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Immunity and Cognitive Function Among Elderly People:

A Cross-Sectional Study

ABSTRACT

Objective: Neuro-inflammation has been found to cause impairments in a variety of cognitive domains, including learning, memory, and attention. Neutrophil Lymphocyte Ratio (NLR) is a reliable marker for peripheral inflammation in various diseases. This study aims to explore the role of systemic, peripheral inflammation in the pathogenesis of cognitive decline in the non-demented healthy elderly by using NLR as an inflammatory marker.

Approach and Results: I conducted a cross-sectional study using multivariate linear regression models to relate individual NLR to performance on two cognitive assessment tests among physically and mentally healthy elderly participants from the National Health and Nutrition Examination Survey 2013-2014 dataset. I also built a model that both controls for confounders and examines different socioeconomic and biological factors that are significantly associated with cognitive function in the study population. In multivariate models adjusted for age, gender, race, and smoking status, socioeconomic and biological factors such as education level, income level, and blood pressure were all associated significantly with performance on cognitive tests.

In stratification analysis, NLR is negatively associated with performance on both cognitive tests among the older age group (>70 years old) but is not in the younger age group (60-70 years old), adjusted for all confounders and significant variables of interest.

Conclusion: A significant association between NLR and cognitive ability was only observed among the older age group. NLR may then be used as an index of potential cognitive impairment which will aid in early diagnosis and suitable management of cognitive dysfunction in the very old population.

INTRODUCTION

The central nervous system has been believed to be immunologically independent from the peripheral immune system, due to the blood brain barrier and no clear indication of the existence of a system of lymphatic vessels. Nevertheless, recent discoveries have suggested that microglia--immune cells in the brains--are not the only cells that trigger brain inflammation, and that neutrophils and monocytes also contribute to cerebral inflammation by infiltrating the injured brain (Jeong et al., 2013).

This interaction of the immune system and the nervous system is suggested to play a role in maintaining homeostasis throughout the body through a vast network of communication pathways (Kipnis et al., 2015). The newly acknowledged existence of the lymphatic vessels that also have been shown to change with age is something worth thinking about (Kipnis, et al. 2015).

It has been established that neuro-inflammatory changes may contribute to cognitive changes. In fact, more associations have been discovered between neuro-inflammation and some neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, and mild cognitive impairment (MCI) (Simen et al., 2011). Microglia perform their mission of getting rid of intruders in the brain. However, they can also cause damage in some cases. It is suggested that

activation of microglia is followed by an increased in expression of some pro-inflammatory cytokines (Pourganji et al., 2014).

It has been widely known that peripheral inflammatory cells induce cytokine production, which then communicate with microglia to trigger behavioral changes known as sickness behavior. In the senescent brain, microglial cells are overly sensitive to messages from the peripheral immune system. Therefore, during an infection in old individuals, microglia may overreact to signals from the peripheral immune system and produce excessive levels of pro-inflammatory cytokines, which in turn can cause serious deficits in cognition (Johnson, 2014).

Neutrophil is a type of phagocyte that is first to respond to and defend against (by ingesting foreign entities) microbial infections. Lymphocyte includes T-cells, which attack foreign cells directly, and B-cells, which differentiate into plasma cells that synthesize antibodies. The neutrophil-lymphocyte ratio reflects the balance between innate (neutrophils) and adaptive (lymphocytes) immune responses, as neutrophil elevation and lymphocyte depletion indicate physiological stress (Gokhan et al., 2013). "Compared with White Blood Cell counts, neutrophil-lymphocyte ratio as an emerging biomarker for systemic immune-inflammation index would reflect more precisely the balance between neutrophil and lymphocyte responses and could be better associated with the severity of the inflammatory response" (Curbelo et al. 2017).

Since they are toxic to neurons, neutrophils are only recruited to cleanse foreign microbial intruders in the brain when an infection is suspected (Jeong et al., 2013). However, peripheral inflammation only leads to higher levels of synthesis of pro-inflammatory cytokines within the brain and, potentially, cognitive decline under circumstances that involve the breakdown of the blood-brain barrier, ischemia, or sepsis (Simen et al., 2011). Nevertheless,

whether neutrophils and lymphocytes are still recruited in sound brains in healthy population is still unknown.

