70.3 (7.6)
Body mass index (kg/m^2)	28.1 (5.2)
Systolic blood pressure (mmHg)	138.0 (21.3)
Diastolic blood pressure (mmHg)	69.8 (15.0)
Total cholesterol (mg/dL)	210.7 (39.5)
HDL cholesterol (mg/dL)	54.0 (17.0)
Glycohemoglobin (%)	5.9 (1.1)
C-reactive protein (mg/dL)[a]	0.24 (0.37)
White blood cell count (10^3/μL)	7.2 (2.2)
Fibrinogen (mg/dL)	391.8 (81.3)
DSST	45.2 (18.1)
BOP (%)[a]	2.56 (6.21)
PISA (mm^2)[a]	4.89 (18)
Tooth number[a]	22 (12)
Categorical variables, n (%)	
Male	382 (49.9)
Non-Hispanic White	468 (61.1)
Education ≥ college[b]	355 (46.3)
Annual household income ≥75,000$[b]	120 (15.7)
Current smoker[b]	69 (9.0)
Alcohol intake > 12 drinks/y[b]	471 (61.5)
Arthritis[b]	317 (41.4)
Hypertension[b]	360 (47.0)
Diabetes mellitus[b]	124 (16.2)
Stroke[c]	38 (5.0)
Heart disease	97 (12.7)
Time since the last dental visit > 1 year	242 (31.6)
CDC/AAP definition[c]	430 (56.1)
EFP/AAP definition[d]	454 (59.3)

Note: AAP = American Academy of Periodontology; BOP = bleeding on probing; CDC = Centers for Disease Control and Prevention; DSST = digit symbol substitution test; EFP = European Federation of Periodontology; HDL = high-density lipoprotein; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; PISA = periodontal inflamed surface area; *SD* = standard deviation.

[a]Non-normal distribution continuous variable, median (IQR). [b]Missing values for total study: education (n = 1; <1%), income (n = 72; <9.4%), smoking (n = 2; <1%), alcohol (n = 15; 2%), arthritis (n = 2; <1%), hypertension (n = 3; <1%), diabetes (n = 23; 3%), and stroke (n = 2; <1%). [c]The CDC/AAP case definition identified no/mild periodontitis as indicating periodontal health (26). [d]The EFP/AAP definition of periodontal health (9).

Inflammation As a Potential Mediator

BOP was significantly associated with DSST when controlling for confounders (β = −0.091, 95% CI = −0.174 to −0.008; Table 2). When the WBC count was added to an initial model, the association between BOP and DSST did not remain significant (β = −0.066, 95% CI = −0.148 to 0.016). The proportion mediated through WBC count for the BOP-DSST association was 27.5%. Regarding the burden of periodontal inflammation, PISA was independently associated with DSST score (β = −0.059, 95% CI = −0.087 to −0.031) in the multivariable linear regression (Table 2). When we introduced the WBC count into an initial model, the association between PISA and DSST remained significant (β = −0.047, 95% CI = −0.075 to −0.019). WBC count acted as a partial mediator and mediated 20.3% of the total association. There was no evidence that CRP and fibrinogen mediated the association between BOP/PISA and DSST (Table 2).

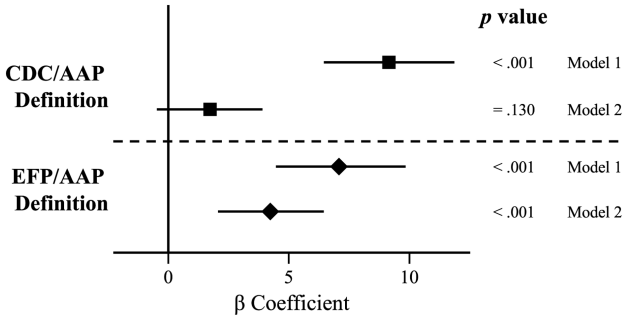

Figure 3. Multivariable linear regression analyses of traditional and new definitions of periodontal health for DSST score (n = 766). Model 1: unadjusted. Model 2: adjusted for sociodemographic variables (age, gender, race, education, and income), health behaviors (smoking habit, alcohol consumption, and dental visit), number of teeth present, and systemic diseases (hypertension, HDL cholesterol, total cholesterol, diabetes mellitus, heart disease, and stroke). Association is deemed to significant if the 95% CI does not include zero. All p values were calculated with a two-sided significance level of .05. AAP = American Academy of Periodontology; CDC = Centers for Disease Control and Prevention; DSST = digit symbol substitution test; EFP = European Federation of Periodontology.

