The Leiden 85-plus Study

Impact of atherosclerosis and inflammation on cognitive function

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Eric van Exel

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Entre le ciel et la terre

Voor Doffie

Contents

Chapter 1

General introduction

General introduction

Alzheimer's disease and vascular dementia are progressive irreversible brain disorders that result in memory loss, personality changes and unusual behaviour. Increased life expectancy in developed countries is expected to leads to a dramatic rise in the number of subjects with Alzheimer's disease and vascular dementia. One out of four 85-year-old subjects suffers from dementia [1,2]. Since no effective cure for dementia is currently present the majority of these subjects will need long-term care.

The accepted view in the field of dementia is that Alzheimer's disease and vascular dementia develop from different aetiologies. It has been suggested that amyloid-beta plays a crucial role in the development of Alzheimer's disease [3], whereas cerebrovascular disease leads to vascular dementia [4]. Definitions of these two disorders should be firm, and the criteria to make a clinical diagnosis of either Alzheimer's disease or vascular dementia should be independent of each other [5]. In the current situation, however, the clinical diagnoses of Alzheimer's disease and vascular dementia are not independent of each other. Since the clinical diagnoses of Alzheimer's disease and vascular dementia are linked by the presence or absence of cerebrovascular disease they are intertwined. The presence of cerebrovascular disease has to be excluded before the clinical diagnosis of Alzheimer's disease can be made, whereas the clinical diagnosis of vascular dementia can only be made when the deterioration in cognitive functioning is accompanied by cerebrovascular disease.

Theoretically the dividing line between Alzheimer's disease and vascular dementia, based on the presence or absence of cerebrovascular disease, does not pose a problem. In the clinical situation, however, it is difficult to determine whether cerebrovascular features, which are frequently detected on imaging, cause dementia. The widespread use of MRI shows that at least 30% of all elderly people have silent cerebral infarctions [6]. However, it is not clear whether these silent infarctions have contributed to cognitive impairment or dementia. The findings from the examination of the brains of subjects diagnosed with Alzheimer's disease and the brains of subjects without dementia throw further doubt on the current criteria to diagnose either Alzheimer's disease or vascular dementia [7,8]. By contrast with the brains of subjects without dementia the brains of subjects with both clinically diagnosed and autopsy-proven Alzheimer's disease often show more multiple vascular lesions. At the same time, the brains of subjects clinically diagnosed with vascular dementia show pathological changes, i.e. amyloid plaques and tau pathology, which are considered to be characteristic of Alzheimer's disease [7,8]. Finally, amyloid plaques and tau pathology are often found in the brains of elderly people who did not suffer from dementia[7].

The post-mortem correlates suggest that the various pathologies of the late-onset type of dementia, both vascular dementia and Alzheimer's disease, are not mutually exclusive. A unifying hypothesis is that atherosclerotic disease causes clinical and subclinical ischaemic diseases in the brain, which contribute to the development of late-onset dementia [9]. Such a multicausal interpretation of the observational data provides an explanation for the findings that in old age treatment of cardiovascular risk factors, such as high serum cholesterol and hypertension, is associated with a decreased risk of vascular dementia as well as Alzheimer's disease [10-12].

The post-mortem findings which suggest that the differences between vascular dementia and Alzheimer's disease are not distinct question whether the currently used definitions of Alzheimer's disease and vascular dementia are correct. It is tempting to speculate and redefine late-onset type of dementia, by using criteria irrespective of a possible cause of cognitive decline. Several studies recognise that there is a transition between the extremes of "optimal" cognitive functioning and dementia [13,14].
These studies, based on the "brain reserve capacity theory", assume that there is a continuum in cognitive functioning. This means that some subjects, i.e. those with less brain reserve capacity, are more likely to surpass the threshold beyond which the presence of dementia becomes clinically apparent[15,16]. The advantage of regarding cognitive functioning as a continuum is that it can be described irrespective of a possible cause of poor cognitive functioning. The clinical classification of dementia, on the contrary, is dependent on criteria such as the presence or absence of cerebrovascular disease, or the presence or absence of amyloid plaques. It is therefore interesting to investigate risk factors contributing to "poor" cognitive functioning, i.e. cognitive impairment, in the general population irrespective of the presence of Alzheimer's disease or vascular dementia. This can be done by using sophisticated neuropsychological tests, which only measure cognitive functioning.

In this thesis the effect of vascular determinants on cognitive functioning will be studied. The focus in this chapter will be on the already known effects on cognitive functioning of "classical" vascular risk factors, such as gender, diabetes, hypertension and high serum cholesterol, and a more recently identified risk factor, that is inflammation.

Gender
Several studies show that the prevalence of dementia and poor cognitive function is higher in women than in men. A high level of education is associated with a higher socio-economic status and reduced mortality. A high level of education is also associated with a lower prevalence of dementia and less cognitive impairment [15,16]. Since in general elderly women have received less formal education than men it has been suggested that a high level of education is a plausible explanation for the finding that the prevalence of dementia and poor cognitive function is higher in women than in men. Another possible explanation for the difference in cognitive functioning between women and men is that post-menopausal oestrogen deficiency leads to cognitive impairment in women. Several studies describe a beneficial effect of oestrogen replacement therapy on cognitive function [17,18]. However, in all observational studies the comparisons suffer from selection on health and it is yet unclear which part of the beneficial effect is real [19]. The ultimate proof lies within randomised-controlled trials of post-menopausal hormonal replacement therapy investigating the effects on cognitive functioning. However, the findings of these trials are conflicting since the hormonal replacement therapy is associated with both a beneficial effect[20] and no effect on cognitive function in subjects with dementia [21,22].

Type-2 diabetes
Both cross-sectional and longitudinal studies show that diabetes is associated with dementia or cognitive impairment [23-25]. There are several mechanisms that can explain this finding. Evidently, clinical stroke,

which is more present in subjects with diabetes [26,27] is associated with a poor cognitive function and dementia [9]. This is not the only plausible explanation. Advanced glycation end products in the brain could also explain the increase in poor cognitive functioning and dementia [28,29], since pathological changes in the glucose metabolism lead to an increase in advanced glycation end products. These irreversible, protease-resistant, cross-linking proteins are formed by a non-enzymatic reaction between glucose and protein amino groups. Several studies suggest that these advanced glycation end products lead to an increased deposit of amyloid-beta in the brain, which subsequently could lead to a poor cognitive function and dementia [28,29]. Finally, experimental animal studies show that hyperglycaemia leads to a reduced endoneurial blood flow resulting in endoneurial hypoxia and nerve conduction deficits [30].

Hypertension

The finding that treatment of isolated systolic hypertension in elderly subjects reduces the incidence of dementia by 50% emphasises the contribution of hypertension to the development of dementia and cognitive impairment [12]. Longitudinal studies have shown that both systolic and diastolic blood pressure had increased 10 to 15 years before the onset of dementia [31,32]. These observational studies have also shown a decline in blood pressure level in the years before dementia becomes clinical apparent. Blood pressure levels were then similar to or lower than those in non-demented individuals. Over a long period of time these findings suggest that hypertension increases the risk of dementia. Since hypertension leads to stroke and subclinical ischaemic events in the brain, which are associated with dementia, it is a very plausible determinant of the development of dementia and cognitive impairment.

Cholesterol

Since both high and low serum cholesterol have been associated with dementia [33-37], the evidence for an association between total cholesterol and cognitive function is ambiguous. Cholesterol may directly affect neurodegeneration [38], since in-vitro studies show that when statins are used cholesterol reduction leads to inhibition of the formation of amyloid-beta [39], the main constituent of amyloid plaques. Case-control studies investigating the effect of statins on the cognitive function also show that the use of statins protects against dementia[10,11]. There is also indirect support for a connection between cholesterol and cognitive function via atherosclerotic disease. Since it has been suggested that atherosclerotic disease is associated with clinical and subclinical ischaemic diseases in the brain, which contributes to the development of late onset dementia [9]. Clearly, studies with longitudinal designs should be done to determine if there is a causal association between cholesterol and cognitive function.

Inflammation

Observational studies have shown that the use of anti-inflammatory drugs lowers the risk for dementia and poor cognitive function [10,11,40-42]. These studies could be biased, since physicians might not prescribe anti-inflammatory drugs to patients with dementia, however they do suggest a relation between chronic inflammation, dementia and poor cognitive function. Randomised clinical trials using anti-inflammatory drugs in subjects with dementia, however, did not show beneficial effects of these

drugs on cognitive function [43,44]. However, these findings cannot refute that inflammation plays a role in dementia and poor cognitive function, since immune system proteins, such as the upregulation of adhesion molecules and pro-inflammatory cytokines in microglia cells, and the deposit of complement and C-reactive protein are found around amyloid plaques[41,42,46-48]. Furthermore, it has been shown that patients with Alzheimer's disease exhibit a pro-inflammatory response upon endotoxin stimulation in whole blood samples[49]. However, the *initiating event* leading to neuro-inflammation, neurodegeneration, poor cognitive function and dementia remains unclear.

The aim of this thesis

The first aim is to test the hypothesis: "Is atherosclerosis the *initiating event* that leads to ischaemia in the brain and subsequently to neuro-inflammation, followed by neurodegenerative processes that ultimately results in cognitive impairment and dementia ?" This hypothesis is based on findings from experimental animal studies investigating the effect of ischaemia and a pro-inflammatory response on stroke lesion. These studies revealed that blocking of the pro-inflammatory response after ligation of the middle cerebral artery markedly reduced the lesion size and improved neurological outcome [50,51]. These findings suggest that the magnitude of ischaemic lesion size in the brain depends on the inflammatory response. It is therefore possible that subjects with atherosclerosis and a pro-inflammatory response have an increased ischaemic lesion size compared to subjects with atherosclerosis and an anti-inflammatory response. The difference in ischaemic lesion size subsequently leads to differences in cognitive function between subjects with a pro- or an anti-inflammatory response.

The second aim of this thesis is to investigate the association between the inflammatory response and atherosclerosis. Since elevated immune system proteins, such as C-reactive protein and fibrinogen are associated with atherosclerosis. The idea is that the inflammatory response directly or indirectly, via lipid and glucose metabolism, contributes to atherosclerosis [52-54]. Since both insulin resistance and dyslipdemia are associated with systemic inflammation and atherosclerosis..

All studies presented in this thesis were performed within the Leiden 85-plus Study, a population based study in 85 year old citizens of Leiden. First, in this thesis the effect of "classical" cardiovascular risk factors and cardiovascular disease on cognitive function is described (figure 1). The effect of gender (chapter 3), high-density lipoprotein (chapter 5) and cardiovascular disease, i.e. atherosclerosis (chapter 4) on cognitive function is described in the so-called "classic" route (figure 1). Then data on the first aim of this thesis are presented in the so-called "alternative" route (figure 1), showing the relation between atherosclerosis, inflammation and cognitive function (chapter 6). Finally, two studies report on the second aim of this thesis, i.e. the association between the inflammatory response and atherosclerosis, by describing the relation between the "classic" route and the "alternative" route (figure 1). The first of these two studies shows the association between interleukin-10, a strong anti-inflammatory cytokine, type-2 diabetes and the metabolic syndrome, i.e. a clustering of cardiovascular risk factors, such as insulin resistance, dyslipidemia and hypertension (chapter 7). The second study describes the relation between atherosclerosis - that is cerebrovascular disease- (chapter 8) and inflammation.

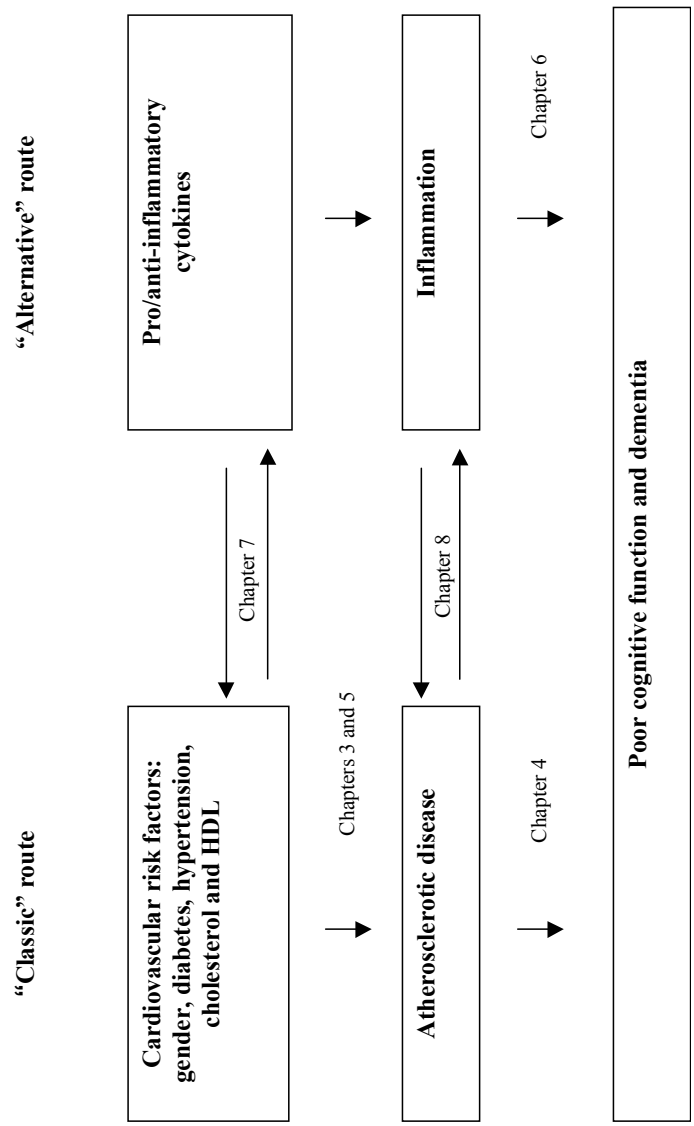

Figure 1 *Atherosclerosis, inflammation and cognitive function*

Definition and measurement of cognitive function and inflammation

Cognitive function

Some basic theoretical issues on cognitive function need to be discussed before exploring the effect of atherosclerosis and inflammation on cognitive function. Areas of cognitive function that tend to change as a result of (clinical) events include general cognitive speed, attention and memory. These are listed among the so-called *fluid abilities* [55]. Many other cognitive domains are far less likely to change, e.g., reading, general knowledge, and language abilities. These are called *crystallised abilities* and, by definition, do not change much [55]. In this thesis fluid abilities were determined, i.e. those cognitive function that tend to change when (clinical) events occur, by measuring cognitive speed and memory, using sophisticated neuropsychological tests. Cognitive speed, consisting of attention and processing speed, is the most discriminative measure because age-related cognitive decline first manifests itself by a decline in attention and processing speed [56,57]. In the elderly, memory remains relatively intact until the late stages of cognitive decline, whereas cognitive speed declines more rapidly.