Study Objective: On the one hand, there have been studies on the relationship between neutrophil-lymphocyte ratio and cardiovascular diseases or cognitive dysfunction among some diseased population (such as diabetes, coronary artery disease, stroke, cancer, etc.) (Sasirekha). However, diseased populations would also naturally possess higher levels of systemic inflammation than normal. In addition, as a biomarker for systemic inflammation, NLR was found to be positively associated with age in the healthy population. The oldest age group possessed the highest NLR and the youngest age group had the lowest NLR (Li et al., 2015). Although the true physiological root of age-related cognitive decline is not entirely known, partially because as human age, more age-related diseases start developing which complicates the establishment of causality, studies have shown that systemic inflammation, specifically through using cytokines as the marker for systemic inflammation, has been linked to the development of cognitive dysfunction as one ages (Simen et al., 2011). I then aim to determine if NLR as another marker for systemic inflammation would be a predictor of cognitive dysfunction in this population. I hypothesized that there would be an inverse association between the neutrophil-lymphocyte ratio and cognitive ability in my study population. In addition, I aimed to determine the effect of other socioeconomic and behavioral risk factors in predicting cognitive function of people aged 60 and above. This cross-sectional study aims to investigate the effects of immunological activities, specifically inflammation (with NLR serving as proxy), on cognitive function among physically and mentally healthy elderly individuals.

This topic matters because of aging populations is an undeniable trend, which may result in an immense healthcare burden. It is thus important that I take measures to maintain a high life

quality despite health problems that accompany longer longevity, such as the inability to live independently due to cognitive dysfunction. This study can contribute to the epidemiology of aging by providing an understanding of how inflammation may contribute to cognitive function decline among physically and mentally healthy elderly people as Ill as possible directions in coming up with interventions that can be developed to target neutrophil counts in older individuals to combat deterioration of cognitive function that could possibly be linked to diseases such as Alzheimer's.

METHODS

1. Study population

This is a cross sectional study. The data were collected through the National Health and Examination Survey (NHANES) 2013-2014. NHANES is a program that aims to assess the health and nutritional status of adults and children in the United States through studies. The data collection methods involve a combination of interviews and physical examinations. Data were randomly selected from across the US, in a top-down process from groups of counties to county to group of households to individual households and finally individuals within each household.

Cognitive function was assessed in participants aged 60 and above who self-reported having good physical health and no depression at the time of questionnaire administration. The original data set included 1,059 observations, with 307 participants being excluded later due to missing data on covariates of interest. As part of NHANES 2013-2014 data collection protocols, documented consent was obtained from all participants.

2) Variables

1. Main predictor variable

NLR was created using the formula:

Segmented neutrophil number (1000 cells/uL) / Lymphocyte number (1000 cells/uL)

2. Primary outcomes

    a. Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD) assesses immediate and delayed learning ability for new verbal information. There are three learning trials in total in one subtest (each ranging from 0 to 9 or 10). In each trial, participants read aloud 10 unrelated words, one by one as they were presented. Participants then were asked to recall as many words as possible. In each learning trial, the order of the 10 words is changed. I added the three scores up to obtain the total CERAD score, which ranges between 0 and 29.

    b. The Digit Symbol Substitution test (DSST) assesses processing speed, attention, and working memory. The participants were given 9 numbers paired with different symbols and were asked to copy the copy the symbols in 133 empty boxes below that adjoin the numbers. The score is the total number of correct matches, which ranges between 0 and 100.

3. Covariates

Age, gender, race, smoking status were identified as confounders based on previous literature. I picked confounders based on three criteria: the variable was shown to cause/contribute to the cause of (1) the outcome and (2) the main exposure, and (3) that the variable is not in the causal pathway of the exposure and the outcome (i.e. is not caused by the exposure).

Neutrophil-to-lymphocyte ratio was found to be positively associated with age in the healthy population (Li et al., 2015). Additionally, aging is also associated with cognitive changes, regardless mild or serious, explained through structural and functional changes observed in aging brains (Harada et al., 2015). I then included age in the adjusted model as a confounder.

A study by Azab et al. (2014) looked at the average values of NLR across races in US adults using aggregate NHANES data from 2007-2010. They found that average NLR is lower in non-Hispanic Black and Hispanic participants than in non-Hispanic White participants. In addition, a study by Sloan and Wang (2005) investigated race-related disparities in cognitive function among the very old population in the US. They found cognitive function is lower in non-Whites compared to Whites, and that among non-Whites, cognitive function is higher among other races compared to Blacks. Thus, race was included as a confounder.

On average, men were shown to possess higher NLR than women (Lin et al., 2016), and women with mild cognitive impairment were shown to have greater progression rates over time into neurodegenerative diseases such as Alzheimer's than men (Lin et al., 2015). This variable was also adjusted for in the adjusted model.

In terms of smoking, since cigarette smoking was proven to have immunomodulatory effects and possess certain microbial components that trigger inflammation and affect the host responses to foreign entities, and at the same time is associated with lower cognitive ability (Hosseinzadeh, 2016), the variable was also picked as a confounder.

I identified blood pressure as a mediator since it is suggested to be lying in the causal pathway of NLR and cognition: “Inflammation participates in many processes that contribute to the development of elevated blood pressure” (Wu et al., 2014).

