Chapter 6

Interaction of atherosclerosis and inflammation in elderly subjects with poor cognitive function: The Leiden 85-plus Study

E. van Exel (1); A.J.M de Craen (1,2); E.J. Remarque (1); J. Gussekloo (1); P. Houx (3); A. Bootsma-van der Wiel (1); Marijke Frölich (4); P.W Macfarlane (5) ; G.J. Blauw (1) ; R.G.J. Westendorp (1).

Gerontology and Geriatrics, Department of General Internal Medicine (1), Clinical Epidemiology, Leiden University Medical Center (2), University Department of Psychiatry and Neuropsychology, University of Limburg (3), Clinical Chemistry (4), Leiden University Medical Center, the Netherlands and University Department of Medical Cardiology, Royal Infirmary, Glasgow, Scotland (5).

Submitted

ABSTRACT

Background Accumulating evidence suggest that atherosclerosis leads to (sub)clinical ischaemic lesions in the brain contributing to poor cognitive function and dementia in old age. Experimental evidence indicates that the size of ischaemic lesions is enhanced by a pro-inflammatory cytokine response. We hypothesise that the combination of atherosclerosis and a pro-inflammatory cytokine response predisposes to poor cognitive function and dementia.

Methods All 85-year-old inhabitants of Leiden (n=599) were visited at their place of residence (response rate 87%). Cognitive speed and memory were determined with four neuro-psychological tests. Innate production of the pro-inflammatory cytokine Tumor Necrosis Factor-α and the anti-inflammatory cytokine Interleukin-10 was assessed in a whole blood assay using lipopolysaccharide as a stimulus. We determined the presence of myocardial infarction or myocardial ischaemia using the electrocardiogram, as a proxy for detecting atherosclerosis.

Results In all subjects the risk of poor cognitive speed and poor memory gradually increased two to threefold over the strata representing an increasing pro-inflammatory cytokine response (all $p<0.01$). In subjects with atherosclerosis, the risk of poor cognitive speed and poor memory, gradually increased four to sixfold over the strata representing an increasing pro-inflammatory cytokine response (all $p<0.01$). In subjects without atherosclerosis, there was only a two fold increase in risk of poor cognitive speed ($p=0.02$) and there was no increase in risk of poor memory ($p=0.5$).

Conclusion Our findings support the hypothesis that a combination of atherosclerosis and a pro-inflammatory cytokine response is associated with poor cognitive function and dementia in the population at large.

Introduction

The difference between Alzheimer's disease and vascular dementia gradually disappears [1], i.e. it has become apparent that signs and symptoms of Alzheimer's disease and vascular dementia overlap. The debate on the potential mechanism of Alzheimer's disease no longer focuses exclusively on the role of beta-amyloid or tau-protein. It is becoming increasingly clear that generalised atherosclerosis contributes to susceptibility to cerebrovascular disease [2] and dementia [3]. Several lines of evidence support this view. Autopsy studies have shown that patients with dementia, Alzheimer's disease included, have significantly more cerebrovascular disease than expected on the basis of age and sex [1,4]. It has also been shown that generalised atherosclerosis strongly interacts with risk factors for Alzheimer's disease such as Apo-E4 [5], the effect of both being stronger than the sum of the two. Moreover patients with generalised atherosclerosis have a tripled risk of developing stroke [2], and patients who suffered from stroke have an increased risk of developing Alzheimer's disease[6]. Finally, cardiovascular risk factors are associated with a higher risk of dementia [7], whereas treatment of cardiovascular risk factors is associated with lower risk of dementia in both observational and experimental studies. Treatment of hypertension in old age has been shown to result in fewer cases of dementia compared to untreated individuals [8], while the use of statins has been shown to protect against dementia in two case-control studies [9,10].

