Associations of blood absolute neutrophil count and cytokines with cognitive function in dementia-free participants: a population-based cohort study

Wenxin Fa [1] MD, Xiaoyan Liang [2] MD, PhD, Keke Liu [1,2,3] PhD, Nan Wang [2] MD, Cuicui Liu [1,2,3] MD, Na Tian [1,2,3] MD, PhD, Min Zhu [1,2,3] MD, PhD, Yixun Ma [1] MD, Lin Song [1,2,3] MD, PhD, Shi Tang [1,2,3] MD, PhD, Lin Cong [1,2,3] MD, PhD, Yongxiang Wang [1,2,3] MD, PhD, Tingting Hou [1,2,3 *] MD, PhD, Yifeng Du [1,2,3 *] MD, PhD, Chengxuan Qiu [2,4] PhD

[1] Department of Neurology, Shandong Provincial Hospital affiliated to Shandong First Medical University, Jinan, Shandong, P.R. China

[2] Department of Neurology, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, P.R. China

[3] Shandong Provincial Clinical Research Center for Neurological Diseases, Jinan, Shandong, P.R. China

[4] Aging Research Center and Center for Alzheimer Research, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet-Stockholm University, Stockholm, Sweden

[*] Correspondence to: Yifeng Du and Tingting Hou, Department of Neurology, Shandong Provincial Hospital affiliated to Shandong First Medical University, No. 324 Jingwuweiqi Road, Jinan, Shandong 250021, P.R. China. Tel./Fax: +86-531-68776354. Email: duyifeng2013@163.com (Yifeng Du); houtingting@sdfmu.edu.cn (Tingting Hou).

Abstract

Background: The relationships of neutrophils and cytokines with cognitive dysfunction are poorly defined. We aimed to investigate the association of peripheral blood absolute neutrophil count (ANC) with cognitive function in older adults and to further explore the mediating role of serum cytokines in this association.

Methods: This population-based cohort study included 1,666 dementia-free participants (age ≥60 years) derived from baseline examinations (March-September 2018) of the Multimodal Intervention to Delay Dementia and Disability in Rural China (MIND-China); of these, 1,087 participants completed follow-up examinations in October-December 2019. We used a neuropsychological test battery to assess episodic memory, verbal fluency, attention, and executive function at the baseline and follow-up examinations. We used Mindray BC-6800 automated hematology analyzer to measure ANC and Meso Scale Discovery to measure serum interleukin-6 (IL-6) and eotaxin-3.

Results: The linear regression analysis of cross-sectional data at baseline (n=1,666) suggested that increased ANC was significantly associated with a lower episodic memory z-score (multivariable-adjusted β coefficient: -0.149, 95% CI: -0.274 to -0.023) and lower long-delayed free recall z-score (-0.216, -0.361 to -0.070). Serum IL-6 and eotaxin-3 could mediate 16.16% to 20.21% and 7.55% to 9.35%, respectively, of these associations. The analysis of longitudinal data (n=1,087) showed a J-shaped relationship of ANC with decline in episodic memory z-score (*p* for nonlinear=0.049), and a U-shaped relationship between ANC and decline in long-delayed free recall z-score (*p* for nonlinear=0.043).

Conclusions: Increased neutrophils are associated with poor cognitive performance and accelerated decline in episodic memory, and the cross-sectional association is partly mediated by serum cytokines.

Keywords: neutrophils; peripheral inflammation; episodic memory; old age; cohort study

Introduction

The homeostatic function of the neuroinflammation deteriorates with the aging process (1-3). Chronic neuroinflammation induced by sustained activation of microglia contributes to synaptic impairment and neuronal death in aging, which may further lead to cognitive impairment (4). However, inflammation in aging is not restricted to the central nervous system (CNS), peripheral inflammatory responses also undergo functional changes that are characterized by increased production of a series of proinflammatory cytokines (5). Following the discovery of the "cross-talk" between peripheral inflammation and neuroinflammation, the relationship between peripheral inflammation and cognition has attracted widespread attention. Robust evidence has emerged that patients with dementia exhibit conspicuous peripheral inflammation (6), and peripheral immunity is associated with dementia (7,8). However, the relationship of peripheral inflammation with cognitive phenotypes in aging is still not well studied. This is important because understanding the role of peripheral inflammation in cognitive aging may facilitate the development of preventive and therapeutic intervention approaches to postponing age-related cognitive decline and the onset of dementia.

Neutrophils, as the essential component of the peripheral innate immune response, are the first responders to tissue damage or pathogens (9). However, neutrophils can increase but display reduced phagocytosis and a slower resolution during the aging process. The increased neutrophils contribute to chronic inflammation through the release of serine proteases and different classes of chemoattractant to activate other immune cells (9). In recent years, several studies have reported the association of

neutrophils with Alzheimer's disease (AD) (7,10,11). In addition, experimental research suggested that depletion of neutrophils in transgenic AD mice could significantly improve memory function (12). However, the potential mechanisms underlying the associations of neutrophils with cognitive dysfunction are still unclear. Peripheral proinflammatory cytokines, such as interleukin-6 (IL-6) and eotaxin-3 secreted directly or indirectly by neutrophils, reflect the inflammatory process in aging and are also linked with cognitive disorders (13-16), which might mediate, at least partly, the association of neutrophils with cognitive impairment in old age.