No relevant literature could be found on the inter-relations between self-reported income levels as Ill as education levels and NLR and cognitive function.

STATISTICAL ANALYSES

Baseline characteristics for the study sample were presented in Table 1. In the analysis, the continuous variable age was converted into a dichotomous variable of age group 60-70 and >70 years old. The distribution of age was not skewed, and the median (which also equals the median) of 70 was picked as the cut-off point. The race variable in my study has 5 levels: Hispanic (including Mexican Americans and other Hispanics), White, Black, Asian, and Other/Multiracial. The variable smoking was self-reported, based on the question if the participant had smoked more than 100 cigarettes in their life. The variable blood pressure was self-reported and based on the question if the participant had been told they had hypertension. There are two levels: Yes and No. Household annual income was re-grouped into three levels according to definition of income class by US Census Bureau: low (<$35,000), medium ($35,000 - $100,000), and high (>$100,000). Education contains five levels: <9th grade, 9th-12th grade, high school diploma, some college/AA degree, and college degree or higher.

I started off with simple linear regression models to look at the crude associations between cognitive ability (with the two cognitive assessment tests serving as proxies) and peripheral inflammation (NLR as the marker) in the study population. I then proceeded with the minimally-adjusted models, accounting for the identified confounders. Variables that are statistically significantly associated with the outcome variable was then introduced into the minimally-adjusted model through the stepwise selection procedure to come up with expanded exploratory models. F-tests were performed on the full and reduced models after each new

covariate was introduced to confirm the significance of the variables. Confounders were kept in the model despite being statistically insignificant to minimize confounding bias. I also incorporated an appropriate interaction term between age and NLR in the expanded model since NLR has been found to be positively associated with age (Li et al., 2015).

Stratification analysis: After stratifying the original study population by age, multivariate linear regression models adjusting for gender, race, smoking, blood pressure, income level, and education level were re-run among each sample.

Exclusion of outliers: Initial exploration of the main predictor variable NLR (mean = 2.06, SD = 1.6) showed 9 outliers lying at least 3 SDs away from the mean. Therefore, I excluded these outliers to obtain a more normally distributed predictor variable and then perform the same statistical analyses.

Statistical analyses were carried out in R, with P-values <0.05 being considered statistically significant.

RESULTS

The final working dataset ended up having 752 observations across 18 variables (49.6% female). Simple linear regression models (Table 2, models 1) and minimally-adjusted multivariate linear regression models (Table 2, models 2) showed significant crude negative associations and adjusted associations, respectively, between the NLR value and both DSST and CERAD scores within the physically and mentally healthy people aged 60 or older. In the expanded models (Table 2, models 3), there were also significant negative associations between the NLR-age interaction term and performances on the two tests of cognitive function.

Covariates such as gender, race, blood pressure, income, and education level were also found to be statistically significant in explaining the variabilities in the outcome variables.

Stratification analysis models were presented in table 4 (models 7, 8, 9, and 10). As suggested by the statistically significant NLR-age interaction term, there were differences in the effects of NLR on cognitive ability between the younger (N = 404) and older group (N = 348). To be more specific, NLR were found to be significantly negatively correlated with the cognitive test scores among the older group, while not being significantly associated with the same outcome variables in the younger group.

However, after excluding NLR outliers, NLR were no longer significantly associated with the outcome variables (as shown in table 3 - models 4, 5, and 6).

DISCUSSION

The main aim of this study was to find out the relationship between systemic inflammation for which NLR was used as a biomarker and cognitive function in physically and mentally healthy elderly individuals in America in 2013-2014. The following observations were demonstrated by my study. Significant correlations were observed between NLR value and CERAD and DSST scores in the stratified older subgroup (aged 70 or older) of the study population.

Limitations: Since my study is cross-sectional, it is hard to determine the temporal relationship between the main predictor and the outcomes of interest. The study also suffers from a few types of biases. Information bias, specifically misclassification bias, occurred when participants self-reported their self-perceived physical health as Ill as mental health condition. This plays an important role in my study since I utilized these two variables to restrict my study

population. Selection bias occurred when participants did not answer/refused to answer certain questions in the survey or questionnaire, which resulted in missing observations for many variables. Hence, external validity of the study results would suffer. Although there have been previous studies that used the same outcome variables (CERAD and DSST scores) as a proxy for cognitive function, and NLR has emerged as a favorable biomarker for systemic peripheral inflammation, they remain mere proxies and have not been systematically and officially proven to be accurate, effective, and standard measurements. Since they are the main predictor and outcome variables in this study, this might also hurt the internal validity of the study.