The link between Alzheimer's disease and atherosclerosis may lie in an inflammation mediated neurodegenerative process [11,12]. The inflammation observed in Alzheimer's disease is caused by the innate immune system, with little or no involvement of adaptive immunity thus it has been coined autotoxicity [11]. Markers of inflammation in and around amyloid plaques are the upregulation of adhesion molecules and pro-inflammatory cytokines in microglia cells, as well as deposits of complement and C-reactive protein [13-15]. In line with these findings we have previously shown that patients with Alzheimer's disease exhibit a pro-inflammatory cytokine response upon stimulation in whole blood samples [16] Various studies have shown that the use of non-steroidal anti-inflammatory drugs protects against dementia [11,17]. Finally, experimental animal models investigating the effect of ischaemia and pro-inflammatory cytokines on stroke lesion revealed that blocking of pro-inflammatory cytokines after ligation of the mid-cerebral artery, markedly reduce the lesion size and improve neurologic outcome [18]

In this paper, we propose the hypothesis that ischaemia in the brain due to generalised atherosclerosis triggers an inflammatory reaction that contributes to a neurodegenerative process, poor cognitive function and dementia. Therefore, in the Leiden 85-plus Study, we have analysed the relation between an innate pro-inflammatory cytokine response and poor cognitive function in elderly individuals with and without atherosclerosis.

Methods

Subjects

The Leiden 85-plus Study is a population-based study of inhabitants of Leiden, the Netherlands. Since 1997, all members of the 1912 to 1914-birth cohort were enrolled in the month of their 85th birthday. There were no selection criteria on health or demographic characteristics. Those who were eligible for the study were informed about the study by mail. They were then contacted by telephone, or were visited at home to ask for informed consent. When the subjects were severely cognitively impaired, informed consent was obtained from a guardian. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center. Subjects were visited three times at their place of residence. During these visits, face to face interviews were conducted, blood samples were collected and an electrocardiogram was recorded.

Cognitive function

The Mini-Mental State Examination [19] was administered in all subjects. To investigate the various domains of cognitive function, we used four neuro-psychological tests that are widely utilised in observational studies and have proven clinical relevance [20]. Cognitive speed was measured with two neuro-psychological tests, the Letter Digit Coding test (processing speed) [21] and a short 40-item version of the Stroop test (attention) [22,23]. For data analysis, we used the third Stroop card showing colour words printed in ink of different colours. Memory was measured with the 12-Word Learning, which assesses immediate and delayed recall test [24,25]. The neuro-psychological tests were not administered in subjects with a Mini-Mental State Examination score of 18 points or lower, because in these subjects neuro-psychological tests can not be accurately assessed [21]. All neuro-psychological tests were administrated by the same trained research nurse, who gave her impression of whether the tests went well and whether the test scores could be trusted to reflect the subject's ability to perform the test at that time.

Good cognitive speed was defined as a score below the median on the Stroop test and a score above the median on the Letter Digit Coding test. Poor cognitive speed was defined as a score above the median on the Stroop or a score below the median on the Letter Digit test. Good memory was defined as a score above the median on both the immediate recall test and the delayed recall test. Poor memory was defined as a score below the median on either the immediate recall test or the delayed recall test. Subjects with Mini-Mental State Examination scores of 18 points or lower and subjects who, for cognitive reasons, were unable to perform the test were classified as having a poor test performance. Subjects who, for other reasons, were unable to complete the tests were excluded from the analyses.

A definite diagnosis of dementia was obtained from the medical records of the subject's general practitioner [26] or the subject's treating physician when the subject lived in a nursing home.

Education was divided into two levels: a lower education level, including individuals without schooling or with primary school education only (with a maximum of 6 years of schooling), and those with a higher education level (equivalent to more than 6 years of schooling).

Atherosclerosis

We used two approximations to determine the presence of atherosclerosis. For the first approximation we used electrocardiograms, which were recorded on a Siemens Siccard 440 and transmitted by telephone to the ECG Core Lab in Glasgow for automated Minnesota coding [27]. Codes 1-1, 1-2, and 1-3 were equated with a diagnosis of myocardial infarction[28] . Codes 4-1, 4-2, 4-3, 5-1, 5-2 and 5-3 represented subjects with myocardial ischaemia [28]. Subjects were classified as having atherosclerosis, when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia.