Inflammation

Tumour-necrosis factor-α (TNF-α) and interleukin-10 (IL-10) are two central inflammatory cytokines, which play a crucial role in the regulation of immune reactivity. TNF-α shows a wide spectrum of biological activities [58]. It is a decisive pro-inflammatory mediator in the host defence to infection by activating the inflammatory host response thus increasing the proliferation of macrophages and lymphocytes. Furthermore, TNF-α inhibits anticoagulatory mechanisms and promotes thrombotic processes and therefore plays an important role in pathological processes such as venous thromboses and atherosclerosis. In the brain it promotes the proliferation of astroglia and microglia and may therefore be involved in neurodegenerative processes such as demyelinisation [58]. IL-10 on the other hand is a central anti-inflammatory cytokine [59] with strong deactivating properties on the inflammatory host response mediated macrophages and lymphocytes. And it potently inhibits the production of pro-inflammatory cytokines such as IL-6 and TNF-α [59].

In this thesis ex vivo whole-blood samples were simulated with 10 ng/ml of endotoxin (lipopolysaccharide) to determine TNF-α and IL-10 production capacity [60]. This has been done since the ex-vivo production of TNF-α and IL-10 shows good reproducible patterns and because the production of both TNF-α and IL-10 is under tight genetic control[61]. Approximately 60% of the variation in TNF-α production and 75% of the variation in IL-10 production is genetically determined.

Atherosclerosis

Two approximations to determine the burden of atherosclerosis were used. For the first approximation electrocardiograms were recorded on a Siemens Siccard 440 and transmitted by telephone to the ECG Core Lab in Glasgow for automated Minnesota coding [62]. Codes 1-1, 1-2, and 1-3 were equated with a diagnosis of myocardial infarction [63]. Codes 4-1, 4-2, 4-3, 5-1, 5-2 and 5-3 represented subjects

with myocardial ischaemia [63]. Subjects were classified as having atherosclerosis, whereas the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia.
The classification from the Second Manifestations of ARTerials disease (SMART) study [64] was used as a second approximation to determine the presence of atherosclerosis. Findings from the SMART study using this classification showed among other things 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 the Leiden 85-plus Study, the history of cardiovascular disease was obtained from the general practitioner or the subject's attending physician. Subjects were classified as having atherosclerosis, whereas 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.

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Chapter 2

A high response is not essential to prevent selection bias: results from the Leiden 85-plus Study

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ABSTRACT

Background We tested the hypothesis that an additional effort to increase the response rate would diminish selection bias in a community-based cohort study.

Methods In the Leiden 85-plus Study, all subjects of the town of Leiden who had reached their 85th birthday were informed of the study by mail and then asked to participate by telephone. In an additional recruitment stage, those subjects who did not participate directly were visited and personally asked to participate. When these subjects refused, some non-response questions were asked. In this way we collected data on the whole source population.

Results Of 691 eligible elderly subjects, 511 subjects (74%) participated directly. Of those who did not participate directly, 88 subjects participated after the additional effort. The response rate increased from 74% to 87%. Compared to the 511 subjects who directly participated, the 88 subjects who entered the study after the additional effort had poorer health and lower survival. The subjects who refused were more healthy and had poorer mood. The direct sample did not differ from the source population with respect to socio-demographics, health, and mortality.

Conclusion We showed that given a moderately high direct response the additional effort was effective in increasing the response rate, but was also selective and was not necessary to prevent selection bias.

Introduction

A highly representative sample of participants is no longer considered essential for generalisability in etiological studies that report risk estimates rather than prevalence estimates[1-3]. Even a minimum of 80 percent response in follow-up studies is debatable[4]. Generalisability depends on the ability to abstract universal scientific hypotheses or theories from a set of observations and not only from the statistical framework of these observations[5,6]. However, many studies in the elderly have a public health goal in addition to more scientific etiological goals. In such community surveys, generating estimates that can be extrapolated to the general population, representativeness is still very important. Furthermore, it is essential to include frail elderly subjects in a study to investigate the determinants of and causal relations with chronic conditions. Refusal to participate due to ill health would surely invalidate results on the impact of chronic conditions in an elderly population[7] .

A high response rate increases the validity of community-based studies, since a low response rate might lead to selection bias[8]. The success of the response depends to a great extent on the way eligible subjects are approached. A high response can be achieved by interviewing and examining elderly subjects in their homes, since frail elderly subjects are less inclined to visit a study site[9]. Other effective strategies to optimise response rates are notification in advance by mail, involvement of expert researchers, and the prospect of a small gift[10]. Another possibility to increase response is to approach eligible subjects who initially declined or did not respond at all[11,12]. Using these strategies surveys among the elderly have been conducted resulting in response rates between 60 and 90 percent[13-24]. Differences in characteristics and associations between the sample of participants and the source population, however, frequently remain unknown.

In the Leiden 85-plus Study, a research nurse visited all subjects who did not participate directly after the first approach by telephone. Through this additional effort more subjects were drawn into the study. Moreover, the nurse asked a few questions to those who refused to participate to get an impression of their health and well-being. In this way we collected data from the whole source population. This provided an excellent opportunity to test the hypothesis that the additional effort to increase the response rate had diminished selection bias.

Methods

The Leiden 85-plus Study is a series of gerontological surveys of the population of the oldest old living in the town of Leiden, the Netherlands. The first survey started in 1986. The present survey is a community-based follow-up study in a delineated cohort of 85-year-olds. Special topics within the Leiden 85-plus Study are atherosclerosis, cognitive function, chronic diseases, disabilities, and well-being.

Study design

Between September 1997 and September 1999, all members of the 1912 to 1914-birth cohort (n = 705) were eligible to participate in the study. Subjects of the source population were informed about

the study by mail in the week after their 85th birthday. Within a month a physician or research nurse contacted them by phone to request their participation. If subjects agreed to participate, they were visited at their place of residence, oral and written information about the study was provided, and oral informed consent was obtained. When participants were severely cognitively impaired, informed consent was obtained from a responsible person.
When subjects hesitated or declined during the first telephone contact or when they could not be reached by phone, they entered the additional recruitment stage. In this stage the research nurse approached the subjects at their place of residence. She managed to visit virtually all subjects of the source population of 85-year-olds. During these visits, she made personal contact and provided oral and written information on the study. After two weeks and after three months, she visited these subjects again to ask them to participate in the study. The Medical Ethical Committee of the Leiden University Medical Center approved the Leiden 85-plus Study, including the approach and informed consent procedures.

Data collection
For all subjects, socio-demographic characteristics such as gender, marital state, and type of housing were available from the municipal registry. Mean income of neighbourhood of residence was used as indication of socio-economic status (SES). Mean income after taxes in the neighbourhood of residence was obtained by postal codes[2]. We classified low-income neighbourhoods as those with an income below the median.
During the main interview with participants, disability in activities of daily living (ADL) was measured with the Groningen Activity Restriction Scale (GARS)[25]. For participants with severe cognitive impairment, information was obtained from a responsible person. Cognitive function was assessed with the Mini-Mental State Examination (MMSE). Severe cognitive impairment was defined as an MMSE score of 18 points or lower[27]. In participants without severe cognitive impairment, depressive symptoms were measured with the 15-item Geriatric Depression Scale (GDS-15)[27]. At the end of the visit to non-participants as well as to all participants, the research nurse recorded her impression of the subject's daily functioning, cognition, and mood in a standardised questionnaire, using a four-point scale (very good, good, poor, very poor). Validation of the nurse impression about daily functioning, cognition, and mood is presented in the appendix.

All subjects were followed up for all-cause mortality until 1 May 2001. Mortality data were obtained from the municipal registry.

Data analysis
Prevalence estimates of health characteristics by participation status (direct sample, additional input, or non-participants) are presented with 95 percent confidence intervals (95% CI) and are compared using Chi-square tests. Differences in prevalence for socio-demographic and health characteristics between the source population (n = 691) and either the direct sample (n = 511) or the total sample (n = 599)

were assessed by comparing the "true" prevalence of the source population with the calculated confidence interval for the prevalence estimate of both samples. Overall survival was calculated from the 85th anniversary to the date of death or to the date of censoring (1 May 2001). Survival was estimated using the Kaplan-Meier product limit method. Survival by participation status was compared with the log-rank test.

Results

Between 1 September 1997 and 1 September 1999, 705 inhabitants of Leiden reached the age of 85. Fourteen inhabitants died before they could be enrolled in the study and thus 691 subjects were eligible to participate in the study. A total of 511 subjects, the direct sample, participated directly after invitation by phone, resulting in a response rate of 74 percent. After the additional recruitment stage another 88 subjects were included after being personally approached by our research nurse. As a result the total number of participants increased to 599 and the response rate to 87 percent. The remaining 92 subjects refused to participate, of whom 11 subjects refused any contact. Reasons for non-participation were "no interest, no time" (25 percent), "too nervous or anxious" (19 percent), "too tired or ill" (9 percent) or "being against surveys in general" (5 percent). Data from self-report and the nurse's impression were available for 680 subjects (599 participants and 81 non-participants), corresponding to 98 percent of the source population.

Table 1 shows the characteristics of the direct sample (n = 511), the additional input (n = 88) and the non-participants (n = 92). In comparison to the direct sample, subjects from the additional input had poorer health and were more often institutionalised. In contrast, non-participants reported less disability and equal or better health. Subjects from both the additional input and the non-participants reported more often a poor mood.

Differences in characteristics disappeared when we compared the direct sample (n = 511, response 74 percent) and the total sample after the additional recruitment stage (n = 599, response 87 percent) with the source population (n = 691), table 2. Socio-demographic and health characteristics in the source population did not differ from the estimates of these characteristics within the direct and the total sample, except the estimated prevalence of a poor mood.

Figure 1 shows survival by participation status. The 88 subjects who entered the study after the additional recruitment stage had a significantly lower survival compared to the 511 subjects who were directly included (p = 0.04). Survival of the 92 non-participants did not differ from the survival of the direct sample. After inclusion of the additional input with the direct sample (resulting in the total sample) survival functions overlapped (figure 2). Survival of the direct sample as well as survival of the total sample was equal with survival of the source population.

Table 1 *Prevalence estimates and 95% confidence intervals of characteristics by participation status, Leiden 85-plus Study (1997-1999)*

	Direct sample (n=511)			Additional input (n=88)			Non-participants (n=92)		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Socio-demographics									
Women	335	66%	60-71	62	70%	61-80	72	78 %	70-87*
Institutionalised	82	16%	13-19	26	30%	20-39†	10	11 %	5-17
Widowed	292	57%	53-61	53	60%	50-70	64	70 %	60-79
Low SES	185	36%	32-40	32	36%	26-46	37	40 %	30-50
Self report‡									
Difficulties ADL	208	41%	37-45	45	53%	41-62	20	25 %	16-34†
Poor Health	136	27%	23-31	34	40%	30-51*	17	21%	12-30
Not satisfied	86	17%	14-21	18	24%	14-34	11	14%	6-21
Nurse's Impression‡									
Poor daily functioning	233	46%	41-50	53	60%	50-70*	36	44%	34-55
Poor cognition	183	36%	32-40	53	60%	50-70†	29	36%	25-46
Poor mood	20	4%	2-6	12	14%	7-21†	23	28%	19-38†

** P<0.05 for difference compared with direct sample using Chi square test. † P<0.005 for difference compared with direct sample using Chi square test. ‡ Prevalence estimates are based on total numbers after correction for missing data (11 non-participants refused any contact); When additional input and non-participants were combined, no significant difference with direct sample existed, except for mood.*

Table 2 *Prevalence estimates and 95% confidence intervals of characteristics of the source population and both samples of participants, Leiden 85-plus Study (1997-1999)*

	Source population (n=691)		Direct sample†, 74% response (n=511)			Total sample, 87 % response (n=599)		
	n	%	n	%	95% CI	n	%	95% CI
Socio-demographics								
Women	463	67%	335	66%	61-70	397	66%	62-70
Institutionalised	118	17%	82	16%	13-19	108	18%	15-21
Widowed	403	58%	292	57%	53-61	345	58%	54-62
Low SES	251	36%	185	36%	32-40	217	36%	32-40
Self report‡								
Difficulties ADL	273	40%	208	41%	37-45	253	42%	38-46
Poor Health	187	28%	136	27%	23-31	170	29%	25-33
Not satisfied	115	18%	86	17%	14-21	104	18%	15-21
Nurse's Impression‡								
Poor daily functioning	322	47%	233	46%	41-50	286	48%	44-52
Poor cognition	265	39%	183	36%	32-40	236	39%	35-43
Poor mood	55	8%*	20	4%	2-6	32	5%	4-7

**Significant difference, "true" estimate beyond 95% confidence interval of the estimates from direct and total sample. † Note that subjects of the direct sample are also part of the total sample. ‡ Prevalence estimates are based on total numbers after correction for missing data (11 non-participants refused any contact).*