Thus, we hypothesize that a higher level of neutrophils, as the hallmark of peripheral low-grade inflammation, may be associated with cognitive deficits in older adults and that peripheral cytokines might partly mediate their association. In this population-based cohort study, we sought to test the hypotheses by examining the association between peripheral blood absolute neutrophil count (ANC) and cognitive function among rural-dwelling dementia-free older adults and exploring the potential meditation effects of serum proinflammatory cytokines in the association.

Methods

Study design and participants

This was a population-based cohort study. Study participants were derived from the Multimodal Interventions to Delay Dementia and Disability in Rural China (MIND-China), as previously reported (17,18). In brief, MIND-China engaged individuals who were aged ≥60

years and living in the rural communities (52 villages) of Yanlou Town, Yanggu County, Western Shandong Province, China. At baseline (March-September 2018), 5,765 individuals were examined for MIND-China. Serum cytokines were measured in a subsample of 1,674 dementia-free participants from 18 villages that were randomly selected using the cluster (village)-based random sampling methods from all the 52 villages. Compared with participants not included in the serum subsample (n=3,735), those in the subsample (n=1,674) were slightly younger, more likely to be women, and had higher levels of education and body mass index (BMI) (p<0.05), but the two groups had no significant differences in the distribution of lifestyle and clinical factors, and in the mean cognitive test z-scores (Supplementary Table 1). Of these 1,674 participants, 8 were excluded due to missing at least one of the cognitive tests (n=7) and missing serum IL-6 data (n=1), leaving 1,666 participants for the analysis of cross-sectional associations of ANC and serum cytokines with cognitive performance at baseline (analytical sample 1).

In October-December 2019, baseline participants in MIND-China who were aged 60-79 and free of dementia and functional disability were invited for assessments to recruit participants for interventions according to the design of the MIND-China study; participants who were aged ≥80 years at baseline were not invited for the follow-up assessment because the interventions were supposed to be less effective and the risk of injuries during the interventions due to, for example, falls was too high for the old-old people. Out of the 1,666 baseline participants, 579 were not available for the follow-up assessments due to age ≥80 years (n=32), death prior to the examination (n=15), severe mental illnesses or functional disability (n=72), did not show up at the date and time for follow-up

examination for unknown reasons (n=425) and incomplete follow-up examinations (n=35), leading to 1,087 persons for the analysis of longitudinal association of baseline ANC with cognitive changes over time (analytical sample 2). Fig. 1 shows the flowchart of the study participants.

The protocol of MIND-China was reviewed and approved by the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong First Medical University, Jinan, China. Written informed consent was obtained from the participants, or in the case of cognitively impaired persons, from an informant (usually a family member). Research within MIND-China has been conducted in accordance with the ethical principles for medical research involving human subjects expressed in the Declaration of Helsinki as well as relevant national guidelines and regulations. MIND-China was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

Data collection and definitions

The procedure of baseline data collection and definitions was described in previous reports (18,19). In brief, all data were collected via face-to-face interviews, clinical and neurological examinations, neuropsychological testing, and laboratory tests by trained research staff. The interview was conducted following a structured questionnaire that covered demographic factors (e.g., age, sex, and education), lifestyles (e.g., smoking and alcohol drinking), health history [e.g., hypertension, diabetes, dyslipidemia, coronary heart disease (CHD), and stroke], use of medications, and cognitive function. Arterial blood pressure was measured on the right arm using an electronic sphygmomanometer

(HEM-7127 J, Omron Corporation, Kyoto, Japan) after at least a 5-min rest. Height and weight were measured in light clothes without shoes. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared (kg/m^2). Educational achievement was classified into no formal schooling, primary school, and middle school or above. Smoking and alcohol consumption were dichotomized as current or not current smoking or drinking alcohol. Hypertension was defined as systolic pressure ≥140 mmHg or diastolic pressure ≥90 mmHg or current use of blood pressure-lowering medication; diabetes as self-reported history of diabetes made by a physician or fasting blood glucose ≥7.0 mmol/L or current use of blood glucose-lowering medication; dyslipidemia as total cholesterol ≥6.2 mmol/L or triglycerides ≥2.3 mmol/L or low-density lipoprotein cholesterol ≥4.1 mmol/L or high-density lipoprotein cholesterol <1.0 mmol/L or having received drug treatment for dyslipidemia. CHD was ascertained according to self-reported history of CHD or electrocardiogram examination, including angina, myocardial infarction, coronary angioplasty, and coronary artery bypass grafting. Stroke was defined according to self-reported history and neurological examination. Apolipoprotein E (*APOE*) genotypes were determined using the multiple polymerase chain reaction through MultipSeqCustom Panel (iGeneTech, Beijing, China) following the manufacturer's recommendations and *Sanger* sequencing (20). The *APOE* genotype was dichotomized into carriers vs. non-carriers of the ε4 allele. Dementia was clinically diagnosed following the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria, in which a 3-step diagnostic procedure was followed, as fully described in the previous studies (17-19).