For the second approximation to determine the presence of atherosclerosis we used the classification from the Second Manifestations of ARTerials disease (SMART) study [29], which among other things showed that the severity of atherosclerosis (intima-media thickness and arterial stiffness) was related with the number of cardiovascular diseases obtained from the subject's medical history. In our study we obtained the history of cardiovascular disease from the general practitioner or the subject's treating physician, i.e. nursing home physician. Subjects were classified as having atherosclerosis, when a positive history of myocardial infarction, angina pectoris, arterial surgery, stroke, or intermittent claudication was present, or if the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia.

Inflammation

Tumor Necrosis Factor-α (TNF-α) and Interleukin-10 production levels were assessed with an *ex vivo* whole blood assay [30]. The methods by which whole-blood samples were simulated with 10 ng/ml of lipopolysaccharide have been described elsewhere, including data on reproducibility [30]. In short, all blood was drawn in the morning and stimulated before 11.00 am to exclude circadian variation. Heparinised whole blood was diluted 2-fold with RPMI-1640. Lipopolysaccharide (endotoxin, 10 ng/ml) was used as primary stimulus. After addition of lipopolysaccharide, samples were incubated for 4 or 24 hours at 37°C and 5% CO_2. After centrifugation, the supernatants were stored at –80° C until assaying for the pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) in the 4 hour samples, and the anti-inflammatory cytokine Interleukin-10 (IL-10) in the 24 hour samples, using standard ELISA techniques.

Production levels were dichotomized around the median. This was done separately for males and females, since females have a lower cytokine production than males. Subjects with an anti-inflammatory response were those with low TNF-α levels (below the median) and high IL-10 levels (above the median). Subjects with a pro-inflammatory response were those with high TNF-α levels (above the median) and low IL-10 levels (below the median).

Data analysis

The primary outcome measure was the score on the neuro-psychological tests that measure cognitive speed (Stroop test and Letter Digit test) and memory (immediate and delayed Word Learning test). These data are presented as medians with corresponding 95% confidence intervals for the median [31],

representing the range of values which include the "true" median. We used non-parametric tests, because the Mini-Mental State Examination has a maximum score of 30 points and the delayed Word Learning test has a maximum of 12 words. Furthermore, the distributions on both tests were skewed to the left. As the non-parametric equivalent of the one-way ANOVA procedure, we used the Jonckheere-Terpstra test [32] to determine the p-value for trend between the scores of the cognitive tests and the strata representing an increasing pro-inflammatory cytokine response.
Univariate odds ratios and 95% confidence intervals were obtained by cross-tabulation. Multivariate odds ratios were obtained by logistic regression analysis and were adjusted for gender. We tested for trend using the log-likelihood statistic with one degree of freedom. In a secondary analysis, we used dichotomous endpoints, poor and good cognitive speed (Stroop and Letter Digit test), poor and good memory (immediate and delayed recall on the Word Learning test). Statistical significance was assessed by the linear association test to determine the p value for trend. Finally, we compared subjects with dementia to subjects who were cognitively intact (Mini-Mental State Examination score $^{3} \geq 28$ points), using a nested case-control design.

Results

Demographics and atherosclerosis

Between 1st September 1997 and 1st September first 1999, 705 inhabitants of Leiden reached the age of 85 years and were eligible to participate in the study. Fourteen inhabitants died before they could be enrolled. The response rate was 87%, i.e. a total of 599 subjects (397 women, 202 men) participated. There were no significant differences between the 92 non-respondents and the 599 respondents with respect to demographic characteristics, apart from a slightly different sex-ratio (72 women refused to participate whereas 61 was expected, p=0.02). Table 1 shows the demographic and clinical characteristics of the subjects in the study. Almost half of all subjects (47%) had atherosclerosis, defined as the presence of myocardial infarction or myocardial ischaemia as recorded by the electrocardiogram. Electrocardiograms were not recorded in 32 subjects, since 7 subjects died before an electrocardiogram could be recorded, in 2 subjects there was a technical failure and 23 subjects refused the recording of an electrocardiogram.