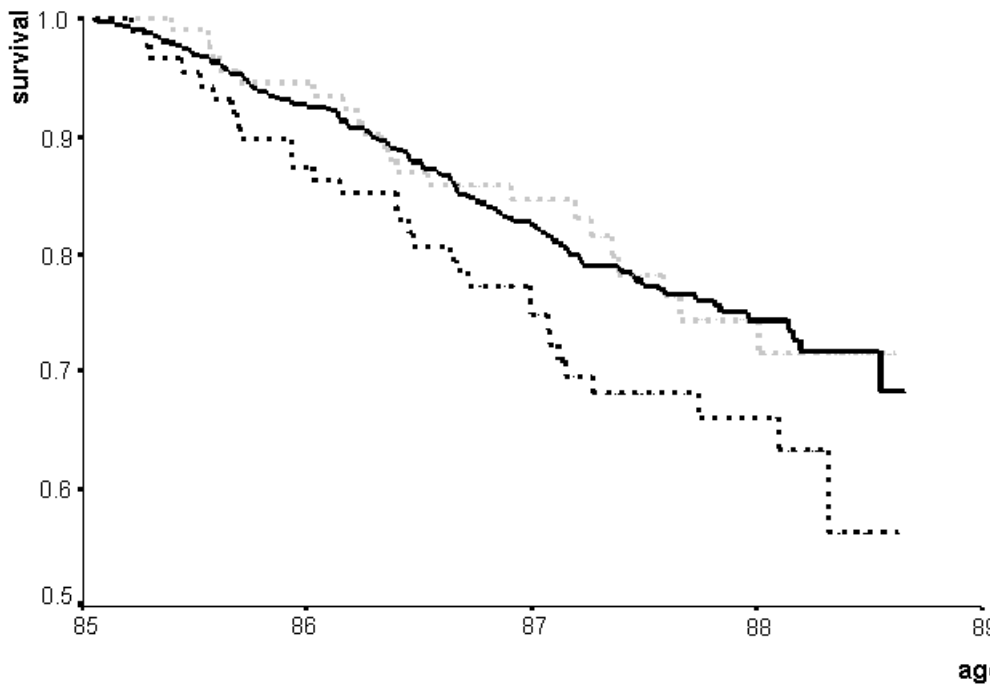

Figure 1 *Cumulative survival for subgroups from the source population.*
Kaplan-Meier estimates of cumulative survival in the participants of the direct sample (n = 511) (continuous line), the additional input (n = 88) (black dotted line) and the non-participants (n = 92) (grey dotted line), Leiden 85-plus Study (1997-1999).

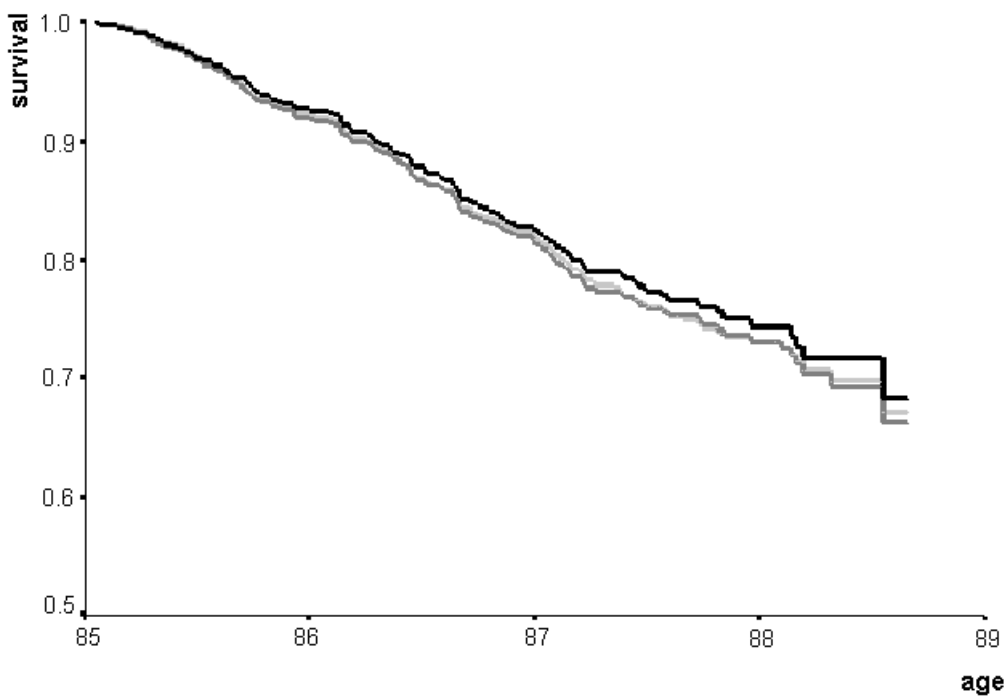

Figure 2 *Cumulative survival for the source population and both samples of participants Kaplan-Meier estimates of cumulative survival in the direct sample (n = 511) (black line), the total sample (n = 599) (dark-grey line) and in the source population (n = 691) (light-grey line), the Leiden 85-plus Study (1997-1999).*

Discussion

The design of our study in which virtually all subjects from the source population were visited at their place of residence, gave us the unique opportunity to compare characteristics of subjects from different samples of participants with all the subjects from the source population, including the non-participants. We tested the hypothesis that the additional effort to increase the response rate would diminish selection bias. We found that the direct sample with a response rate of 74 percent was representative for the source population on baseline characteristics and mortality. With the additional recruitment stage we included frail subjects as shown by a lower survival rate. However, the total sample with a response rate of 87 percent remained representative of the source population. We found that the additional effort to increase the response rate from 74 to 87 percent did not necessarily prevent selection bias. On the contrary, we found that selection bias might have been induced by this effort.

Using data from the Leiden 85-plus Study, we showed that after achieved a representative direct sample with a moderate high response rate, the additional input was a selection of more frail elderly. We used rather crude outcome measures to compare the samples not only on demographic, but also on disabilities, health, and well-being. Using more sensitive measures would not have altered our conclusions that in this population of oldest old additional effort was not necessary to prevent selection bias.

Few studies have mentioned the representativeness of a first wave of recruitment[12] and the possibility of selective additional input[10,28]. Most studies find that particularly frail elderly participate less often in health surveys. Non-participants are described as having a higher age, lower social economic status, lower health status, more depressive mood, lower cognition, and higher morbidity and mortality[1,7,19,29,30]. We found that non-participants were more often depressed but on other characteristics had equal or better health. One could argue that the nurse impression of the mood of non-participants was biased through disappointment and that validation of this impression was done in participants only. However, the high prevalence of poor mood in non-participants is supported by a high proportion (19 percent) of the non-participants who reported depressive symptoms like being too nervous or anxious as the reason for not participating. Moreover, the finding of equal or better health of non-participants might be biased by socially desirable answers[36], since non-participants may have used good health as a reason to support their decision not to participate.

We invested much time and effort in obtaining a very high response rate. The high response rate in our study was due to the personal approach, but other factors of our study design also contributed. Due to the wide publicity our study received, inhabitants of the municipality of Leiden anticipated their 85th birthday letter and felt privileged to belong to the "oldest old". Other factors that might have increased the direct response rate were the involvement of medical staff and nurses instead of lay interviewers, face-to-face interviews at the place of residence[9,10], and an oral informed consent. We think it is conceivable that subjects perceive a written informed consent as a binding contract and therefore refuse to sign anything[31]. The self-confidence and skill in using appropriate heuristics of our expert research

nurse[10], her knowledge of the local situation, and her experience in home care for the elderly were very helpful in achieving a high additional response. Also the enthusiasm of a small research team, where a refusal was felt as a failure might have contributed to the high response rate.

Our design was very time consuming and enabled us to obtain essential information from virtually all eligible subjects. Moreover, survival also appeared to be a good measure to compare subjects by participation status. Mortality is an unbiased outcome that is easily available for both participants and non-participants. Survival rates gave us insight into health differences and comparability of the different samples[16,32,33]. We therefore suggest a wider use of survival as a measure to compare the study population with the source population.

In conclusion, the approach of eligible subjects and the inclusion procedure of population studies are the crux of valid research. We demonstrated that an additional intensive and personal approach is rewarding for drawing more frail elderly subjects into a study. However, this effort will only diminish selection bias if the frail elderly are underrepresented in the direct sample. With an appropriate and conscientious approach the direct sample might already be representative, making additional efforts to increase the response rate to very high levels not necessary.

Appendix

The research nurse recorded her impression on a subject's daily functioning, cognition and mood in a standardised questionnaire at the end of the visit. We compared the scores from these four-point scales (very good, good, poor, very poor) with the scores of the corresponding validated questionnaires on daily functioning, cognition and mood from the main interview as assessed by another member of the medical staff. As the distributions of data were skewed to the left, groups were compared with non-parametric tests that do not assume an underlying normal distribution of the data. As the non-parametric equivalent of the one-way ANOVA procedure, we used the Jonckheere-Terpstra test to determine the p-value for trend between the scores of the questionnaires and the four categories (very good, good, poor, very poor) of the nurse's impression.Results are shown in figures 3A,3B and 3C. The median score for each validated measure showed a gradual and significant ($p < 0.001$) decline or rise over the four categories of the corresponding nurse's impression.

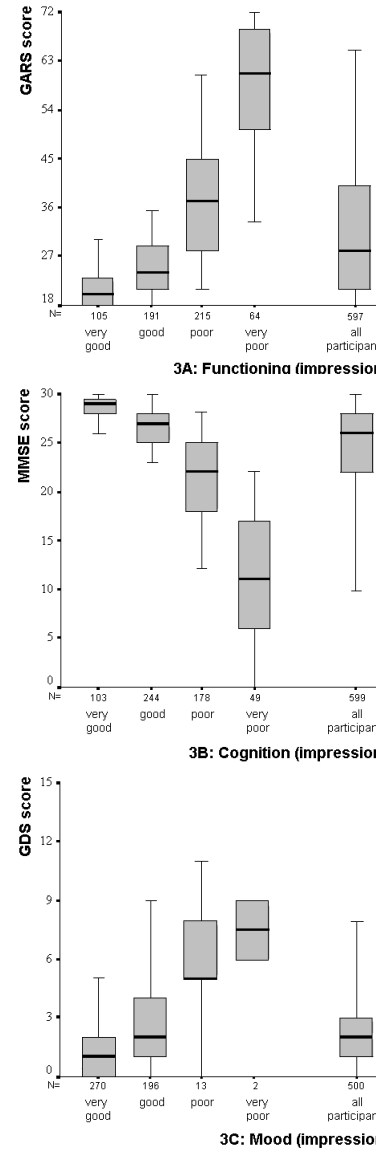

Figure 3 *Comparison between nurse's impression and validated questionnaires*
Comparison of the nurse's impression about daily functioning (A), cognition (B) and mood (C) with test scores on corresponding validated questionnaires within the total sample of participants in the Leiden 85-plus Study (1997-1999). The boxplots show the median (thick line), interquartile range (box) and all values within 5th and 95th percentile.

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Chapter 3

Cognitive function in the “oldest old”: Women perform better than men

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ABSTRACT

Background Limited formal education is associated with poor cognitive function. This could explain gender differences in cognitive function in the oldest old. We explored if limited formal education explains differences on cognitive function between elderly women and men.

Methods The Leiden 85-plus Study is a population-based study investigating all 85-year-old inhabitants of Leiden with an overall response rate of 87%. A sample of 599 participants were visited at their place of residence. The Mini-Mental State Examination was completed by all participants. Cognitive speed and memory were determined with four neuro-psychological tests in participants with a Mini-Mental State Examination score higher than 18 points.

Results The proportion of women with limited formal education was significantly higher than that of men (70% versus 53%, p=0.001), but women had better scores for cognitive speed and memory than men ($p<0.05$). After adjustment for differences in limited formal education and the presence of depressive symptoms, the odds ratio for women to have a higher cognitive speed than men was 1.7 (95% CI; 1.0 to 2.6), and for them to have a better memory the odds ratio was 1.8 (95%CI; 1.2 to 2.7).

Conclusion We found that women have a better cognitive function than men, *despite* their lower level of formal education. We therefore conclude that limited formal education alone can not explain the differences in cognitive function in women and men. These findings support our alternative hypothesis that biological differences, such as atherosclerosis, between women and men account for the gender differences in cognitive decline

Introduction

The impressive body of knowledge on cognitive function that has been accumulated leaves many questions on the effect of gender on cognitive function unresolved. One explanation for a possible effect of gender on cognitive function could be that more elderly women have received a limited formal education when compared to men. A limited formal education is associated with less cognitive function. The "brain reserve capacity theory" argues that subjects with less cognitive function i.e. less brain reserve capacity, are more likely to surpass the threshold beyond which cognitive decline becomes clinically apparent[1,2]. An alternative explanation could be that different biological mechanisms cause differences on cognitive function in elderly women and men.

By measuring cognitive speed and memory, cognitive function in elderly persons can reliably be assessed. Cognitive speed, consisting of attention span and processing speed, is the most sensitive measure because age-related cognitive decline first manifests itself by a decline in attention span and processing speed[3,4]. In old persons memory remains relatively intact until late stages of cognitive decline, while cognitive speed declines more rapidly[4].

We measured cognitive speed and memory in a population-based sample of women and men aged 85 years. Our aim was to explore whether there is an effect of gender on cognitive function and whether differences in limited formal education explain differences on cognitive function between elderly women and men. If this hypothesis is true we would expect women to have a poorer cognitive function than men because of the limited formal education they have received.