Assessments of cognitive function

Cognitive function was assessed both at baseline and the follow-up examinations by trained research staff via in-person interviews using a neuropsychological test battery that has been validated among Chinese rural adults (21,22). In brief, we assessed function of four specific cognitive domains: episodic memory (Auditory Verbal Learning Test [AVLT]-immediate recall, long-delayed free recall, and long-delayed recognition), verbal fluency (Verbal Fluency Test [VFT]-categories of animals, fruits, and vegetables), attention (Digit Span Test [DST]-forward and Trail Making Test [TMT] A), and executive function (DST-backward and TMT B) (17). The raw test score for each of cognitive tests in certain cognitive domain was standardized into z score, and then the composite z score for the cognitive domain was calculated by averaging the z scores of the tests for that domain. The cognitive z scores at the follow-up were computed by subtracting the mean of cognitive test scores at baseline from cognitive test scores at the follow-up, and then dividing by the standard deviation (SD) of the baseline cognitive test scores, as previously described (23). Then, the changes of cognitive z-score from baseline to the follow-up period were defined as the difference between baseline and follow-up cognitive z scores (i.e., baseline cognitive z-score – follow-up cognitive z score).

Measurements of peripheral blood absolute neutrophil count (ANC)

After an overnight fast, peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA)-coated tubes. The ANC ($\times 10^9$/L) was assayed with

a Mindray BC-6800 automated hematology analyzer (Mindray Medical International Ltd., Shenzhen, Guangdong, China) in the clinical laboratory of Yanlou Town Hospital.

Measurements of serum cytokines

The serum samples were stored at −80 °C and thawed immediately before quantitative analysis. Serum IL-6 and eotaxin-3 were quantified using commercial kits from Meso Scale Discovery (MSD V-PLEX® Assays) according to the manufacturer's protocol and instructions. Every assay plate had two pooled sample controls to monitor the intra- and inter-plate coefficients of variation (CVs). Intra-class coefficients or inter-class coefficients of variations were all below 15%.

Statistical analysis

Baseline characteristics of study participants were compared using the chi-square test for categorical variables and the Mann-Whitney U test or Student's t-test for continuous variables. ANC and serum cytokines were analyzed both as continuous (ln-transformed) and categorical (quartiles) variables. General linear regression models were used to estimate the β coefficient and 95% confidence interval (CI) of the baseline cognitive z-score associated with ANC or serum cytokines. We reported the main results from two models: Model 1 was controlled for age, sex, and education, and Model 2 was additionally controlled for BMI, alcohol drinking, smoking, diabetes, hyperlipidemia, hypertension, CHD, stroke, and *APOE* genotype. We also evaluated the mediating effects of serum cytokines IL-6 and eotaxin-3 on the across-sectional association between ANC and cognition after adjusting for all covariates. We used bootstrapping method (based on

10,000 resamples) to estimate the corresponding 95% CI. The mediation effect was considered statistically significant if the bootstrap 95% CI of β-coefficient did not include zero. In the longitudinal sample, we used restricted cubic spline regression to assess the nonlinear relationship between ANC and decline in cognitive z-score. If nonlinear relationship was suggested, ANC was analyzed as quartiles in Model 1 and Model 2 with additionally adjusting duration of follow-up. All analyses were performed using Stata Statistical Software, Release 14 (Stata Corp., College Station, TX, USA) and R packages (i.e., "mediation", "rms", and "ggplot2") for Windows (version 4.1.0, R Core Team, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org). Two-tailed $p<0.05$ was considered statistically significant.

Results

Baseline characteristics of study participants (n=1,666)

Of the 1,666 participants, the mean age at baseline was 69.63 (SD, 4.63) years, 58.58% were women, and 35.71% had no formal schooling. Out of these, 1,087 (65.24% of baseline participants) undertook the follow-up examination in October-December 2019, with the mean follow-up time being 1.45 years (SD, 0.16). Compared to subjects who did not participate in the follow-up examination, those who did were younger, more likely to be women, had a higher z-score of episodic memory, verbal fluency, attention, and executive function, and a lower level of serum IL-6 ($p<0.05$). The two groups did not differ significantly in the distribution of lifestyles (smoking and alcohol drinking), health

history (hypertension, diabetes, dyslipidemia, CHD, and stroke), *APOE* genotype, ANC, or serum eotaxin-3 concentration (Table 1).