Cognitive function

One third of all subjects (34%) had a Mini-Mental State Examination score of 28 points or more, Table 1. We categorised subjects as having a good or poor cognitive function based on test scores dichotomised around the median in order to further explore the effect of atherosclerosis and inflammation on cognitive function. Subjects with Mini-Mental State Examination scores of 18 points and lower and subjects who for, cognitive reasons, were unable to perform the test were classified as having a poor test performance. Subjects who for other reasons were unable to complete the tests were excluded from the analyses. A poor cognitive speed was present in 67% of all participating subjects and a poor memory in 63%. A total of 79 (13%) subjects suffered from dementia.

Table 1 *Demographic and clinical characteristics of subjects in the Leiden 85-plus Study*

Characteristic	(n=599)
Gender (female / male)	397 / 202
Low level of education	386 (64%)
Use of non-steroidal anti-inflammatory drugs	162 (27%)
Prevalence of cardiovascular diseases	
Myocardial infarction *	99 (17%)
Myocardial ischaemia †	214 (36%)
Atherosclerosis ‡	
Present	214 (36%)
Absent	353 (59%)
Cognitive function	
MMSE ≥28 points	206 (34%)
Poor cognitive speed	401 (67%)
Poor memory	380 (63%)
Dementia	79 (13%)

** ECG diagnosis using Minnesota codes 1-1,1-2 and 1-3.† ECG diagnosis using Minnesota codes 4-1, 4-2, 4-3, 5-1, 5-2 or 5-3. ‡ Numbers do not add up to 599 as, for some subjects, there was missing data.*

Inflammation

Cytokine responses could not be obtained in 7 subjects as they died before a blood sample could be drawn while 30 subjects refused to give a blood sample. Under unstimulated conditions, 9 subjects had detectable TNF-α concentrations (TNF-α >100 pg/ml) and were excluded from the analyses. Upon stimulation with endotoxin (lipopolysaccharide) in whole blood samples (n=553), the median production of TNF-α concentration was 11541 pg/ml (inter quartile range 8556-14945 pg/ml) in men and 9615 pg/ml (inter quartile range 6763-12651 pg/ml) in women (Mann-Whitney test, p <0.0001). The median IL-10 concentration was 839 pg/ml (inter quartile range 529-1244 pg/ml) in men and 736 pg/ml (inter quartile range 465-1007 pg/ml) in women (Mann-Whitney test, p=0.001). Based on the median values of both TNF-α and IL-10, men and women were classified separately in inflammatory groups.
76 (14%) of the 553 subjects had an anti-inflammatory cytokine response (low TNF-α production and high IL-10 production) and 77 (14%) subjects had a pro-inflammatory response (high TNF-α production and low IL-10 production). 400 subjects had an intermediate cytokine response of which 201 (36%) subjects had a high TNF-α production and a high IL-10 production and 199 (36%) subjects had a low TNF-α production and a low IL-10 production.

Inflammation and cognitive function

Table 2 shows the median scores of the Mini-Mental State Examination and the neuro-psychological tests in the four strata of cytokine production varying from an anti-inflammatory response (low TNF-α production and high IL-10 production) to a pro-inflammatory response (high TNF-α production and low IL-10 production). The scores of the Mini-Mental State Examination and the neuro-psychological tests measuring cognitive speed and memory gradually declined over the strata representing a pro-inflammatory cytokine response (all p for trend < 0.05). The strength of the association was not affected when subjects on non-steroidal anti-inflammatory drugs were excluded from the analysis (data not shown). The association was equally strong in both men and women, and in subjects with high and low levels of education (data not shown).