Discussion

The main findings of our study were that individuals considered periodontally healthy based on the EFP/AAP definition exhibited better cognitive performance than those with poorer periodontal health. In the multivariable regression analyses, BOP and PISA as markers of periodontal inflammation were negatively associated with the DSST score. Systemic inflammation (as measured by WBC count) was an explanatory mediator of these associations (27.5% for BOP and 20.3% for PISA). This study shows the role of systemic inflammatory factors in the relationship between periodontal inflammation and cognitive function among the community-dwelling U.S. older adults.

We compared traditional and newly proposed definitions of periodontal health to assess whether inducing gingival bleeding affected the overall association between periodontal health and cognitive function. EFP/AAP definition employed BOP as one of the parameters and remained strongly associated with cognitive performance after controlling all covariables. However, there was no significant association between the CDC/AAP case definition (no/mild vs moderate/severe periodontitis) and DSST scores. Further, moderate/severe periodontitis individuals with BOP ≥10% showed significantly lower DSST scores than no/mild those with BOP <10%. The active inflammation of periodontium might to be more related to cognitive performance compared to the accumulative deficiencies. Although considering the presence of BOP to predict periodontal disease remains controversial, absence of BOP could be regarded as a valid predictor of a stable periodontium (32,33). Based on the inflammatory mechanisms in the pathogenesis of cognitive impairment (4,5), BOP might act as an informative and precise indicator for outcomes due to infectious exposure. Alternatively, we used PISA, incorporating BOP and PPD/CAL, to quantify the inflammatory burden of periodontitis. Our study found that PISA value, as a continuous variable, showed a notable association with DSST, as expected based on previous evidence (34). The periodontal burden measured by the PISA has also been associated with other systemic diseases, including diabetes (35), rheumatoid arthritis (36), decreased kidney function (37), and ischemic stroke (38). In brief, it would be difficult to comprehensively and accurately evaluate the association between periodontitis

Table 2. Mediation Analysis of the Effects of Systemic Inflammatory Marks on the Association of PISA With DSST Score (n = 766)

Coefficient (95% CI)	Path a	Path b	Path c (direct effect)	Total effect	Proportion mediated
BOP (%)					
CRP (mg/dL)	−.001 (−.006 to .008)	−.204 (−1.173 to .765)	−.091 (−.174 to −.008) *	−.091 (−.174 to −.008) *	NA
WBC count (10^3/μL)	.019 (.006 to .032)**	−1.391 (−1.883 to −.900) ***	−.066 (−.148 to .016)	−.091 (−.174 to −.008) *	27.5%
Fibrinogen (mg/dL)	−.121 (−.601 to .360)	.001 (−.012 to .015)	−.090 (−.173 to −.006) *	−.091 (−.174 to −.008) *	NA
PISA (mm^2)					
CRP (mg/dL)	.000 (−.002 to .002)	−.204 (−1.173 to .765)	−.059 (−.087 to −.031) ***	−.059 (−.087 to −.031) ***	NA
WBC count (10^3/μL)	.010 (.005 to .014) ***	−1.391 (−1.883 to −.900) ***	−.047 (−.075 to −.019)**	−.059 (−.087 to −.031) ***	20.3%
Fibrinogen (mg/dL)	.079 (−.083 to .241)	.001 (−.012 to .015)	−.059 (−.087 to −.031) ***	−.059 (−.087 to −.031) ***	NA

Notes: BOP = bleeding on probing; CI = confidence interval; CRP = C-reactive protein; NA = not appropriate; PISA = periodontal inflamed surface area; WBC = white blood cell. Potential mediator explained a mediated proportion of the total effect. All models were adjusted for sociodemographic variables (age, gender, race, education, and income), health behaviors (smoking habit, alcohol consumption, and dental visit), number of teeth present, and systemic diseases (hypertension, HDL cholesterol, total cholesterol, diabetes mellitus, heart disease, and stroke).

*p < .05; **p < .01; ***p < .001.

and cognitive function without assessing an individual's activity and burden of periodontal inflammation.

Mediation analysis suggested that the negative associations between periodontal inflammatory activity/burden and cognitive performance could be explained, at least in part, by the WBC count (27.5% for BOP and 20.3% for PISA). Our study used a mediation model to explore the process that underlies the association between periodontal inflammation (exposure) and cognitive function (outcome) via the inclusion of the presumed "mediator" (systemic inflammatory marker). The results well confirm our initial theoretical framework (ie, inflammatory hypothesis), which expects the decreased cognitive test scores through exposure to systemic inflammatory factors and leads us to explore the possible mechanisms of the association. The neuroinflammation pathogenesis of dementia gives biological plausibility to these mediating effects (39,40). The periodontal infection leads to leakage of various pathogenic products (ie, host inflammatory factors, pathogenic oral bacteria, and its products) through the gingival epithelium into the bloodstream in the active phases of the disease (6). At the same time, the chronic inflammatory process contributes to activates blood–brain barrier permeability, which results in the entry of the systemic inflammatory products and the pathogen into the brain leading to synaptic and cognitive dysfunction (41).