Cross-sectional associations of ANC with cognitive function at baseline (n=1,666)

As a continuous variable, a higher ANC was significantly associated with a lower episodic memory z-score in both the demographic-adjusted and multivariable-adjusted models (Table 2). When the ANC was analyzed as quartiles, compared to the first quartile, the fourth quartile was significantly associated with lower episodic memory z-score, even in the multivariable-adjusted model (Table 2).

We further investigated the associations of ANC with three episodic memory-associated tests (i.e., immediate recall, long-delayed free recall, and long-delayed recognition). In the demographic-adjusted model, a higher ANC was significantly associated with lower z-scores of immediate recall and long-delayed free recall, and the associations remained statistically significant even in the fully-adjusted model (Table 2). There was no significant association of ANC with the z-score of long-delayed recognition test.

There was no significant cross-sectional association of ANC with the z-score of verbal fluency, attention, or executive function test (Supplementary Table 2).

Mediation of serum IL-6 and eotaxin-3 in the associations of episodic memory with ANC at baseline (n=1,655)

The correlation coefficients of ANC with serum IL-6 and eotaxin-3 were 0.234 and 0.139 (both $p<0.001$), respectively (Supplementary Figure 1). ANC was also significantly associated with serum IL-6 (demographic-adjusted β coefficient=0.573, 95% CI: 0.458 – 0.688, $p<0.001$; multivariable-adjusted β coefficient=0.547, 95% CI: 0.430 – 0.664, $p<0.001$) and eotaxin-3 (0.229, 0.115 – 0.343, $p<0.001$; 0.219, 0.103 – 0.334, $p<0.001$). In addition, higher serum IL-6 and eotaxin-3 concentrations were significantly associated with lower z-scores of episodic memory and long-delayed free recall tests (Supplementary Table 3).

The mediation analysis suggested that the association between ANC and episodic memory z-score was partially mediated by serum IL-6 and eotaxin-3, with the proportion of mediation being 20.21% and 9.35%, respectively (Fig. 2). Similarly, the mediating effects of serum IL-6 and eotaxin-3 on the association of ANC with long-delayed free recall were statistically significant, with the corresponding proportion of mediating effect being 16.16% and 7.55%, respectively (Fig. 2).

Longitudinal associations of baseline ANC with cognitive decline (n=1,078)

The restricted cubic spline regression analysis suggested a J-shaped relationship between ANC and change in episodic memory z-score (p for nonlinear=0.049) and a U-shaped association between ANC and change in long-delayed free recall z-score (p for nonlinear=0.043, Supplementary Figure 2). Thus, we analyzed ANC as quartiles using the

third quartile as the reference group. The analysis suggested that in the demographic-adjusted model, the first quartile of ANC (vs. the third quartile) was significantly associated with a faster decline in the long-delayed free recall z-score and that the second and fourth quartiles of ANC were significantly associated with the accelerated decline in both episodic memory z-score and long-delayed free recall z-score (Table 3). In addition, the mediation analysis of longitudinal associations suggested that the serum IL-6 and eotaxin-3 did not show significant mediation in the association of ANC with changes of episodic memory z-score and long-delayed free recall z-score (p>0.05) (data not shown).

Discussion

In this population-based cohort study of rural-dwelling dementia-free older adults in China, we found a strong cross-sectional association between high ANC and low performance in episodic memory test, especially long-delayed free recall test, independent of sociodemographic, behavioral, metabolic, clinical characteristics, and *APOE* genotype. Serum IL-6 and eotaxin-3 could partly mediate these associations. In addition, we found a U-shaped association between ANC and decline in long-delayed free recall test score such that both higher and lower ANC were associated with an accelerated decline in episodic memory function. Taken together, these results suggest that neutrophils might be implicated in cognitive aging and that ANC could be a marker of accelerated memory decline.

The potential associations of neutrophils with cognitive impairment and decline have been reported previously (11,24). Cross-sectional data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) showed that an elevated ANC was associated with poor performance in global, memory, and executive function (25), which is in line with findings from our population-based cross-sectional study. In addition, the analysis of longitudinal data in our cohort revealed a nonlinear relationship between ANC and episodic memory decline (J-shaped), especially long-delayed free recall (U-shaped). The discrepancies in the results between cross-sectional and longitudinal associations were not fully understood. Previous studies have suggested that the immune homeostasis is beneficial for maintaining cognitive health (1,2). Thus, hypoactive or hyperactive peripheral immunity reaction might be deleterious for age-related cognitive decline in older adults.