Methods

Subjects and procedures

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 study in the month of their 85th birthday. Those who were eligible for the study were informed about the study by mail. Then they were 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.
Socio-demographic characteristics and living arrangements were obtained for all subjects eligible to participate in the study. The Mini-Mental State Examination[5] was administered to screen for cognitive impairment. Subjects were classified as severely cognitive impaired defined by a Mini-Mental State Examination score of 18 points or lower[6]. Education was divided into two levels: a lower education level, including participants 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). Since depression could lead to cognitive impairment, we used the Geriatric Depression Scale (GDS-15)[7] to adjust for the effect of depressive symptoms on cognitive function. A score of four points or above on the Geriatric Depression Scale indicates that the presence of depression is likely.

To further investigate the various domains of cognitive function we used four neuropsychological tests that are widely used in observational studies in and outside the Netherlands. These tests proved to have clinical relevanc[8]. Cognitive speed was measured with two neuropsychological tests, the abbreviated 40-item version Stroop test (attention)[9,10] and the Letter Digit Coding test (processing speed)[11] For data analysis we made use of the third Stroop card showing color words printed in ink of different colors. Memory was measured with the 12-Word Learning test[12,13], testing immediate and delayed recall. All neuro-psychological tests were administrated by the same trained research nurse, who gave her impression whether the tests went well and whether the test scores could be trusted to reflect the subjects ability to perform the test at that time.
The Geriatric Depression Scale and the neuropsychological tests were not administered in subjects with a Mini-Mental State Examination score of 18 points or lower, because in these subjects neither depressive symptoms, nor cognitive speed or memory can be accurately assessed[6,11].

Data analysis
Data are presented as medians and interquartile ranges. Groups were compared with non-parametric tests (Chi square test and Mann Whitney test) that do not assume an underlying distribution of the data, since the test scores on the Mini-Mental State Examination and the delayed Word Learning test were skewed to the left. Confidence intervals for differences between medians were calculated assuming that the data in groups were both skewed in a similar direction[14]. Univariate odds ratios and 95% confidence intervals were obtained by cross-tabulation.

We compared elderly persons with a good cognitive speed with those who had a poor cognitive speed, and we compared elderly persons with a good memory to those who had a poor memory, using dichotomous variables, good and poor cognitive speed and good and poor memory. Good cognitive speed was defined as a score above the median on both the Stroop test and the Letter Digit Coding test. Poor cognitive speed was defined as a score below the median on either the Stroop or 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 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 for the analyses.

Multivariate odds ratios were obtained by logistic regression analysis, adjusting for unequal distributions of the number of depressive symptoms and level of education, between women and men. In all analyses speed and memory, as dichotomized variables, were the dependent variables. Gender, level of education and the presence of depressive symptoms were the independent variables

Results

Between September first 1997 and 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 in the study. The response rate was 87%, a total of 599 subjects (397 women, 202 men) participated. There were no significant differences between the 92 non-respondents and the 599 respondents for various demographic characteristics apart from a slightly skewed sex-ratio (72 women refused whereas 61 was expected, p=0.02)

Table 1 shows the demographic and clinical characteristics of the participants. Women were significantly more institutionalised (p=0.01), more often widowed (p=0.001) and had a lower formal education level than men (p=0.001). The median score on the Mini-Mental State Examination was 26 points and similar in women (interquartile range 21 to 28) and men (interquartile range 23 to 28). Significantly more women than men (20% versus 9%) had severe cognitive impairment, defined as a Mini-Mental State Examination score of 18 points or lower. The distribution of depressive symptoms was similar in women and men.

316 women and 184 men had a Mini-Mental State Examination higher than 18 points or more and were further characterised for cognitive function using the neuro-psychological tests. In 27 women (8.5%) and 27 men (14.7%) the neuropsychological tests to measure cognitive speed and memory could not be completed. 18 subjects did not complete the tests because of visual impairment, 20 subjects refused to execute the neuropsychological tests, and 16 subjects did not understand the instructions as given by the research nurse, due to cognitive impairment. There were no demographic or clinical differences between the participants who were able and those who were unable to complete the neuropsychological tests (data not shown).

Table 2 presents the data on cognitive speed for women and men. Women completed the Stroop test more quickly than men (p=0.01). The median test score on the Letter Digit test was similar for women and men. Table 3 presents data on memory. Women remembered more words than men on the immediate Word Learning test (p=0.001). Women had the same test score as men on the delayed Word Learning test. Participants with a higher level of education had significantly higher scores on the tests measuring cognitive speed (p<0.001). Participants without depressive symptoms scored significantly better on all tests (p<0.001). The effects of formal education and depression on cognitive function were similar in women and men (data not shown).

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

Characteristic	Women (n=397)	Men (n=202)
Living arrangements*		
Independent	312 (79%)	177 (88%)
Institutionalised †	85 (21%)	25 (12%)
Marital status*		
Married	70 (18%)	128 (63%)
Unmarried	28 (7%)	10 (5%)
Widowed	283 (71%)	62 (31%)
Divorced	16 (4%)	2 (1%)
Education*		
Low level	279 (70%)	107 (53%)
High level	114 (29%)	93 (46%)
Missing	4 (1%)	2 (1%)
MMSE score *		
MMSE 19-30 points	316 (80%)	184 (91%)
MMSE 0-18 points	81 (20%)	18 (9%)
Depressive symptoms		
GDS ≤ 3 points	241 (61%)	140 (69%)
GDS ≥ 4 points	75 (19%)	44 (22%)
GDS not administered ‡	81 (20%)	18 (9%)

MMSE; Mini Mental State Examination, GDS; Geriatric Depression Scale. Chi square test $p< 0.05$. † Instiutionalised were those living in a home for the elderly or those living in a nursing home. ‡ GDS not administered in subjects with a MMSE score of 18 or lower.*

Table 2 *Effect of various determinants on cognitive speed*

	Stroop (seconds)	Letter Digit Test (no. of letters)
Gender		
Female (n=289)	71.8 (58.2 to 95.5)	16.0 (12.5 to 21.0)
Male (n=157)	79.1 (64.8 to 104.2)	16.0 (12.0 to 22.0)
Median difference (95% CI)	-7.3 (-11.7 to –1.3)*	0.0 (-2.0 to 1.0)
Education		
High (n=178)	65.3 (54.9 to 86.4)	19.5 (15.0 to 25.0)
Low (n=268)	81.7 (62.7 to 104.0)	14.0 (10.0 to 18.0)
Median difference (95% CI)	-16.4 (-17.1 to –7.3)*	5.5 (5.0 to 7.0)*
Depression		
No (n=349)	72.3 (57.7 to 92.8)	17.0 (13.0 to 22.0)
Yes (n= 97)	85.9 (65.1 to 115.8)	14.0 (9.0 to 18.0)
Median difference (95% CI)	-13.6 (-20.8 to –7.3)*	3.0 (2.0 to 5.0)*

*Median scores and interquartile ranges. 95% CI: 95 % Confidence interval. * p <0.05*

Table 3 *Effect of various determinants on memory*

	Immediate Word Learning Test (no. of words)	Delayed Word Learning Test (no. of words)
Gender		
Female (n=289)	26.0 (21.0 to 29.0)	9.0 (8.0 to 11.0)
Male (n=157)	23.0 (20.0 to 27.0)	9.0 (7.0 to 10.0)
Median difference (95% CI)	3.0 (1.0 to 3.0)*	0.0 (0.0 to 1.0)
Education		
High (n=178)	25.0 (21.0 to 29.0)	9.0 (8.0 to 11.0)
Low (n=268)	25.0 (21.0 to 28.0)	9.0 (7.0 to 11.0)
Median difference (95% CI)	0.0 (0.0 to 2.0)	0.0 (0.0 to 1.0)
Depression		
No (n=349)	26.0 (22.0 to 29.0)	9.0 (8.0 to 11.0)
Yes (n= 97)	22.0 (18.0 to 26.5)	8.0 (6.0 to 10.0)
Median difference (95% CI)	4.0 (1.0 to 4.0)*	1.0 (1.0 to 2.0)*

*Median scores and interquartile ranges. 95% CI: 95 % Confidence interval. * p <0.05*

To further explore the gender differences in cognitive function we categorised participants as having a good or poor cognitive function based on test scores dichotomised around the median. Good cognitive speed was found in 33% of the women and 28% of the men. 41% of the women and 29% of the men had a good memory. Table 4 shows the crude and adjusted odds ratios for good cognitive speed and memory in women versus man. Odds ratios were obtained in participants with a Mini-Mental State examination score higher than 18 points. The differences between women and men became more apparent and statistically significant after adjustment for unequal distributions of depressive symptoms and formal education. Marital status could not explain for the differences between the sexes. Similar odds ratios were obtained when the sample was further restricted to participants with Mini-Mental State Examination scores between 28 and 30 points (data not shown). When we evaluated all participants Mini-Mental Examination Score between 0 and 30 points, attributing a poor cognitive speed and memory to those who for cognitive reasons were unable to perform the neuropsychological tests, we also obtained similar crude odds ratios.

Table 4 *Odds ratios for good cognitive speed and good memory in women versus men.*

Test	Crude odds ratio (95%Confidence interval)	Adjusted odds ratio* (95%Confidence interval)
Cognitive Speed	1.3 (0.8 to 1.9)	1.7 (1.0 to 2.6)
Memory	1.6 (1.1 to 2.5)	1.8 (1.2 to 2.7)

*Odds ratios obtained in participants with a MMSE score >18 points. * Adjusted for the level of education and the presence of depressive symptoms.*

Discussion

The aim of the present study was to explore whether there is an effect of gender on cognitive function and whether a limited formal education explains differences on cognitive function between elderly women and men. We found that women have a better cognitive function than men, *despite* their lower level of formal education. This effect is far greater than the sex differences that are generally reported at an earlier age[15]. We therefore conclude that limited formal education alone can not explain the differences in cognitive function in women and men. These findings support our alternative hypothesis that biological differences between women and men could account for the gender differences in cognitive impairment.

Previous studies have described associations between limited formal education, poor cognitive function, and susceptibility to develop dementia[2]. Within the Leiden 85-plus Study, participants with low levels of education also had poorer test scores on the neuropsychological tests. We have earlier reported that elderly persons with poor cognitive function are characterised by an accelerated decline in cognitive function[16]. In line with the "brain reserve theory" these persons are thus more likely to develop dementia. However, the brain reserve theory cannot explain the gender differences in cognitive decline as elderly women have better preserved their cognitive function than men.

The neuropsychological tests that were used in the present study could not be administered to participants with severe cognitive impairment. To ascertain that our findings also hold when the population is studied as a whole, we attributed a poor cognitive speed or memory to those who for cognitive reasons were unable to perform the neuropsychological tests. The results were not affected. We also studied the subgroup of participants, who were clinically free from cognitive impairment, i.e. Mini-Mental State Examination scores from 28 to 30 points. We again found that women had a better cognitive speed and memory.

It is tempting to speculate that biological mechanisms, such as atherosclerosis could account for the gender differences in cognitive decline. Cerebrovascular disease, a late stage of systemic atherosclerosis, is highly prevalent among elderly persons. Several studies have suggested that atherosclerosis causing subclinical, ischaemic events in the brain contribute to cognitive decline at old age[17-20]. In this respect it is noteworthy that the accelerated increase of cardiovascular disease at old age starts some ten years later in women than in men. This delay is reflected by the difference in life expectancy between women and men[21]. The greater life expectancy for women indicates that in comparison with men, elderly women of the same age are relatively free from cardiovascular disease[22]. We hypothesise that the relative absence of cardiovascular disease may explain the better cognitive functioning of old women.

Several population-based studies have shown that the prevalence of dementia in women older than 80 is higher than that in men[23-25]. The higher prevalence of dementia can be explained by the finding that the mortality rate in patients with dementia is lower in women than that in men[26]. The lower mortality rate in women who suffer from dementia, explains why we found a higher proportion of women with severe cognitive impairment among the oldest.

In conclusion, our study shows that despite a lower level of education women have better cognitive function than men. Differences in the level of education in women and men can not explain the differences in cognitive function. The better cognitive function in women is more likely to be explained by a biological mechanism, such as atherosclerosis.

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Chapter 4

Atherosclerosis and cognitive impairment are linked in the elderly. The Leiden 85-plus Study

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In press Atherosclerosis

ABSTRACT

Background Post-mortem analyses suggest that atherosclerosis more often contributes to late-onset dementia than hitherto expected. We set out to further unravel the relation between atherosclerosis and cognitive impairment. We therefore tested the hypothesis that number the of cardiovascular pathologies is positively associated with cognitive impairment in elderly subjects, and that the smaller number of cardiovascular pathologies in women explains for the better cognitive function of elderly women.

Methods Within the Leiden 85-plus Study, we assessed the atherosclerotic burden by counting the number of cardiovascular pathologies in the medical histories of a population-based sample of 599 subjects aged 85 years (response 87%).

Results Significantly more men than women had a history of cardiovascular pathologies (67% compared to 59%, $p<0.001$). In addition, cognitive function was assessed. All subjects completed the Mini-Mental State Examination (MMSE). Cognitive speed and memory were determined with specific neuro-psychological tests in those with a MMSE-score above 18 points. There was a highly significant dose-response relationship between the number of cardiovascular pathologies and cognitive impairment for both men and women. The median MMSE-score was 26 points in subjects without cardiovascular disease and decreased to 25 points for subjects who had two or more cardiovascular pathologies (p for trend =0.003). Similar associations were found for cognitive speed but not for memory.

Conclusion Our data confirm that at old age atherosclerosis significantly contributes to cognitive impairment. Since treatments for atherosclerosis appear to be particularly effective in elderly people, we consider our finding of utmost clinical importance to possibly preventing cognitive impairment and late-onset dementia.