Table 2 *Neuro-psychological test scores in relation to inflammatory responses*

	Anti-inflammatory	Intermediate-inflammatory response		Pro-inflammatory	
Characteristic	TNF-α low IL-10 high	TNF-α high IL-10 high	TNF-α low IL-10 low	TNF-α high IL-10 low	p for trend
All subjects (n)	76	201	199	77	
Global cognitive function					
MMSE (points)	27 (25-28)	26 (25-27)	26 (25-27)	26 (24-26)	0.05
Neuro-psychological tested subjects (n)	62	158	153	61	
Cognitive speed					
Stroop (seconds)	62.8 (57.0-70.8)	73.7 (68.6-8.4)	78.4 (73.0-84.0)	79.1 (66.0-88.8)	0.004
Letter Digit (digits/minute)	19 (16-20)	16 (15-18)	15 (15-17)	15 (14-18)	0.03
Memory					
Immediate Word Learning (words)	26 (24-29)	25.5 (24-26)	24 (23-25)	24 (22-27)	0.02
Delayed Word Learning (words)	10 (9-11)	9 (9-10)	8 (8-9)	9 (8-10)	0.04

Data are presented as medians and corresponding 95% confidence intervals. TNF-α; Tumor Necrosis Factor-α. IL-10; Interleukin-10.

Atherosclerosis, inflammation and cognitive function

We studied the association between cognitive function and the inflammatory response in subjects with and without atherosclerosis apart to test the hypothesis that atherosclerosis triggers an inflammatory reaction leading to poor cognitive function. This is illustrated in figure 1 depicting global cognitive function as measured with the Mini-Mental State Examination. Subjects with atherosclerosis showed a significant decline on the Mini-Mental State over strata representing an increasing pro-inflammatory cytokine response (p for trend =0.04). Such a decline was absent in subjects without atherosclerosis (p for trend =0.4). Different associations between cytokine responsiveness, and cognitive function in subjects with and atherosclerosis were also present for the Stroop test, the Letter-Digit test, as well as the immediate and the delayed Word Learning test (Table 3).