Elevated serum proinflammatory cytokines are the hallmarks of peripheral inflammation. Serum IL-6, a frequently studied cytokine, has been associated with cognitive impairment in population-based studies (26,27). We found an association of high serum IL-6 concentration with poor memory function, independent of demographic, lifestyle, and clinical factors. This is consistent with other well-designed population-based studies. For instance, the Sacramento Area Latino Study of Aging (SALSA) study suggested that poor cognitive performance was linked to higher serum IL-6 concentration (26). In addition, the population-based Epidemiology of Hearing Loss Study of middle-aged and older adults in Beaver Dam, Wisconsin, USA also showed that elevated serum IL-6 was related to a greater risk of cognitive impairment (28). By contrast, the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) in patients with pre-existing vascular

disease or at increased risk showed that increased levels of serum IL-6 were associated with poor performance in executive function but not in memory function (29). These discrepant findings might be partly attributed to diverse features of the study populations and the different methods used to assess cognitive function across studies. We further found that higher serum eotaxin-3 was related to lower performance in memory function. Eotaxin-3, a C-C motif chemokine, is mainly expressed by tissue-resident macrophages and epithelial cells (30). Although serum eotaxin-3 is involved in the inflammatory response, its potential association with cognitive function in old age has not been well explored. Previously, clinical-based studies found that eotaxin-3 in the brain was increased in non-demented older individuals with cortical amyloid-beta neuropathology than those without (31), and that increased CSF eotaxin-3 was associated with accelerated progression of AD (32). Our population-based study extended these previous findings from the clinical-based studies by showing that a higher level of eotaxin-3 was associated with worse cognitive performance among rural-dwelling older adults who were free of dementia and functional disability.

Our study further revealed that serum cytokines (i.e., IL-6 and eotaxin-3) could partly mediate the association of higher ANC with cognitive deficits, which represents an important contribution of our study to the current literature. Elevated peripheral IL-6 secreted by neutrophils could cross the blood brain barrier and activate microglia in CNS (33). Then, the activated microglia could produce proinflammatory cytokines such as IL-6, interleukin-1beta (IL-1β), and tumor necrosis factor alpha (TNF-α) (33). These cytokines in turn modulate the peripheral immune system and help recruit more neutrophils from the

peripheral blood to CNS, thereby forming a vicious positive feedback loop to exaggerate neuroinflammation and lead to cognitive impairment (12,33). In addition, neutrophils can also indirectly adhere to capillaries to obstruct cerebral blood flow, which is known to be involved in cognitive dysfunction (34,35).

Our population-based cohort study engaged rural-dwelling dementia-free older adults, a demographic group that has been rarely studied in research of cognitive aging. Our study also has limitations. First, the relatively short follow-up period does not allow us to assess the long-term association of ANC with cognitive function. Future long-term follow-up data collected at multiple time-points will enable us to better define the temporal associations of neutrophil counts with cognitive consequences. Furthermore, participants in the analytical sample were relatively younger and healthier than those who were lost to follow-up, which might result in an underestimation of the true association between high neutrophils and poor cognition. Third, our study involved hypothesis testing with multiple outcomes, which could increase the probability of type I error. Future large-scale prospective cohort studies are warranted to replicate the findings in different populations. Finally, our study sample was derived from the rural areas in western Shandong province where a considerable proportion of people had no or very limited education and relatively poor socioeconomic status, which should be kept in mind when generalizing our findings to other populations, even rural populations in China.

Conclusion

In conclusion, our cohort study supports the associations of an increased ANC with poor cognitive performance and accelerated decline in episodic memory among rural-dwelling Chinese older adults and further reveals a partial mediation effect of serum cytokines on the cross-sectional association between high ANC and poor memory function. Future long-term prospective cohort studies are warranted to clarify the longitudinal associations of neutrophils with subsequent function of multiple cognitive domains in different populations as well as the potential role of inflammatory pathways in the association.

Abbreviations

ANC: absolute neutrophil count; IL: interleukin; MSD: Meso Scale Discovery; CNS: central nervous system; AD: Alzheimer's disease; MIND-China: Multimodal Interventions to Delay Dementia and Disability in Rural China; BMI: body mass index; CHD: coronary heart disease; *APOE*: Apolipoprotein E gene; SD: standard deviation; CI: confidence interval.

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Authors' contributors

W.F., T.H., Y.D., and C.Q. designed the study. W.F., X.L., K.L., N.W., C.L., N.T., M.Z., Y.M., L.S., S.T., C.L., and Y.W. contributed to data collection and assessment. W.F. analyzed the data and drafted the manuscript. T.H. and C.Q. contributed to manuscript preparation and editing. T.H., Y.D., and C.Q. supervised this study. All authors made critical comments on the manuscript and approved the final version of the manuscript.

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Declarations of interest

None.

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Table 1. Baseline characteristics of the study participants

Characteristics[a]	Total sample (n=1,666)	Follow-up		
		Yes (n=1,087)	No (n=579)	*P*-value
Age, years	69.63 (4.63)	69.45 (4.13)	69.97 (5.42)	0.03
Women, n (%)	976 (58.58)	656 (60.35)	320 (55.27)	0.04
Education, n (%)				
No formal schooling	595 (35.71)	395 (36.34)	200 (34.54)	
Primary school	761 (45.68)	490 (45.08)	271 (46.80)	0.75
Middle school or above	310 (18.61)	202 (18.58)	108 (18.65)	
Body mass index, kg/m^2	25.09 (3.58)	25.03 (3.47)	25.20 (3.79)	0.37
Current smoking, n (%)	337 (20.23)	214 (19.69)	123 (21.24)	0.45
Current drinking, n (%)	509 (30.55)	332 (30.54)	177 (30.57)	0.99
Diabetes, n (%)	248 (14.89)	166 (15.27)	82 (14.16)	0.55
Hyperlipoidemia, n (%)	398 (23.89)	255 (23.46)	143 (24.70)	0.57
Hypertension, n (%)	1133 (68.63)	744 (69.02)	389 (67.89)	0.64
Coronary heart disease, n (%)	323 (19.39)	214 (19.69)	109 (18.83)	0.67
Stroke, n (%)	229 (13.75)	137 (12.60)	92 (15.89)	0.06