Introduction

The risk of dementia is increased in patients who have suffered a stroke [1,2]. Moreover, recent post mortem analyses suggest that cerebrovascular disease more often contributes to late-onset dementia than hitherto expected [3,4]. Examination of brains of patients diagnosed with late-onset dementia, as well as the brains of patients with autopsy proven Alzheimer's disease, often show multiple vascular lesions when compared to brains of subjects without dementia. Furthermore, amyloid plaques and tau pathology are often found in the brains of elderly people who did not suffer from dementia [4]. Taken together, these post mortem correlates suggest that the various entities of late-onset type dementia are not mutually exclusive and that the differences between vascular dementia and Alzheimer's disease are not distinct. A unifying hypothesis is that atherosclerotic changes of heart, peripheral and cerebral arteries cause clinical and subclinical ischemic disease in the brain contributing to the development of late onset dementia [5]. Such a multicausal interpretation of the observational data provides an explanation for the experimental finding that treating systolic hypertension at old age decreases the risk of both clinically diagnosed vascular dementia and Alzheimer's disease [6].

In an earlier study, we found that elderly women had better cognitive function than men [7]. This is highly remarkable as women born at the beginning of the 20th century have, in general, lower levels of formal education than men, which increases the risk of late-onset dementia [8]. Women have on average a lower atherosclerotic burden compared to men of the same age [9,10]. Evidence indicates that elderly women and men with atherosclerosis are more likely to be cognitively impaired [11-14]. Therefore, differences in atherosclerotic burden between women and men might explain the gender differences in cognitive function at old age.

To further unravel the relation between atherosclerosis and cognitive impairment at old age, we assessed the atherosclerotic burden by counting the number of cardiovascular pathologies in the medical history and assessed cognitive function in a large population-based sample of 85 year olds. We also analyzed the data to test the hypothesis that a lesser atherosclerotic burden in women may explain for the better cognitive function of women at old age.

Methods

Subjects

The Leiden 85-plus Study is a population-based study of inhabitants of Leiden, in 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 related to health or demographic characteristics. Subjects were visited three times at their place of residence. During these visits, face-to-face interviews were conducted and an electrocardiogram was recorded. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Cognitive impairment

The Mini-Mental State Examination (MMSE) was administered in all subjects [15]. We defined good cognitive function as a score on the MMSE equal or above 28 points; cognitive impairment was defined as an MMSE-score below 28 points. A cut-off point of 28 points on the MMSE was used, since there is strong evidence that subjects aged 85 years with MMSE scores of 28 points or higher are cognitively intact [16].

To further investigate the various domains of cognitive function, we used a set of four neuro-psychological tests that are widely utilized in observational studies and have proven clinical value [17]. Cognitive speed was measured with two neuro-psychological tests, the Letter Digit Coding test (processing speed) and an abbreviated 40-item version of the Stroop test (attention). Memory was measured with the 12-Word Learning test, assessing immediate and delayed recall. The neuro-psychological tests were not conducted in subjects with an MMSE score of 18 points or lower, because in these subjects neuro-psychological tests cannot be accurately administered [18]. All neuro-psychological tests were conducted by the same trained research nurse, who gave her impression of whether the tests went well and whether the test scores reflected the subject's ability to perform the test at that time.

As described earlier, subjects were defined as having a good cognitive speed when they had a score below the median on the Stroop test and a score above the median on the Letter Digit Coding test [7]. Subjects were defined as having poor cognitive speed when they had a score above the median on the Stroop test 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 MMSE scores of 18 points and lower and subjects who, for cognitive reasons, were unable to complete some of the neuro-psychological tests were also classified as having a poor cognitive speed and memory [7]. Subjects who, for other reasons such as low vision, or physical disabilities, were unable to complete the set of neuro-psychological tests were excluded from the analyses.

Atherosclerosis

All subjects' general practitioners or subject's nursing home physicians were interviewed to assess the medical history for number of cardiovascular pathologies for each subject. In addition, electrocardiograms were recorded on a Siemens Siccard 440 and transmitted by telephone to the ECG Core Lab in Glasgow for automated Minnesota coding [19].

In line with the Second Manifestations of Arterial disease (SMART) study [20] the atherosclerotic burden of each subject was expressed by the total number of cardiovascular pathologies. Each cardiovascular pathology was classified into one of five categories: (1) arterial surgery, (2) stroke, (3) intermittent claudication, (4) myocardial infarction, or (5) angina pectoris or myocardial ischemia. Arterial surgery,

stroke, and intermittent claudication were considered present when there was a positive medical history. Myocardial infarction was considered present when there was a positive medical history of myocardial infarction or when the ECG revealed myocardial infarction (Minnesota codes 1-1, 1-2, or 1-3) [20]. Subjects with a medical history of angina pectoris or myocardial ischemia on the ECG (Minnesota codes 4-1, 4-2, 4-3, 5-1, 5-2 and 5-3) [21] were also classified as having one cardiovascular pathology. In addition, subjects with both a medical history of myocardial infarction and myocardial ischemia on the ECG were classified as having myocardial infarction only, not myocardial ischemia [21]. Transient ischemic attacks were not included in the cardiovascular pathologies, since they may not always be distinguished from other disorders such as migraine, epilepsy, or syncope.

We classified the atherosclerotic burden of subjects by counting the total number of cardiovascular pathologies they had; i.e. subjects without cardiovascular pathology, subjects with one cardiovascular pathology, and subjects with two or more cardiovascular pathologies.

Possible confounders

Socio-demographic characteristics such as gender or level of education were considered as possible confounders. 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). We used the 15-item Geriatric Depression Scale [22] to adjust for the effect of depressive symptoms on cognitive function. A score of four points or above on the Geriatric Depression Scale indicates that the presence of depression is likely. The Geriatric Depression Scale was not administered in subjects with a Mini-Mental State Examination score of 18 points or lower, because in these subjects depressive symptoms cannot be accurately assessed [23]. We also had to ascertain that our findings were not due to the use of cardiovascular drugs or non-steroidal anti-inflammatory drugs. These drugs, which are mainly prescribed for cardiovascular disease and arthritis, may also affect cognitive function [24,25]. The use of cardiovascular drugs (diuretics, calcium channel blockers, beta blockers, digoxin, nitrates) and non-steroidal anti-inflammatory drugs was obtained from the records of subjects' pharmacists.

Data analysis

Because the MMSE has a maximum score of 30 points and the delayed Word Learning test has a maximum of 12 words, the distributions of both tests were skewed to the left. Data are thus presented as medians with corresponding confidence intervals for the median. A confidence interval for the median represents the range of values that include the "true" median [26]. Groups were compared with non-parametric tests that do not assume an underlying normal distribution of the data. As the non-parametric equivalent of the one-way ANOVA procedure, we used the Jonckheere-Terpstra test [27] to determine the p-value for trend between the scores of the cognitive tests and the strata representing an increasing number of cardiovascular pathologies. In a secondary analysis, we used dichotomous endpoints, respectively cognitive impairment (MMSE score <28 points) versus good cognitive function (MMSE score ≥ 28 points), poor versus good cognitive speed (Stroop and Letter Digit test) and poor versus the

MMSE score 28 points or higher), poor versus good cognitive speed (Stroop and Letter Digit test) and poor versus good memory (immediate and delayed recall on the Word Learning test).]. In these analyses we compared the cognitive function of elderly subjects over the strata representing an increasing number of cardiovascular pathologies. Statistical significance was assessed by the linear association test to determine the p value for trend. Univariate odds ratios and 95% confidence intervals were obtained by cross-tabulation. Multivariate odds ratios were obtained by logistic regression analysis, adjusting for possible confounders.

Results

Between 1 September 1997 and 1 September 1999, 705 inhabitants of Leiden reached the age of 85 years and became eligible for inclusion in the study. Fourteen inhabitants died before they could be enrolled. Of the remaining 691 subjects, a total of 599 subjects participated (response 87%). The proportion of subjects with high education was 46% in men and 29% in women ($p<0.001$). There were no significant differences for various demographic characteristics between the 599 participants and the source population.

For three subiects we had no information on the presence of the cardiovascular pathologies in medical history. Of the remaining 596 subjects, table 1 shows the prevalence of cardiovascular pathologies. More than half of all subjects (62%) had a medical history of cardiovascular disease. Men had significantly more cardiovascular pathologies than women (table 1).

Table 1 *Prevalence of cardiovascular pathologies in participants of the Leiden 85-plus Study.*

Characteristic	All (n=596)	Men (n=201)	Women (n=395)
Myocardial infarction	139 (23%)	59 (29%)	80 (20%)*
Angina or myocardial ischemia †	277 (46%)	89 (44%)	188 (48%)
Stroke	62 (10%)	22 (11%)	40 (10%)
Arterial surgery	40 (7%)	26 (13%)	14 (4%)*
Intermittent claudication	37 (6%)	22 (11%)	15 (4%)*
Number of cardiovascular pathologies			
No cardiovascular pathology	227 (38%)	66 (33%)	161 (41%)**
one cardiovascular pathology	204 (34%)	65 (32%)	139 (35%)
two or more cardiovascular pathologies	165 (28%)	70 (35%)	95 (24%)

*† diagnosed by a positive medical history of angina pectoris (n=108) or ECG revealed ischemia (Minnesota codes 4-1, 4-2, 4-3, 5-1, 5-2, 5-3). * Chi square tests between men and women, df=1, p <0.05.** Chi square tests between men and women, df=2, p <0.05*

One might argue that physicians tend to diagnose cardiovascular disease more frequently in men than in women. For that reason, we also compared the proportion of men and women with a silent myocardial infarction, i.e. a myocardial infarction that was not recorded in subjects' medical history, but was revealed by ECG only. There was no significant difference in the proportion of men and women with silent myocardial infarctions (16% vs. 13%, chi-square p=0.4).

Severe cognitive impairment (MMSE ≤ 18 points) was present in 99 (16.6%) subjects, whereas the other 497 subjects had a MMSE score higher than 18 points. The set of neuro-psychological tests to measure cognitive speed and memory could not be completed in 53 subjects because of visual impairment (n=17), technical failure (n=1), refusal (n=31) and persistent misunderstanding of the instructions as given by the research nurse (n=4). The median scores for the tests of cognitive speed and memory showed that, after stratification for level of education, women had better cognitive function than men (Table 2) .

Table 2 *Gender differences in cognitive function, stratified for level of education.*

Characteristic	Men (n=156)	Women (n=288)	p-value
Low level of education (n)	80	187	
Cognitive speed			
Stroop Test (seconds)	89.6 (77.9-101.5)	78.4 (72.3-83.4)	0.008
Letter Digit Test (digits/minute)	14 (12-15)	14 (14-15)	0.2
Memory			
Immediate Word Learning test (words)	23 (21-24)	26 (24-26)	0.003
Delayed Word Learning test (words)	8 (8-9)	9 (9-10)	0.05
High level of education (n)	76	101	
Cognitive speed			
Stroop Test (seconds)	72.6 (64.7-79.1)	63.1 (60.4-66.5)	0.07
Letter Digit Test (digits/minute)	20 (18-23)	19 (18-22)	0.9
Memory			
Immediate Word Learning test (words)	24 (23-26)	27 (24-28)	0.05
Delayed Word Learning test (words)	9 (8-10)	10 (9-10)	0.4

Data are presented as medians and corresponding 95% confidence intervals.

The median MMSE score showed a gradual decline over the number of cardiovascular pathologies. Subjects without cardiovascular pathology had a median MMSE score of 26 points, whereas subjects with two or more cardiovascular pathologies had a MMSE score of 25 points (Table 3, p for trend = 0.003). This decline in MMSE score was also present when we stratified for gender and for level of education. The decline remained similar when we excluded subjects who had suffered a stroke (n=62).

Table 3 *Mini-Mental State Examination scores in relation to number of cardiovascular pathologies.*

	Number of cardiovascular pathologies			
	0	1	≥2	p for trend
All subjects (n=596)	26 (26-27)	26 (26-27)	25 (24-27)	0.003
Gender				
Female (n=395)	26 (25-27)	26 (25-27)	24 (23-27)	0.005
Male (n=201)	26.5 (26-28)	27 (26-28)	26 (25-27)	0.1
Education †				
Low (n=384)	25 (24-26)	25 (24-26)	24 (22-26)	0.02
High (n=206)	28 (27-28)	28 (27-29)	27 (26-28)	0.4

Data are presented as medians and corresponding 95% confidence intervals.