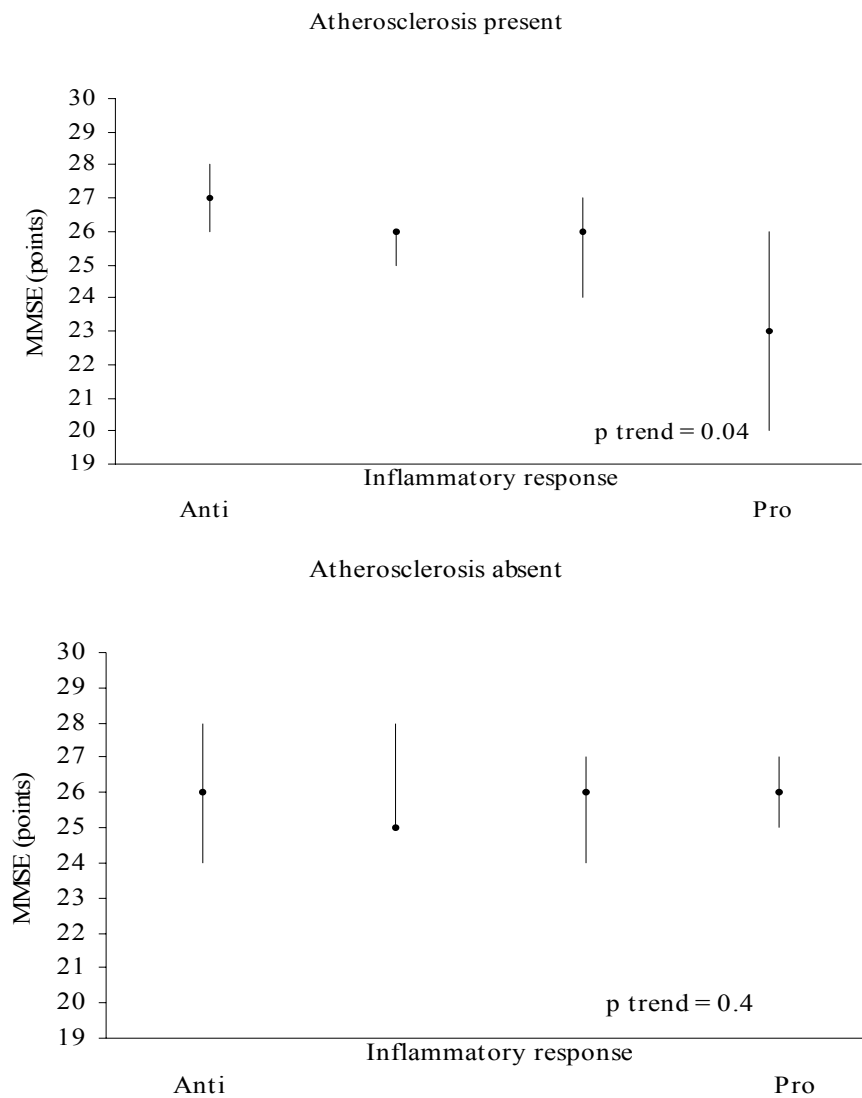

Figure 1 *Mini-Mental State Examination score in relation to inflammatory response and atherosclerosis.Data are presented as medians and corresponding 95% confidence intervals.*

Table 3 *Neuro-psychological test scores in relation to atherosclerosis and inflammatory responses*

	Atherosclerosis present*			Atherosclerosis absent*		
Characteristic	Anti-inflammatory (n=31)	Pro-inflammatory (n=32)	p value	Anti-inflammatory (n=45)	Pro-inflammatory (n=44)	p value
Cognitive speed						
Stroop (seconds)	64.3 (54.6-79.2)	82.1 (64.1-92.3)	0.06	61.3 (56.2-73.3)	79.1 (64.3-89.5)	0.05
Letter Digit (digits/minute)	19 (18-24)	14 (10-18)	0.005	16 (12.5-20.5)	15 (14-18)	0.9
Memory						
Immediate Word Learning (words)	27 (26-30)	21.5 (18-29)	0.02	24 (22-29)	25 (23-27)	0.8
Delayed Word Learning (words)	10 (9-11)	8 (5-9)	0.01	9 (9-10)	10 (9-10)	0.6

** Subjects were classified as having atherosclerosis, when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia. P values are determined using the Mann-Whitney test. Data are presented as medians and corresponding 95% confidence intervals. Anti-inflammatory response; low Tumor Necrosis Factor-α production and high Interleukin-10 production. Pro-inflammatory response: high Tumor Necrosis Factor-α production and low Interleukin-10 production.*

Table 4 presents the odds ratios for poor cognitive speed and memory in relation to inflammatory responses, stratified for atherosclerosis. In subjects with atherosclerosis, the odds ratio for poor memory was 5.9 (95% CI; 1.7-20.0) when subjects with a pro-inflammatory or an anti-inflammatory cytokine response were compared. To ascertain that our findings were not due to subjects who have suffered a stroke, we did an additional analysis in which subjects with a history of stroke were excluded. The results were unaffected.

We re-analysed the data, to ascertain that our approximation of atherosclerosis using electrocardiographic signs of myocardial infarction and myocardial ischaemia was valid. We therefore used a classification based on the SMART study [29] to determine the prevalence of atherosclerosis. Atherosclerosis was considered present when subjects had a positive history of myocardial infarction (n=63, 11%), or angina pectoris (n=108, 18%), or arterial surgery (n=37, 6%), or stroke (n=40, 7%), or intermittent claudication (n=37, 6%), or if the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia (n=214, 36%). The prevalence of atherosclerosis was 62% (n=369), based on this classification The effects of increasing pro-inflammatory cytokine response and atherosclerosis on cognitive function remained similar when we used this classification to determine the presence of atherosclerosis.

Atherosclerosis, inflammation and dementia

We analysed the data using the definite diagnosis of dementia as a clinical endpoint. To this end, we performed a nested case-control study. 79 subjects had a definite diagnosis of dementia. In 72 out of those 79 subjects, an inflammatory cytokine response could be obtained. We compared subjects with dementia to subjects who were cognitively intact (n=194). Since it is known that general practitioners under-report dementia [26], we defined the control group as subjects who on screening with Mini-Mental State Examination, scored 28 points or more and were therefore unlikely to suffer from dementia [19]. Table 5 shows that the odds ratios for dementia gradually increased to 3.5 (CI 95; 1.1-11.6) over the strata representing a mounting pro-inflammatory cytokine response. In subjects with atherosclerosis, i.e. those with electrocardiographic signs of myocardial infarction or myocardial ischaemia, the odds ratio was 15.2 (95% CI; 1.5-157) when subjects with either a pro-inflammatory or an anti-inflammatory cytokine response were compared. The odds ratio was not significantly increased in subjects without atherosclerosis. The odds ratios for dementia remained similar when we used the classification for atherosclerosis based on the subject's cardiovascular history.