CONCLUSION

Through this study, I discovered a significant association between NLR level and performance on cognitive assessments among elderly individuals who were self-perceived to be healthy and non-depressed, adjusted for potential confounders and other socio-economic and behavioral covariates. Furthermore, the effects were significant in the older subset of the study population compared to the younger one. This signifies some relationship between peripheral immunity and cognitive function, and the role aging plays in impacting this relationship. Considering that NLR is potentially a reliable marker for cognitive decline in this study population, there is room for development of new technology that makes use of this index to facilitate early diagnosis or interventions that target neutrophil counts in older individuals to better manage deterioration of cognitive function at its onset in the healthy elderly population.

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Table 1. Sample Demographics (n=752)

Variable	Value*
Women, n (%)	49.6%
Race, n (%)	
Hispanics	13.3%
White	60.1%
Black	16.1%
Asian	9.7%
Other/Multi-Racial	0.8%
BMI, n (%)	
Normal	28.6%
Overlight	39.6%
Obese	31.8%
Income, n (%)	
Low	36.7%
Medium	43.4%
High	19.9%
Education, n (%)	
Less than 9th Grade	4.4%
9th to 11th Grade	8.9%
High School Graduate	22.6%
Some College	32.6%
College Graduate/Above	31.6%
Smoker, n (%)	45.5%
Diabetes, n (%)	17.3%
High Blood Pressure, n (%)	57%

Table 2. Relations of NLR on average cognitive assessment test score outcomes (N = 752)

Cognitive function Outcomes	Crude Model[1]		Adjusted model [2]		Expanded Model [3]	
	B	*P*	B	*P*	B	*P*
ERAD Score	-0.36	<0.001	-0.20	<0.05	-0.39*	0.051
SST Score	-1.00	<0.01	-0.83	<0.05	-1.19*	<0.05

[1] Crude models are simple linear regressions of cognitive function outcomes on main predictor variable NLR

[2] Adjusted models adjusted for confounders age, sex, race, and smoking level

[3] Expanded models adjusted for age, sex, race, smoking level, education level, income level, blood pressure, and the interaction betIen age and NLR

* point estimate of the interaction term betIen age and NLR

Table 3. Relations of NLR on average cognitive assessment test score outcomes (N = 743)

Cognitive function Outcomes	Crude Model[4]		Adjusted model [5]		Expanded Model [6]	
	B	*P*	B	*P*	B	*P*
ERAD Score	-0.38	<0.05	-0.1312	0.41	-0.398*	0.19
SST Score	-0.8209	0.14	-0.6123	0.24	-1.29053*	0.15

[4] Crude models are simple linear regressions of cognitive function outcomes on main predictor variable NLR

[5] Adjusted models adjusted for confounders age, sex, race, and smoking level

[6] Expanded models adjusted for age, sex, race, smoking level, education level, income level, blood pressure, and the interaction betIen age and NLR

* point estimate of the interaction term betIen age and NLR

Table 4. Stratification analysis expanded regression models

VARIABLES	(7) DSST score	(8) CERAD score	(9) DSST Score	(10) CERAD score
NLR	-0.30	-0.05	-1.39***	-0.35**
	(0.47)	(0.15)	(0.41)	(-0.13)
Gender	4.34**	1.34**	4.26**	2.13***
	(1.35)	(0.44)	(1.46)	(0.49)
Race				
White	6.60***	1.44*	7.61*	-0.31
	(1.8658)	(0.61)	(3.07)	(1.05)
Black	-3.10	1.47*	-1.39	0.99
	(2.20)	(0.72)	(3.46)	(1.17)
Asian	3.23	0.09	3.57	-1.77
	(2.52)	(0.83)	(3.91)	(1.33)
Other/Multiracial	13.20	4.10	6.08	0.54
	(13.15)	(4.31)	(6.57)	(2.24)
Smoking	0.36	-0.10	0.19	-0.37
	(1.35)	(0.44)	(1.48)	(0.50)
Education				
9^{th} -11^{th} grade	9.15*	-0.58	16.55***	2.84*
	(4.10)	(1.35)	(4.04)	(1.38)
High school diploma	16.45***	0.62	16.02***	3.07*
	(3.71)	(1.23)	(3.71)	(1.26)
Some college/AA	22.83***	1.53	21.53***	4.02**
	(3.63)	(1.20)	(3.65)	(1.24)
College grad & above	26.51***	2.36	25.05***	5.06***
	(3.67)	(1.25)	(3.65)	(1.27)
Blood Pressure	-0.54	-0.27	-2.99*	-0.65
	(1.33)	(0.44)	(1.46)	(0.50)
Sample Size	N =404	N =404	N = 348	N =348

Standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1
(7) + (8): younger subgroup aged 60-70; (9) + (10): older subgroup aged > 70
Note: Omitted groups: *Race*: Hispanics. *Education*: <9^{th} grade