** Cardiovascular pathologies; myocardial infarction, angina or ischemia, stroke, arterial surgery, and intermittent claudication. † Level of education was missing for 6 subjects (all had MMSE-scores ≤ 18 points).*

Figure 1 presents the neuro-psychological test scores related to the number of cardiovascular pathologies. Subjects with cardiovascular pathologies performed significantly worse on the tests measuring cognitive speed (Stroop test and Letter Digit Coding test). The median time needed to complete the Stroop test increased from 69.9 seconds in subjects without cardiovascular pathology to 84.2 seconds in subjects with two or more cardiovascular pathologies (p for trend < 0.001). The median number of digits scored on the Letter Digit test in one minute was 17 in subjects without cardiovascular pathology and 15 in subjects with two or more cardiovascular pathologies (p for trend = 0.005). Subjects with cardiovascular pathologies performed not different on the memory tests (immediate and delayed Word Learning test).

increasing number of cardiovascular pathologies. Statistical significance

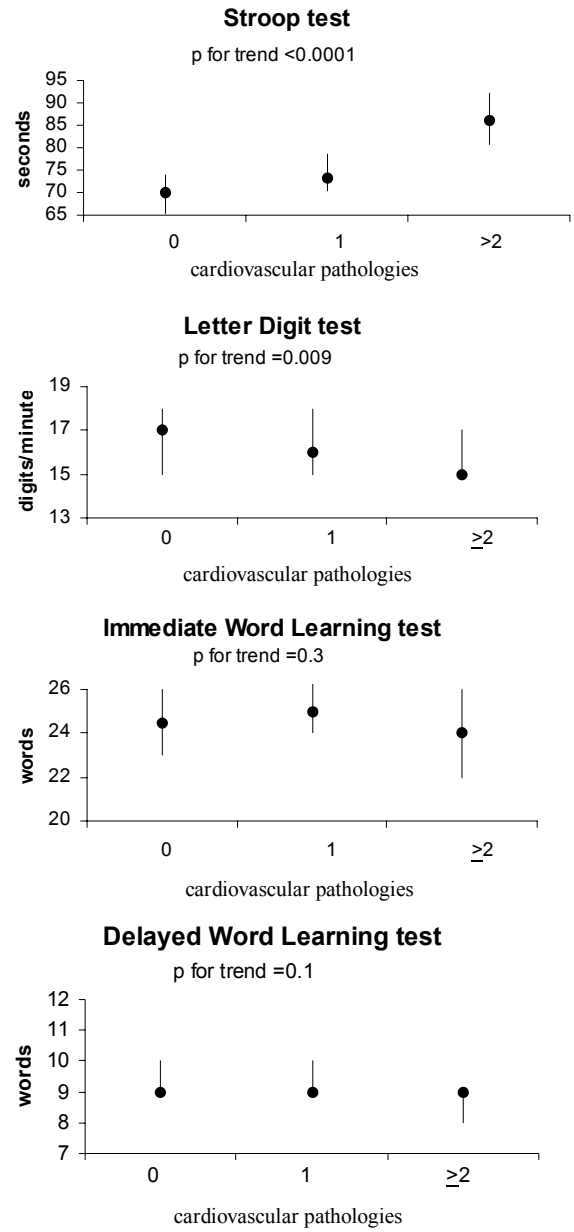

Figure 1 *Test scores of neuro-psychological tests measuring cognitive speed (Stroop test, Letter Digit test) and memory (Immediate and Delayed Word Learning test) dependent on the number of cardiovascular pathologies. Data are presented as medians and corresponding 95% confidence intervals. A confidence interval for the median represents the range of values with a 95% probability of containing the "true" median.*

The odds ratios of cognitive impairment (MMSE <28) and poor cognitive speed gradually increased to 2.0 (95% CI 1.3-3.0) and 2.1 (95% CI 1.0-3.5) over strata of increasing number of cardiovascular pathologies (Table 4). Comparable odds ratios were found for both men and women. Adjusting for possible confounders such as education, depressive symptoms, use of non-steroidal anti-inflammatory drugs, and use of cardiovascular drugs did not alter the odds ratios (data not shown). Memory was not affected by the number of cardiovascular pathologies (Table 4). Since it could be argued that the observed cognitive impairment in subjects with cardiovascular disease was due only to subjects with a medical history of stroke, we did an additional analysis excluding these subjects (n=62). The odds ratio for cognitive impairment (MMSE<28) and poor cognitive speed gradually increased to 1.7 (95% CI 1.0-2.6) and 1.8 (95% CI 1.1-3.2) respectively over increasing number of cardiovascular pathologies.

Table 4 *Odds ratios to have cognitive impairment (MMSE ≤28), poor cognitive speed, or poor memory dependent on the number of cardiovascular pathologies.*

	Number of cardiovascular pathologies			
	0	1	≥2	p for trend
MMSE <28				
All (n=596)	1*	1.2 (0.8-1.8)	2.0 (1.3-3.0)	0.003
Men (n=201)	1*	1.1 (0.6-2.2)	2.1 (1.0-4.3)	0.02
Women (n=395)	1*	1.3 (0.8-2.1)	2.0 (1.1-3.5)	0.04
Poor cognitive speed†				
All (n=547)	1*	1.2 (0.7-1.8)	2.1 (1.2-3.5)	0.07
Men (n=177)	1*	1.6 (0.7-3.5)	3.2 (1.3-7.6)	0.009
Women (n=370)	1*	1.0 (0.6-1.7)	1.8 (0.9-3.4)	0.1
Poor memory‡				
All (n=547)	1*	0.8 (0.5-1.2)	0.7 (0.4-1.2)	0.3
Men (n=177)	1*	1.2 (0.5-2.7)	1.7 (0.7-3.8)	0.6
Women (n=370)	1*	0.6 (0.3-1.1)	0.5 (0.3-0.9)	0.2

Data are presented as crude odds ratios with corresponding 95% confidence intervals.
** Reference category. † Poor cognitive speed (n=303); a score above the median on the Stroop or a score below the median on the Letter Digit test or a MMSE score ≤ 18 points (n=99) or testfailure due to cognitive impairment (n=4). ‡Poor memory (n=281); a score below the median on either the immediate recall test or the delayed recall test or a MMSE score ≤ 18 points (n=99) or testfailure due to cognitive impairment (n=4).*

Discussion

The primary aim of this study was to determine whether elderly subjects with increasing number of cardiovascular pathologies are more likely to be cognitively impaired. We showed that there is a dose-response relationship between the number of cardiovascular pathologies and cognitive impairment in both men and women. The findings of our population-based study confirm the post mortem findings that brains of patients with late-onset dementia more often have multiple vascular lesions [3,4]. It is noteworthy that our findings were unaffected when we excluded subjects with a history of stroke.

The association between atherosclerotic burden and cognitive impairment we present here is far greater than previously reported [12,13]. Studies published so far have primarily tested subjects at a younger age. At age 70, for example, cognitive impairment is relatively rare. Incidence of cognitive impairment and dementia particularly starts rising at 70 years of age and exponentially increases far beyond 80 years [28]. All these findings corroborate the outcomes of studies that associate peripheral atherosclerosis with white matter lesions in the brain, which are presumed to be of vascular origin, leading to cognitive impairment [29-31].

The association between atherosclerotic burden and cognitive impairment was found to be the same for men and women, and similar for those with low and high level of education. However, there were important differences in the atherosclerotic burden between women and men. Men significantly had more often cardiovascular pathologies compared to women. Hence the differences in the *prevalence* of cardiovascular pathologies can thus explain why 85-year old women have better cognitive function than 85-year old men, despite a lower level of education [7]. Paradoxically, the difference in the prevalence of cardiovascular pathologies between women and men may also explain the higher incidence of dementia in elderly women than in men [28,32]. Since women of the same age as men are relatively free from cardiovascular disease, women can only "catch up" with men by surviving them. The higher *incidence* of cardiovascular disease in women aged 80 years and over compared to men in the same age range could therefore contribute to the higher incidence of late onset dementia in women.

Based on findings from the Second Manifestations of ARTerial disease (SMART) study [20], we assessed the atherosclerotic burden by counting the number of cardiovascular pathologies in medical history. In the SMART study, it was demonstrated that presence of cardiovascular pathologies in the medical history was related to intima-media thickness and arterial stiffness, both well-accepted markers of generalized vascular pathology. This apparent association, although elegantly visualized, is not principally different from classifications used in former observational studies [33].

Areas of cognitive function that tend to change as a function of (clinical) events include memory, attention, and general cognitive speed. These are listed among the so-called *fluid abilities* [34]. In the elderly, memory remains relatively intact until later stages of cognitive decline. Cognitive speed, consisting of attention and processing speed, is the most sensitive measure because age-related cognitive decline first manifests itself by a decline in attention and processing speed [35,36]. We found that

atherosclerotic burden was associated with cognitive speed, but not with memory, which is in line with the sequence of cognitive decline. An alternative explanation might be that scores on memory tests typically show larger variances, which makes demonstrating significant differences more difficult. As this study is no exception, with this reduced probability of detecting group differences, it remains possible that age related decline of memory is also associated with atherosclerosis.

A question that may arise is whether 85 years is a proper age to study the effect of atherosclerosis on cognitive impairment. Subjects aged 85 are survivors from a far larger birth cohort and present a selection of the population at large. However, it is most important to know the determinants of cognitive impairment in the population aged 85 and over, since the largest increase in cognitive impairment and dementia occurs after this age. We therefore argue that 85-year-old individuals are an optimal population to study causes of cognitive impairment.

In conclusion, our study shows that there is an apparent dose-response relationship between the number of cardiovascular pathologies and cognitive impairment at old age. We suggest that generalized atherosclerosis at old age significantly contributes to cognitive impairment and late-onset dementia. Atherosclerotic disease, and especially cerebrovascular disease, becomes more and more amenable to medical treatment. Since these treatments appear to be particularly effective in elderly people [37,38], we consider our finding of utmost clinical importance to possibly preventing cognitive impairment and late-onset dementia.

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Chapter 5

Association between high-density lipoprotein and cognitive impairment in the oldest old

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ABSTRACT

Background Low HDL-cholesterol is associated with an increased risk for cardiovascular disease and stroke. At the same time, cardiovascular disease and stroke are important risk factors for dementia. We assessed the association between total- and fractionated cholesterol and cognitive impairment, and explored whether observed associations were dependent or independent of atherosclerotic disease.

Methods In a population-based study, total-cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol were measured in 561 subjects aged 85 years, and grouped in three equal strata representing decreasing serum concentrations. History of cardiovascular disease and stroke was determined. All subjects completed the Mini-Mental State Examination (MMSE) and presence of dementia was determined.

Results Median MMSE scores were significantly lower in subjects with low HDL-cholesterol (25 *vs.* 27 points, $p < 0.001$). No differences in MMSE scores were found for other lipids and lipoproteins. MMSE scores in subjects with and without cardiovascular disease was 26 and 27 points ($p=0.007$) and in subjects with and without stroke 21 and 26 points ($p<0.001$). The associations between low MMSE scores and low HDL-cholesterol remained significant after excluding subjects with cardiovascular disease or stroke. Comparing subjects with low HDL-cholesterol with subjects with high HDL-cholesterol, the odds ratio for dementia was 2.3 (95% CI 1.2-4.3) and in subjects without cardiovascular disease or stroke it was 3.3 (95% CI 1.1-10.3). All odds ratios were unaffected by education, LDL-cholesterol, triglycerides, and survival.

Conclusion Low HDL-cholesterol is associated with cognitive impairment and dementia. At least part of the association between HDL-cholesterol and cognitive function is independent of atherosclerotic disease.

Introduction

Several risk factors for cardiovascular disease, such as hypertension and diabetes mellitus, are linked with cognitive impairment[1,2]. The association of serum total cholesterol concentrations and cognitive impairment, however, has yielded conflicting results[3-7].The amount of evidence relating to a possible association of triglycerides, low-density lipoprotein-cholesterol (LDL-cholesterol), and high-density lipoprotein-cholesterol (HDL-cholesterol) on cognitive impairment is even more limited[5,6,8].

Lipids and lipoproteins may directly affect neurodegeneration. In-vitro studies have shown that both cholesterol and HDL-cholesterol can influence the formation of amyloid-beta[9-12], the main constituent of amyloid plaques. There is also indirect support for a link between HDL-cholesterol and cognitive function via atherosclerotic disease. It has been shown that atherosclerotic disease is associated with clinical and subclinical ischemic diseases in the brain, which contributes to the development of late onset dementia[13,14]. At the same time low serum concentrations of HDL-cholesterol have been associated with an increased risk of stroke[15,16] and patients who suffered a stroke have an increased risk of developing Alzheimer's disease[17].

The primary goal of our study was to examine the association between adverse lipid profiles and cognitive impairment. Furthermore, we explored whether observed associations between individual components of the adverse lipid profiles and cognitive impairment were dependent or independent of atherosclerotic disease.

Methods

Subjects

The Leiden 85-plus Study is a population-based study of inhabitants of Leiden, the Netherlands. There are no selection criteria for health or demographic characteristics. Between September 1997 and September 1999 all members of the 1912 to 1914-birth cohort were contacted by mail in the month after their 85th birthday. They were then contacted by telephone and subsequently visited at home. Subjects were visited three times at their place of residence. At the first two visits face to face interviews were administered and an electrocardiogram was obtained. At the third visit a venous blood sample was drawn. All subjects gave informed consent to participate in the study. For cognitively impaired subjects informed consent was obtained from a guardian. The Medical Ethical Committee of the Leiden University Medical Center approved the study.

Medical history

In the Netherlands general practitioners provide medical care for people of all ages within a small catchment area. Virtually all inhabitants living in the Netherlands register with a general practitioner, who acts as the gatekeeper to further medical care. Hence the general practitioner has a complete medical history of all of his or her patients. The only exception are institutionalised subjects who are treated by a nursing home physician. Five percent of 85-year-olds in Leiden live in a nursing home. All subjects' general practitioners and nursing home physicians were interviewed to obtain a full medical history.

Lipid profile

Serum total cholesterol and triglycerides concentrations were analysed on a fully automated Hitachi 747. HDL-cholesterol was measured with a Hitachi 911. LDL-cholesterol was estimated using the Friedenwald equation: LDL-cholesterol = total cholesterol – HDL-cholesterol – (triglycerides/2.2), whereby 5 subjects with a triglyceride concentration higher than 5 mmol/l were excluded. Concentrations of serum lipids and lipoproteins were grouped into three equal strata representing decreasing concentrations of lipids and lipoproteins. This was done separately for women and men, since women have higher lipids and lipoproteins concentrations than men.

Cognitive function

The Mini-Mental State Examination (MMSE)[18] was administered in all subjects. Cognitive impairment was classified as a MMSE score of 18 points and lower[18]. Clinical diagnosis of dementia was obtained from the medical records of subjects' general practitioner or nursing home physician[19].

Possible confounders

History of cardiovascular disease, history of stroke, and educational level were considered possible confounders. Subjects were classified as having cardiovascular disease when they had a positive history of myocardial infarction, angina pectoris, arterial surgery, or intermittent claudication, as obtained form the medical records. Coronary artery disease was also considered present when the ECG, performed at the home visit, revealed a myocardial infarction (Minnesota codes 1-1, 1-2, and 1-3)[20] or myocardial ischaemia (Minnesota codes 4-1, 4-2, 4-3, 5-1, 5-2 and 5-3)[20]. Subjects were classified as having a history of stroke, when the medical records of the general practitioner or nursing home physician indicated a history of stroke. Subjects were divided into two educational levels: a lower education level (subjects without schooling or with primary school only) and a higher education level (more than 6 years of schooling).