Table 4 *Odds ratios for poor cognitive function in relation to inflammatory responses and atherosclerosis*

	Anti-inflammatory	Intermediate-inflammatory response		Pro-inflammatory	
	TNF-α low IL-10 high	TNF-α high IL-10 high	TNF-α low IL-10 low	TNF-α high IL-10 low	p for trend
All subjects (n)	73	191	191	74	
Poor cognitive speed	1*	1.6 (0.8-2.9)	2.8 (1.5-5.3)	2.8 (1.2-6.2)	0.001
Poor Memory	1*	1.6 (0.8-2.7)	2.5 (1.4-4.5)	1.8 (0.9-3.7)	0.01
Atherosclerosis present (n) †	31	97	88	30	
Poor cognitive speed	1*	3.1 (1.3-7.7)	4.7 (1.8-12.0)	4.0 (1.1-14.0)	0.007
Poor Memory	1*	2.7 (1.1-6.4)	4.0 (1.6-9.7)	5.9 (1.7-20.0)	0.001
Atherosclerosis absent (n) †	42	94	103	44	
Poor cognitive speed	1*	0.9 (0.4-2.2)	1.9 (0.8-4.8)	2.3 (0.8-6.9)	0.02
Poor Memory	1*	1.1 (0.5-2.4)	1.9 (0.8-4.1)	1.0 (0.4-2.3)	0.5

** Reference category. Odds ratios (95% CI) are adjusted for gender and education. TNF-α; Tumor Necrosis Factor-α. IL-10; Interleukin-10.† Subjects were classified as having atherosclerosis, when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia.*

Table 5 *Odds ratios for dementia in relation to inflammatory responses and atherosclerosis in a nested case control design*

	Anti-inflammatory	Intermediate-inflammatory Response		Pro-inflammatory	
	TNF-α low IL-10 high	TNF-α high IL-10 high	TNF-α low IL-10 low	TNF-α high IL-10 low	p for trend
Cases: dementia †	7 (17%)	27 (27%)	26 (28%)	12 (38%)	
Controls: MMSE > 27 points	35 (83%)	73 (73%)	66 (72%)	20 (62%)	
Odds ratios					
All subjects (n=266)	1*	2.0 (0.7-5.7)	2.4 (0.9-6.6)	3.5 (1.1-11.6)	0.04
Atherosclerosis present (n=118) ‡	1*	6.6 (0.8-56.2)	4.8 (0.5-42.5)	15.2 (1.5-157)	
Atherosclerosis absent (n=148) ‡	1*	1.1 (0.3-3.8)	2.0 (0.6-6.8)	1.4 (0.2-7.5)	

Odds ratios (95% CI) are adjusted for gender and education. TNF-α; Tumor Necrosis Factor-α. IL-10; Interleukin-10. Reference category. † Cases are subjects with a definite diagnosis of dementia, controls are subjects who are cognitively intact (Mini-Mental State Examination score > 27 points). ‡ Subjects were classified as having atherosclerosis, when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia.*

Discussion

This analysis of the Leiden 85-plus Study shows that an innate pro-inflammatory cytokine response is associated with poor cognitive function and dementia in old age. The association was especially strong in subjects with myocardial infarction or ischaemia, a proxy for atherosclerosis, and persisted after exclusion of those with a history of stroke. Our findings show an apparent dose-response relationship between an increasing pro-inflammatory cytokine response and poor cognitive function, which is fully consistent with the hypothesis that atherosclerosis triggers an inflammatory response contributing to neurodegeneration, poor cognitive function and dementia [1,4].