APOE ε4 carrier, n (%)	244 (14.87)	165 (15.36)	79 (13.93)	0.44
Absolute neutrophil count, 10^9/L	3.69 (1.36)	3.68 (1.42)	3.70 (1.23)	0.47
Episodic memory z-score	-0.02 (0.89)	0.10 (0.81)	-0.26 (0.99)	<0.001
Verbal fluency z-score	-0.001 (0.81)	0.08 (0.76)	-0.15 (0.87)	<0.001
Attention z-score	-0.04 (0.85)	0.005 (0.83)	-0.13 (0.88)	0.003
Executive function z-score	-0.10 (0.92)	-0.05 (0.87)	-0.19 (1.00)	0.003
Interleukin-6, pg/ml	2.75 (48.07)	1.53 (5.31)	5.06 (81.21)	<0.001
eotaxin-3, pg/ml	17.78 (138.42)	19.01 (155.57)	15.48 (98.53)	0.09

Notes: Data are mean (standard deviation), unless otherwise specified.

[a] The numbers of subjects with missing values are 9 for body mass index, 15 for hypertension, and 25 for *APOE* genotype. In subsequent analyses, categorical variables with missing values were replaced with a dummy variable, and continuous variables with missing values were replaced with the mean value.

Table 2. Cross-sectional associations of absolute neutrophil count with episodic memory function at baseline

Absolute neutrophil count (ANC, 10^9/L)	No. of subjects	β coefficient (95% confidence interval)[a], cognitive z-score	
		Model 1[b]	Model 2[b]
Episodic memory z-score (n=1,655)			
ANC (ln), continuous	1,655	-0.149 (-0.274, -0.023)*	-0.144 (-0.271, -0.017)*
ANC (quartiles)			
Q1 (<3.0)	478	0.000 (reference)	0.000 (reference)
Q2 (3.0 - 3.5)	393	0.019 (-0.094, 0.131)	0.026 (-0.086, 0.139)
Q3 (3.6 - 4.3)	395	-0.042 (-0.155, 0.070)	-0.041 (-0.154, 0.072)
Q4 (>4.3)	389	-0.140 (-0.254, -0.026)*	-0.138 (-0.253, -0.022)*
p for linear trend		0.012	0.014
Immediate recall z-score (n=1,655)			
ANC (ln), continuous	1,655	-0.114 (-0.256, 0.028)	-0.115 (-0.258, 0.029)
ANC (quartiles)			
Q1 (<3.0)	478	0.000 (reference)	0.000 (reference)

Q2 (3.0 - 3.5)	393	0.085 (-0.042, 0.212)	0.089 (-0.038, 0.216)
Q3 (3.6 - 4.3)	395	0.022 (-0.105, 0.149)	0.025 (-0.102, 0.152)
Q4 (>4.3)	389	-0.135 (-0.264, -0.006)*	-0.140 (-0.270, -0.010)*
p for linear trend		0.040	0.036
Long-delayed free recall z-score (n=1,602)			
ANC (ln), continuous	1,602	-0.216 (-0.361, -0.070)†	-0.215 (-0.363, -0.067)†
ANC (quartiles)			
Q1 (<3.0)	462	0.000 (reference)	0.000 (reference)
Q2 (3.0 - 3.5)	379	-0.074 (-0.204, 0.057)	-0.069 (-0.200, 0.061)
Q3 (3.6 - 4.3)	387	-0.150 (-0.280, -0.020)*	-0.154 (-0.284, -0.024)*
Q4 (>4.3)	374	-0.191 (-0.323, -0.059)†	-0.195 (-0.329, -0.061)†
p for linear trend		0.002	0.002
Long-delayed recognition z-score (n=1,608)			
ANC (ln), continuous	1,608	-0.070 (-0.215, 0.076)	-0.052 (-0.199, 0.095)
ANC (quartiles)			
Q1 (<3.0)	464	0.000 (reference)	0.000 (reference)
Q2 (3.0 - 3.5)	385	0.062 (-0.068, 0.193)	0.078 (-0.052, 0.209)

Q3 (3.6 - 4.3)	381	-0.001 (-0.132, 0.130)	0.006 (-0.124, 0.137)
Q4 (>4.3)	378	-0.041 (-0.173, 0.091)	-0.024 (-0.158, 0.109)
p for linear trend		0.432	0.567

Notes: [a] β coefficient represents the average increase (positive values) or decrease (negative values) in cognitive z-score in comparison with the reference group.