Statistical analysis

Distribution of MMSE-scores was skewed to the left, therefore data are presented as medians with corresponding 95% confidence intervals (95% CI)[21]. Such intervals represent the range of plausible values that include the "true" median. Groups were compared with non-parametric tests that do not assume an underlying normal distribution of the data. As the non-parametric equivalent of the one-way ANOVA procedure, we used the Jonckheere-Terpstra test[22] to determine the p-value for trend between MMSE-scores and the strata representing decreasing lipid and lipoprotein concentrations. Univariate and multivariate odds ratios were obtained by logistic regression analysis.

First, we determined the association between MMSE-scores over strata of decreasing concentrations of lipids and lipoproteins. Second, we determined whether the observed associations were independent of atherosclerotic disease by restricting the analysis to subjects without cardiovascular disease and/or subjects without stroke. Third, the presence of cognitive impairment (MMSE score < 18 ponits and lower) or clinical diagnosis of dementia were used as dichotomous endpoints in a logistic regression

model. All odds ratios were adjusted for level of education. We tested for trend using the log-likelihood statistic with one degree of freedom. In a final analysis we created a restricted sample of subjects who survived the first year of follow-up, and repeated all previous statistical analyses.

Results

Between September 1997 and September 1999 a total of 705 inhabitants of Leiden reached the age of 85 years and were thus eligible for inclusion in the study. Fourteen subjects died before they could be contacted. Of the remaining 691 subjects, 599 subjects participated (response rate 87%). There were no significant differences for various demographic characteristics between the 599 respondents and the source population.

The lipid profile of 38 subjects could not be determined because seven subjects died before a blood sample could be obtained and 31 subjects refused to give a blood sample. Table 1 shows the demographic and clinical characteristics of the remaining 561 subjects. More than half of all subjects (60%) had a history of cardiovascular disease, while 10% had a history of stroke. The median HDL-cholesterol concentration was 1.23 mmol/l in subjects with a history of cardiovascular disease compared to 1.31 mmol/l in those without a history of cardiovascular disease (Mann-Whitney, p=0.01). HDL-cholesterol concentrations in subjects with and without stroke were 1.14 mmol/l and 1.29 mmol/l, respectively (Mann-Whitney, p=0.004). There were no differences when concentrations of total cholesterol, LDL-cholesterol, or triglycerides were compared.

Table 1 *Demographic and clinical characteristics of study participants.*

Characteristic	Total (n=561)	
	No.	%
Female	373	67%
Low level of education *	333	63%
History of cardiovascular disease	335	60%
History of stroke	56	10%
Cognitive impairment		
MMSE $\leq$ 18 points	91	16%
Dementia	73	13%

** Low level of education is defined as subjects without schooling or those who finished primary school only. All subjects were aged 85 years. MMSE; Mini-Mental State Examination.*

Table 2 shows the median MMSE scores for the three strata of decreasing serum concentrations of lipids and lipoproteins. There was no association between MMSE scores and serum concentrations of total cholesterol, triglycerides, and LDL-cholesterol. A significant association was observed for HDL-cholesterol and MMSE score. Subjects with a low HDL-cholesterol concentration had a median MMSE score of 25 points compared to 27 points for subjects with a high HDL-cholesterol concentration (p for trend <0.001). The association between HDL-cholesterol and MMSE scores was equally strong in men and women, and in subjects with a high and a low level of education.

Table 2 *MMSE scores in strata of lipid and lipoprotein concentrations.*

	Strata of lipid or lipoprotein *			
	High (n=188)	Intermediate (n=185)	Low (n=188)	p for trend
Total cholesterol (median, mmol/l)	6.79	5.73	4.64	-
MMSE score (median, 95% CI)	26 (25-27)	26 (26-27)	26 (25-27)	0.4
Triglycerides (median, mmol/l)	2.21	1.34	0.90	-
MMSE score (median, 95% CI)	26 (25-26)	26 (25-27)	26 (26-27)	0.2
LDL-cholesterol (median, mmol/l)	4.57	3.64	2.75	-
MMSE score (median, 95% CI)	26 (25-27)	26 (26-27)	26 (25-27)	0.4
HDL-cholesterol (median, mmol/l)	1.65	1.28	0.93	-
MMSE score (median, 95% CI)	27 (26-27)	26 (25-27)	25 (24-26)	<0.001

* *Concentrations of serum lipids and lipoproteins were grouped into three equal strata representing decreasing concentrations of lipids and lipoproteins. This was done separately for women and men, since women have higher lipids and lipoproteins concentrations than men.*

MMSE scores of subjects with and without cardiovascular disease were 26 versus 27 points, respectively (Mann-Whitney, p=0.007). MMSE scores of subjects with and without a history of stroke were 21 versus 26 points, respectively (Mann-Whitney, p< 0.001). Since we found an association between HDL-cholesterol and cardiovascular disease and history of stroke, and an association between MMSE scores and cardiovascular disease and history of stroke, we explored whether the observed relation between low HDL-cholesterol concentration and cognitive impairment was due to the presence of cardiovascular disease and stroke. When subjects with cardiovascular disease (n=335) were excluded from analysis, the decrease on the MMSE score over strata of HDL-cholesterol

concentrations was still significant (table 3). When subjects with a history of stroke (n=56) were excluded, the decrease on the MMSE score over the three strata still remained significant. After excluding subjects with either a history of cardiovascular disease or stroke (n=356), the trend over strata of HDL-cholesterol concentrations was still observed. When the same analyses were carried out for total cholesterol, triglycerides, and LDL-cholesterol, no such associations were observed.

Table 3 *MMSE scores in strata of HDL-cholesterol concentrations.*

	Strata of HDL-cholesterol			
	High	Intermediate	Low	p for trend
All subjects				
N	188	185	188	
MMSE score (median, 95% CI)	27 (26-27)	26 (25-27)	25 (24-26)	<0.001
Subjects without CVD				
N	86	70	70	
MMSE score (median, 95% CI)	27 (26-28)	26 (24-28)	26 (24-27)	0.03
Subjects without history of stroke				
N	178	161	164	
MMSE score (median, 95% CI)	27 (26-27)	27 (26-27)	26 (25-26)	0.002
Subjects without CVD and without history of stroke				
N	83	62	62	
MMSE score (median, 95% CI)	27 (26-28)	27 (25-28)	26 (25-27)	0.1

CVD ; cardiovascular disease

Table 4 presents odds ratios for cognitive impairment (MMSE score < 19 points) and clinical diagnosis of dementia in relation to decreasing concentrations of HDL-cholesterol. The odds ratios are adjusted for level of education and not distorted by gender or age since stratification was dependent on gender and all subjects were 85 years. The odds ratio for cognitive impairment increased to 2.4 (95% CI 1.3-4.5) when subjects with a low concentration of HDL-cholesterol were contrasted with subjects with a high concentration of HDL-cholesterol. The odds ratios were unaffected by excluding subjects with cardiovascular disease or history of stroke. The odds ratio for subjects with a clinical diagnosis of dementia gradually increased over decreasing strata of HDL-cholesterol to 2.3 (95% CI 1.2-4.3).

The odds ratio was 3.3 (95% CI 1.1 to 10.3) when subjects with cardiovascular disease or history of stroke were excluded. All odds ratios for cognitive impairment or a clinical diagnosis of dementia were unaffected when we adjusted for LDL-cholesterol and triglyceride concentrations.

Finally, all analyses were carried out in a restricted sample where subjects who died within the first year of follow-up (n=36) were excluded. All observed associations between HDL-cholesterol and cognition remained similar.

Table 4 *Odds ratios for cognitive impairment and clinical diagnosis of dementia in relation over strata of HDL-cholesterol concentrations*

	Strata of HDL-cholesterol		
	High (n=188)	Intermediate (n=185)	Low (n=188)
Cognitive impairment			
All subjects	1*	1.7 (0.9-3.3)	2.4 (1.3-4.5)
Subjects without CVD	1*	1.6 (0.6-4.3)	2.5 (1.0-6.4)
Subjects without history of stroke	1*	1.4 (0.7-2.9)	2.2 (1.1-4.4)
Subjects without CVD and without history of stroke	1*	1.5 (0.5-4.2)	2.5 (0.9-6.6)
Dementia			
All subjects	1*	1.1 (0.6-2.3)	2.3 (1.2-4.3)
Subjects without CVD	1*	1.6 (0.5-5.0)	3.6 (1.3-10.0)
Subjects without history of stroke	1*	1.0 (0.5-2.2)	2.0 (1.0-4.0)
Subjects without CVD and without history of stroke	1*	2.2 (0.7-7.2)	3.3 (1.1-10.3)

** Reference category. Poor cognitive function, defined as MMSE ≤ 18 points. All odds ratios are adjusted for level of education. CVD ; cardiovascular disease*

Discussion

The aim of the present study was to explore the association between adverse lipid profiles and cognitive impairment. We found that low serum concentration of HDL-cholesterol was linked with cognitive impairment and dementia. Serum concentrations of cholesterol, triglycerides, and LDL-cholesterol showed no association with cognitive impairment or dementia. Serum HDL-cholesterol concentration and cognitive function was lower in subjects with cardiovascular disease and in subjects with stroke. In subjects without cardiovascular disease or stroke, low serum concentration of HDL-cholesterol was still associated with poor cognitive function and dementia. The specific association of low HDL-cholesterol and cognitive impairment was not confounded by level of education, LDL-cholesterol, triglycerides, or survival.

We measured cognitive function in a population based study using the Mini-Mental State Examination and obtained a clinical diagnosis of dementia from the medical records of the subjects' general practitioners and subjects' nursing home physicians. General practitioners tend to underreport dementia[20]. We therefore may have classified subjects as free from dementia, while in fact they were demented. This possible misclassification results in underestimates of the association between HDL-cholesterol and dementia.

It is difficult to infer causality from cross-sectional studies because it is unable to establish the temporal relationship and it is possible that the subjects' HDL-cholesterol serum concentrations show a decrease as a result of the cognitive impairment. However, when underlying disease in subjects with poor cognitive function or dementia would be the explanation for the observed association, we would have expected that the association between HDL-cholesterol and cognitive impairment to disappear when subjects who died early were excluded from the analysis. Since the association between low HDL-cholesterol and cognitive impairment was still present in subjects who survived one year, we think that the association can be regarded as causal and independent of underlying disease.

A possible explanation of the association between HDL-cholesterol and cognitive function may lie in the unifying hypothesis that atherosclerotic disease, i.e. cardiovascular disease and cerebrovascular disease, causes clinical and subclinical ischaemic lesions in the brain which contribute to the development of dementia[13]. Many studies showed an association between high total cholesterol, high LDL-cholesterol, high triglycerides, and low HDL-cholesterol and the risk of cardiovascular disease. These associations were absent or much weaker for high total cholesterol, high LDL-cholesterol, and high triglycerides and stroke. In line with other studies[15,16] we found that only low HDL-cholesterol is associated with stroke. Data from a randomised controlled trial, studying the effects of gemfibrozil, strongly suggests that increasing HDL-cholesterol decreases the risk for stroke[23]. These findings might explain why we found a specific association between low HDL-cholesterol concentrations and cognitive impairment, and why no associations were found when we studied the relation between total cholesterol, LDL-cholesterol, and triglycerides and cognitive impairment.

As expected, subjects with cardiovascular disease or stroke had lower MMSE scores compared with subjects without cardiovascular disease. When analysing subjects without cardiovascular disease or history of stroke, the association between HDL-cholesterol and cognitive function, however, was unaffected. This indicates that the observed association of HDL-cholesterol and cognition was not only due to a history of cardiovascular disease or stroke. When HDL-cholesterol is related to cognitive impairment via the occurrence of atherosclerotic disease, one would have expected odds ratios close to unity when excluding subjects with cardiovascular disease or stroke. Nevertheless, we cannot exclude that the relation we found between low HDL-c and cognitive impairment in subjects without cardiovascular disease or stroke is due to subclinical stroke.
There are two other hypotheses that may explain the observed association between HDL-cholesterol and cognitive impairment. First, HDL-cholesterol is the predominant lipoprotein in the human brain[11], where it can prevent aggregation and polymerisation of amyloid beta protein[11,12]. This might slow or even prevent the development of dementia. Second, HDL-cholesterol has anti-inflammatory properties[24,25]. Inflammatory responses are increasingly recognised as being important in neurodegenerative processes[26]. Markers of inflammation are found in and around amyloid plaques[26,27]. Moreover, various studies have shown that use of anti-inflammatory drugs protect against dementia[26,28,29].

In conclusion, in very old subjects low serum concentration of HDL-cholesterol is associated with cognitive impairment and clinical diagnosis of dementia. Serum concentrations of cholesterol, triglycerides, and LDL-cholesterol were not associated with cognitive impairment and dementia. The association between low HDL-cholesterol and cognitive impairment was not merely due to the presence of cardiovascular disease and stroke. These findings are of great clinical importance since they suggest that increasing HDL-cholesterol rather than lowering total cholesterol might prevent the development of cognitive impairment and dementia. New preventive and therapeutic strategies should identify the effects of HDL-cholesterol on cognitive function in the elderly.

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Chapter 6

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

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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).