Notably, the inclusion of the WBC count (mediator) made the association between BOP and DSST be not significant, indicating WBC count might act as a full mediator of the association. That might be because the marker of acute pathogenesis of inflammation (BOP) efficiently translates into systemic inflammatory factors (WBC). PISA, the incorporation of chronic disease parameters (ie, PPD/CAL), might reflect a historical and long-lasting process of periodontal inflammation compared to acute inflammation (42). In contrast, we did not detect the effect of CRP and fibrinogen on the relationship between BOP/PISA and DSST scores. It may be due to DSST scores measure psychomotor cognitive performance and reflect the frontal-executive functions. The executive functions are based on the integrity of frontal-subcortical circuits, and all types of vascular lesions result in executive dysfunctions (43). WBC count is independently associated with vascular and nonvascular mortality in the participants aged 85 years (44). These results implied that elevated WBC count related to periodontitis could be involved in the process of vascular damage in the brain. Briefly, increased WBC count might support an infection role of periodontal inflammatory activity/burden in the pathogenesis of cognitive impairment.

Several limitations of the current study should be considered. First, NHANES 2001–2002 employed an RHMP. Compared to full-mouth examination of six sites on all teeth, it might account for underestimating the prevalence and severity of periodontal disease (45,46). Smokers have more sites with PPD ≥ 5 mm than nonsmoker, especially on the lingual surfaces (47). To reduce the bias caused by RHMP, we used a half-reduced CDC/AAP case definition (25). In regard to the extent of BOP, the underestimation was seen in the RHMP with three buccal sites (−11.7%) compared to full-mouth examination in periodontal patients (48). PISA could be underestimated using RHMP correspondingly. In addition, amyloid, tumor necrosis factor, and interleukin would likely have been better alternatives, as they are the cognitive-specific inflammatory factors to explore the causal inflammatory mediates (49). Moreover, we employed a single measure to assess cognition since only the DSST test was available in the NHANES data set. Although the DSST test was able to describe visuospatial abilities and episodic memory decline well, several other cognitive deficits, including perceptual speed, attention, and executive function, should be comprehensively assessed in future research (50). Another limitation is that data on oral hygiene behavior (ie, toothbrushing, interdental cleaning, and use of mouthwash) were not available in NHANES 2001–2002, so the influence of these variables on the associations could not be explored. However, the potential confounding effect of dental services utilization was taken into account by adjusting the regression models for the time since the last dental visit. Finally, the cause–effect relation identified in the current cross-sectional study was ambiguous. When exploring the relationship and mediated effect, we should interpret the association cautiously. Long-term prospective cohort studies and treatment-related randomized control studies are required to reveal the causal association between periodontitis and cognitive disease.

In summary, the cross-sectional association between periodontal inflammatory activity/burden and cognitive function was found to have a mediated effect by WBC count in the U.S. elder population. The results may provide some valuable insights into the impact of oral infection on cognitive dysfunction via a systemically inflammatory pathway. Further longitudinal cohort studies are warranted to explore the pathogenetic mechanism underlying the neuro-inflammatory disease. From a preventive strategic view, maintaining a healthy periodontium may substantially reduce or delay the onset of cognitive impairment. Also, health workers

should be aware of how to make corresponding adjustments to cognition-impaired older adults' dental care patterns to improve their life quality.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

A.L. and Y.C. are Ph.D. students conducting the research in the University Medical Center Groningen with funding from the China Scholarship Council studentship (No. 201708440245 and No. 201706100193). And the authors acknowledge funding support from the University of Groningen.

Conflict of 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. Ethical approval was not required.

Acknowledgments

The authors thank the NHANES staff and investigators. A special thanks to all who participated in the NHANES survey for making this study possible through their participation. The authors would also like to thank the anonymous reviewers and editors for their constructive comments that significantly improved the paper.

Author Contributions

A.L., the first author, was responsible for study conception and design, data analysis and interpretation, statistical analysis, and drafted manuscript; Y.C., a statistical consultant, contributed to the study design, statistical analyzing, and drafted manuscript; L.S. contributed to interpretation and critical revision of the manuscript; A.S. contributed to interpretation and critical revision of the manuscript; G.-H.T., contributed to the study conception and design, data analysis and interpretation, and critical revision of the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work to ensure integrity and accuracy.

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