[b] Model 1 was adjusted for age, sex, and education; and in Model 2, additional adjustment was made for body mass index, smoking, alcohol consumption, diabetes, hyperlipidemia, hypertension, coronary heart disease, stroke, and *APOE* genotype.

[*] $p<0.05$; [†] $p<0.01$.

Table 3. Longitudinal associations of baseline absolute neutrophil count with episodic memory decline during the follow-up period

Absolute neutrophil count (quartiles, 10^9/L)	No. of subjects	β coefficient (95% confidence interval)[a], change of cognitive z-score	
		Model 1[b]	Model 2[b]
Episodic memory z-score (n=1,078)			
Q1 (<3.0)	316	0.070 (-0.058, 0.198)	0.044 (-0.067, 0.154)
Q2 (3.0 - 3.5)	252	0.199 (0.064, 0.334)†	0.138 (0.022, 0.254)*
Q3 (3.6 - 4.3)	271	0.000 (reference)	0.000 (reference)
Q4 (>4.3)	239	0.145 (0.008, 0.282)*	0.150 (0.032, 0.268)*
Long-delayed free recall z-score (n=1,032)			
Q1 (<3.0)	301	0.236 (0.070, 0.402)†	0.138 (0.001, 0.276)*
Q2 (3.0 - 3.5)	240	0.201 (0.027, 0.375)*	0.108 (-0.037, 0.252)
Q3 (3.6 - 4.3)	266	0.000 (reference)	0.000 (reference)
Q4 (>4.3)	225	0.274 (0.096, 0.452)†	0.225 (0.078, 0.371)†

Notes: [a] β coefficient represents the change in cognitive z-score during the follow-up period in comparison with the reference group. The positive value of β coefficient indicates a greater rate of decline in cognitive z-score.

[b] Model 1 was adjusted for age, sex, education, and duration of follow-up and Model 2 was additionally adjusted for body mass index, smoking, alcohol consumption, diabetes, hyperlipoidemia, hypertension, coronary heart disease, stroke, *APOE* genotype, and baseline cognitive z-score.

[*] $p<0.05$; [†] $p<0.01$.

Figure legends

Fig. 1 Flowchart of the study participants

Notes: Abbreviation. MIND-China, Multimodal Interventions to Delay Dementia and Disability in Rural China; IL, Interleukin.

Fig. 2 Mediations of serum cytokines in the cross-sectional associations at baseline

Notes: A. Association of absolute neutrophil count with episodic memory z-score mediated by serum interleukin-6 and eotaxin-3; B. Association of absolute neutrophil count with long-delayed free recall z-score mediated by serum interleukin-6 and eotaxin-3. The β coefficients (95% confidence intervals) in all pathways were adjusted for age, sex, education, body mass index, smoking, alcohol consumption, diabetes, hyperlipidemia, hypertension, coronary heart disease, stroke, and *APOE* genotype.

$^{*}p<0.05$; $^{\dagger}p<0.01$.