The present data could suggest that the association between a pro-inflammatory response, as measured with TNF-α production and IL-10 production, and poor cognitive function is causal. We have previously shown associations between pro-inflammatory cytokine responsiveness and systemic diseases such as infection [33] and lupus erythematosus infection [34] and associations between pro-inflammatory cytokine responses and brain diseases like multiple sclerosis [35] and Alzheimer's disease [16]. In some of these studies, we used a family design in which the cytokine response of the patient was estimated in first-degree relatives. Such an analysis favours a possible causal interpretation of the association, as the cytokine response cannot be the result of disease. The fact that heritability of a pro-inflammatory cytokine responsiveness can be demonstrated when studying families and twins [33] makes a possible causal relation even more likely. Furthermore, findings from the Karolinska institute suggest that the cytokine response in whole blood induces the same effects in the brain across the blood-brain barrier [36].

Subjects with coronary disease have an increased risk to develop cerebral ischaemia [2]. This finding strongly suggests that the presence of coronary disease, which is primarily caused by atherosclerosis, also reflects the presence of atherosclerosis in the brain. We used two different strategies to measure the presence of atherosclerosis. In the first classification we used electrocardiographic signs of myocardial infarction or myocardial ischaemia. In the second classification, we used the cardiovascular history of the subjects to measure the presence of atherosclerosis [29]. The effect of a pro-inflammatory cytokine response on cognitive function was present when we used both classifications of atherosclerosis.

We measured two domains of cognitive function, namely cognitive speed and memory. Cognitive speed, consisting of attention and processing speed, is the most sensitive measure because age-related cognitive decline is thought to manifests itself first by a decline in attention and processing speed infection [37,38]. In the elderly, memory remains relatively intact until late stages of cognitive decline, while cognitive speed declines more rapidly [37]. When we tested cognitive speed and memory, we found that they were both affected when atherosclerosis and a pro-inflammatory cytokine response were simultaneously present.

The effect of a pro-inflammatory cytokine response on poor cognitive speed and memory was especially strong in subjects with myocardial infarction or ischaemia, as a proxy for atherosclerosis,

providing evidence in favour of our hypothesis that atherosclerosis triggers an inflammatory reaction which contributes to a neurodegenerative process, poor cognitive function and dementia. Suggesting that there is biological interaction between atherosclerosis and inflammation and it's effect on cognitive function. Our findings are in line with experimental animal studies, which showed that by blocking the actions of pro-inflammatory cytokines, stroke lesion size can be significantly reduced [18]. Alternative explanations may be that inflammation favours atherosclerosis, which in turn leads to poor cognitive function, or that atherosclerosis and inflammation are independent risk factors for poor cognitive function.

We had to ascertain that our findings were not due to the use of non-steroidal anti-inflammatory drugs. These drugs, which are prescribed for cardiovascular disease and arthritis, may affect the inflammatory response. Therefore, we did an additional analysis excluding subjects who used non-steroidal anti-inflammatory drugs. This did not affect our results. Also, the use of lipid lowering drugs in this age group was virtually absent.

The association between atherosclerosis, inflammation and cognitive function described here provides a plausible mechanism of how non-steroidal anti-inflammatory drugs and anti-hypertension treatment prevent poor cognitive function and dementia. Non-steroidal anti-inflammatory drugs may have a beneficial effect by altering the inflammatory response either directly [39] or indirectly via inhibiting platelet aggregation and subsequently in ischaemia-induced inflammatory brain response. Anti-hypertensive treatment prevents the progression of atherosclerosis, reduces arterial thrombosis and ischaemia-induced inflammatory response and may thus explain the preservation of cognitive function [8].

In conclusion, our study shows that there is an apparent dose-response relationship between an increasing pro-inflammatory cytokine response, atherosclerosis and poor cognitive function. This is clinically important, as it implies that both prevention of atherosclerosis and the use of drugs that impair a pro-inflammatory response could prevent poor cognitive function and dementia in the elderly. Preventive strategies should be aimed at elderly having the highest risk, i.e. those with a combination of atherosclerosis and a pro-inflammatory response.

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