Figure 1

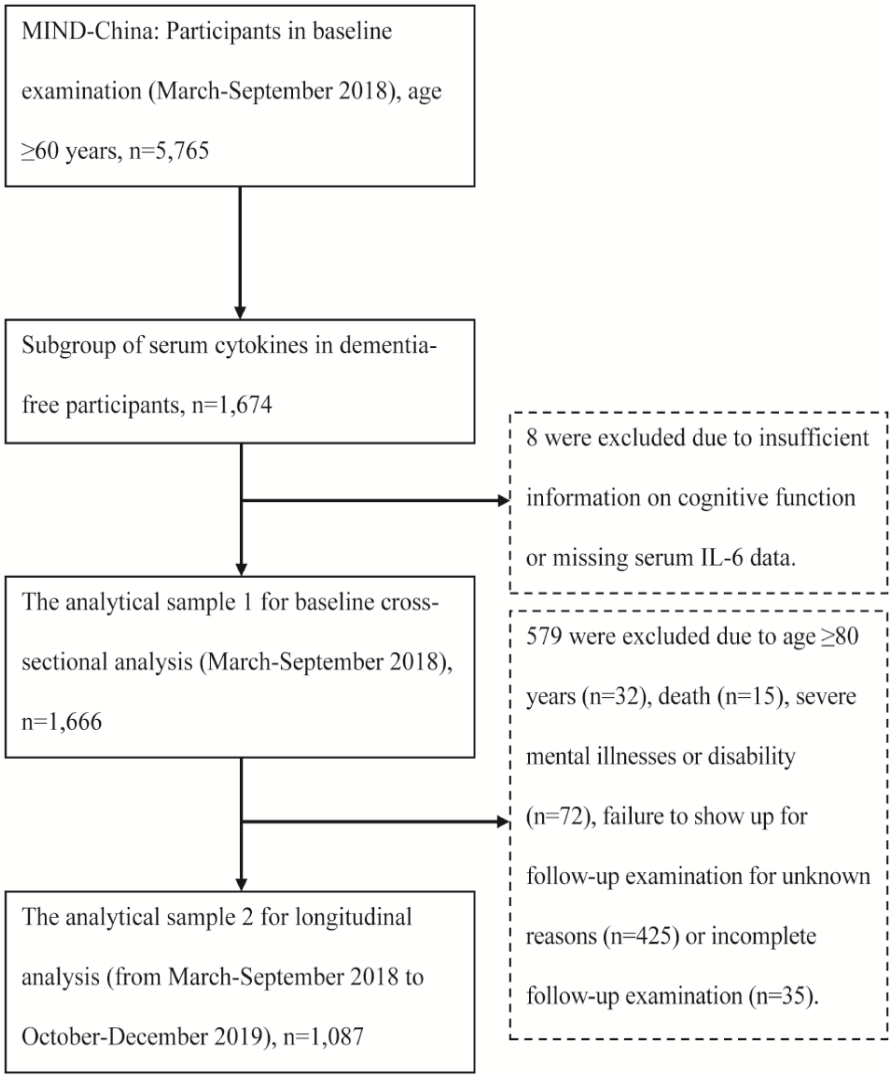

Figure 2A

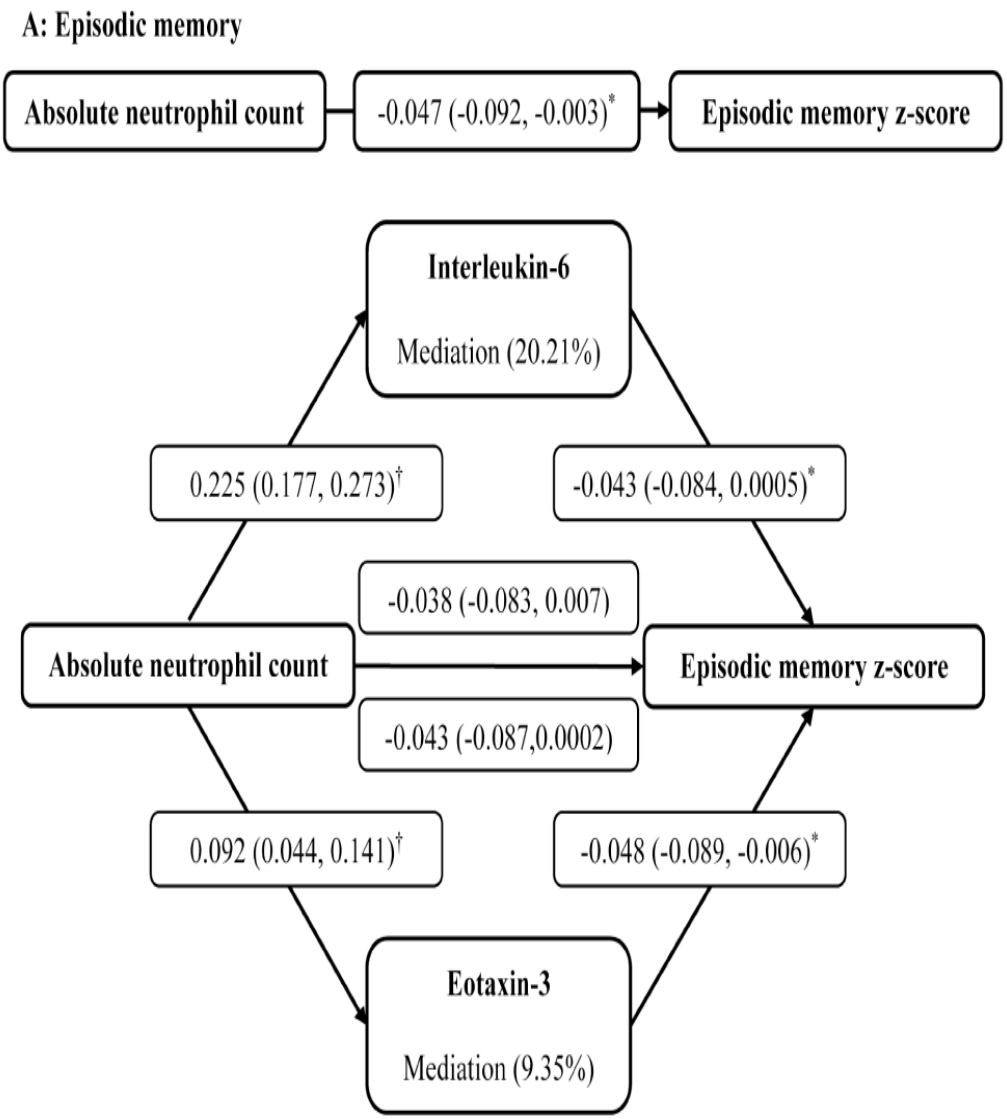

Figure 2B