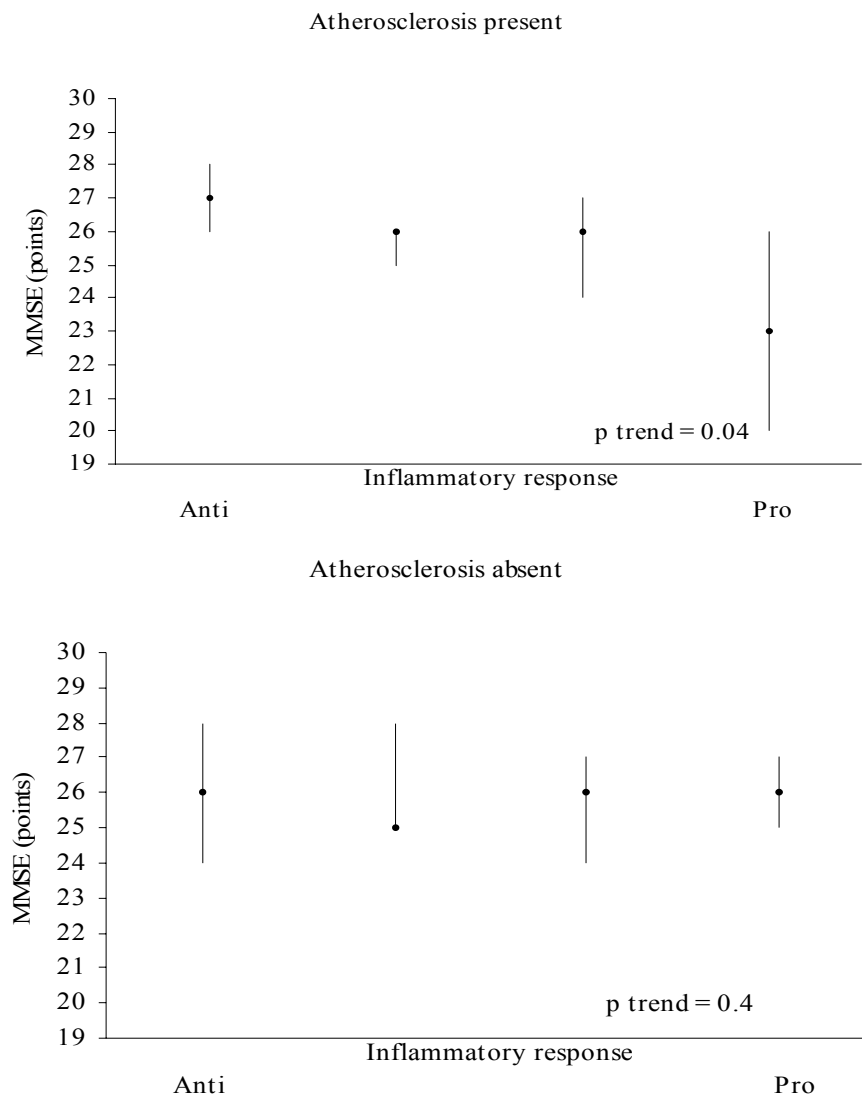

Figure 1 *Mini-Mental State Examination score in relation to inflammatory response and atherosclerosis.Data are presented as medians and corresponding 95% confidence intervals.*

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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Chapter 7

Low production capacity of Interleukin-10 is associated with the metabolic syndrome and type-2 diabetes

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ABSTRACT

Background It has been suggested that the metabolic syndrome and type-2 diabetes are manifestations of the inflammatory host response. This host response is orchestrated by the production of pro and anti-inflammatory cytokines that are under genetic control. We therefore hypothesized that a low production capacity of interleukin-10 (IL-10), a centrally operating cytokine with strong anti-inflammatory properties, associates with the metabolic syndrome and type-2 diabetes in old age.

Methods Five hundred-ninety-nine 85-year-old inhabitants of the city of Leiden were visited at their place of residence. Production capacity of the anti-inflammatory cytokine IL-10 was assessed in a whole blood assay whereby lipopolysaccharide was used as a stimulus. Serum concentrations of lipids, lipoproteins, glucose and HbA1c were determined, and a history of type-2 diabetes was obtained.

Results Serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, glucose and HbA1c gradually decreased over strata representing higher IL-10 production capacity, whereas the concentration of HDL-cholesterol gradually increased (all p for trend <0.01). The odds ratio for type-2 diabetes was 2.7 (CI 95% 1.5-4.9) when subjects with the highest IL-10 production capacity were compared to those with the lowest IL-10 production capacity.

Conclusion These findings show that low IL-10 production capacity, i.e. a pro-inflammatory response, is associated with the metabolic syndrome and type-2 diabetes.

Introduction

The metabolic syndrome is a convergence of dyslipidemia, impaired glucose tolerance and hypertension [1]. About 70 percent of all obese adults have at least one of these major characteristics of the syndrome [2]. Since long it has been recognized that clustering of these risk factors carries an increased risk of type-2 diabetes and cardiovascular disease[3,4].

Insulin resistance has been proposed as the underlying cause for this metabolic and cardiovascular syndrome, although it's molecular basis has not yet been identified [3]. One of the biological mechanisms that may be involved is the innate immune system [5,6]. Several studies have shown that markers of inflammation, such as C-reactive protein [7], fibrinogen[8] , and pro-inflammatory cytokines such as Interleukin-6 [9-11] and Tumor Necrosis Factor-α [15-17] associate with the metabolic syndrome, type-2 diabetes and dyslipidemia. In particular, it has been suggested that TNF-α is associated with insulin resistance and type-2 diabetes, since TNF-α down-regulates the tyrosine kinase activity of the insulin receptor [5,12,13]. Infusion of anti TNF-α antibodies in patients with type-2 diabetes, however, had no effect on their insulin sensitivity [14], and raises doubt on the contribution of TNF-α to type-2 diabetes and the metabolic syndrome.

Interleukin-10 (IL-10) is a centrally operating anti-inflammatory cytokine, which plays a crucial role in the regulation of the innate immune system. It has strong de-activating properties on the inflammatory host response mediated by macrophages and lymphocytes, and potently inhibits the production of pro-inflammatory cytokines such as IL-6 and TNF-α [15-17]. IL-10 is produced by T cells, B cells, monocytes and macrophages and is under tight genetic control, with heritability estimates as high as 75 percent [18]. We therefore propose the hypothesis that low IL-10 production capacity is associated with the metabolic syndrome and type-2 diabetes. To this end, in the Leiden 85-plus Study, we have analyzed the relation between IL-10 production capacity, using a standardized whole blood assay, dyslipidemia and parameters of glucose metabolism.

Methods

Subjects

The Leiden 85-plus Study is a population-based study of inhabitants of Leiden, the Netherlands. From 1997-1999, all members of the 1912 to 1914-birth cohort (n=705) 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 by mail. They were then contacted by telephone, or were visited at home to ask for informed consent. When subjects were cognitively impaired, informed consent was obtained from a guardian. The Medical Ethical Committee of the Leiden University Medical Center approved the study. Subjects were visited three times at their place of residence. At these visits, face-to-face interviews were administered, and an electrocardiogram and body mass index (BMI) was obtained. All blood samples were collected early in the morning under non-fasting conditions. In addition, information on the use of medication was obtained from the subject's pharmacist.

Production capacity of Interleukin-10 and Tumor Necrosis Factor-α

Production capacity of IL-10 and TNF-a were assessed with a standardized whole blood assay [19]. The methods by which whole-blood samples were simulated with 10 ng/ml of lipopolysaccharide have been described elsewhere, including data on reproducibility [19]. In short, 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 TNF-a in the 4-hour samples, and the anti-inflammatory cytokine IL-10 in the 24-hour samples. Production capacity of IL-10 and TNF-α were assayed using standard ELISA techniques. Unstimulated baseline samples were obtained to serve as a control for contamination. Subjects with detectable TNF-α under unstimulated conditions (TNF-α >100 pg/ml) were excluded from further analysis [19,20]. The coefficients of variation for the day-to-day variation in the whole blood stimulation ranged from 8% to 12%. The intra-individual variation was 15% for TNF-α production capacity and 19% for IL-10 production capacity [19].

All subjects were grouped in three equal strata representing decreasing IL-10 production capacity or increasing TNF-α production capacity. This was done separately for women and men, since women have lower IL-10 and TNF-α production capacity than men. The advantage of this stratification is that it intrinsically adjusts for differences in gender.

Lipids and lipoproteins

Total cholesterol and triglycerides levels were analyzed on a fully automated Hitachi 747. High density lipoprotein (HDL) was measured using a Hitachi 911. Low density lipoprotein (LDL) was estimated using the Friedenwald equation [21], whereby five subjects with a triglyceride concentration higher than 5 mmol/l were excluded.

Glucose metabolism and type-2 diabetes

HbA1c and glucose concentration were determined in serum. Subjects were classified as having type-2 diabetes when they met at least one of the following criteria; (1) history of type-2 diabetes obtained from the general practitioner, or the subject's treating physician; (2) use of sulphonylureas, biguanides or insulin, obtained from subject's pharmacist, eight subjects used insulin and were classified as having type-2 diabetes, since they were diagnosed, by there general practitioner, as having diabetes mellitus at a median age of 72 years (range 61 years to 82 years); (3) non fasting glucose of 11.1 mmol/l or higher. Ten subjects, with non fasting glucose of 11.1 mmol/l or higher, were newly diagnosed as having type-2 diabetes. However, these newly diagnosed subjects did not fulfill all the criteria of the American Diabetes Association to diagnose type-2 diabetes, since it was unknown whether these subjects had symptoms of diabetes [22].

Data analysis

The primary outcome measures were the serum concentrations of glucose, HbA1c, lipids and lipoproteins, expressed as means with corresponding 95% CI. We used the one-way ANOVA procedure to determine the p-value for trend over strata of IL-10 production capacity. Univariate odds ratios for type-2 diabetes over strata of IL-10 production capacity and the corresponding 95% confidence intervals were obtained by cross-tabulation. Multivariate odds ratios were obtained by logistic regression analysis. We tested for trend using the log-likelihood statistic with one degree of freedom.

Results

Between 1st September 1997 and 1st September 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 in the study. The response rate was 87 percent, i.e. a total of 599 subjects (397 women, 202 men) participated. There were no statistical significant differences between the 599 participating subjects and the source population with respect to the following characteristics, gender, marital status, socio-economical status, and mortality.

IL-10 production capacity could not be determined in seven subjects as they died before a blood sample could be drawn, while 30 subjects refused to give a blood sample. Under unstimulated conditions, nine subjects had detectable TNF-α concentrations (TNF-α >100 pg/ml) suspect for contamination of the whole blood system and were therefore excluded from the analyses[19,20] . Table 1 shows the demographic and clinical characteristics of the 553 subjects included in the present analysis. Upon stimulation with lipopolysaccharide (LPS) in whole blood samples, the mean IL-10 concentration was 945 pg/ml (CI 95% 861-1030 pg/ml) in men and 799 pg/ml (CI 95% 750-884 pg/ml) in women (Student t-test, p =0.002).

Table 1 *Clinical characteristics of the study sample*

	Subjects (n=553)
Female/Male (n)	370/183
Age (year)	85
Type-2 diabetes	
All	89 (16%)
Use of sulphonylureas or biguanides	49 (9%)
Use of insulin	8 (1%)
BMI (Mean; CI 95%; kg/m^2)	27.2 (26.8-27.7)
Use of non-steroidal anti-inflammatory drugs	152 (28%)

Interleukin-10, lipids, lipoproteins, glucose and HbA1c

We divided subjects in three equal strata dependent on their IL-10 production capacity, for men and women separately, to study the relation between IL-10 production, and lipids, lipoproteins, glucose and HbA1c (Table 2). The serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, glucose and HbA1c gradually decreased over strata representing increasing IL-10 production capacity, whereas the concentration of HDL-cholesterol gradually increased (all p for trend <0.01). There was no association between IL-10 production capacity and BMI. In an additional analysis, we excluded subjects with type-2 diabetes to ascertain that our findings were not only due to the effect of type-2 diabetes on IL-10 production capacity. The trends between IL-10 production capacity, lipids, lipoproteins, glucose and HbA1c remained similar (all p for trend <0.05). The trend between IL-10 production capacity and parameters of the metabolic syndrome could also be distorted by the use of non-steroidal anti-inflammatory drugs that are often prescribed at old age. In a restricted sample of 401 subjects who did not use non-steroidal anti-inflammatory drugs, however, we could still obtain the statistical significant trends as presented in table 2. The trends as shown in table 2 also remained statistical significant when we adjusted for TNF-a using linear regression.

Table 2 *Effect of IL-10 production on lipids, lipoproteins and glucose levels*

	IL-10 production			
	Low (n=184)	Intermediate (n=185)	High (n=184)	p for trend
IL-10 pg/ml	377 (358-396)	763 (746-780)	1402 (1331-1474)	-
BMI (kg/m^2)	27.2 (26.4-27.9)	27.6 (26.8-28.4)	27.0 (26.3-27.6)	0.7
Total cholesterol (mmol/l)	5.95 (5.77-6.12)	5.67 (5.53-5.84)	5.55 (5.41-5.71)	0.001
LDL-cholesterol (mmol/l)	3.86 (3.71-4.02)	3.67 (3.53-3.80)	3.55 (3.42-3.69)	0.002
Triglycerides (mmol/l)	1.77 (1.63-1.91)	1.61 (1.48-1.74)	1.36 (1.28-1.44)	<0.001
HDL-cholesterol (mmol/l)	1.28 (1.21-1.34)	1.29 (1.23-1.34)	1.39 (1.33-1.44)	0.009
Glucose (mmol/l)	7.50 (7.09-7.91)	7.08 (6.68-7.48)	6.31 (6.00-6.61)	<0.001
HbA1c (%)	6.01 (5.83-6.19)	5.82 (5.65-6.00)	5.55 (5.46-5.65)	<0.001

Data are presented as means and 95% confidence intervals. Production capacity of IL-10 as assessed in a whole blood stimulation and were grouped in three equal strata. This was done separately for women and men, since women have lower IL-10 production than men.

In figure 1 we present the mean concentrations of triglycerides and HbA1c over strata of IL-10 production capacity as well as TNF-α production capacity, to determine whether low, intermediate and high TNF-α production capacity had an additional effect on these metabolic outcomes. In each stratum of TNF-α production capacity there was a significant decrease of triglycerides and HbA1c when IL-10 production capacity increased (p for trend in each stratum <0.05). There was no significant increase of triglycerides and HbA1c when TNF-α production increased in any of the strata of IL-10 production capacity (p for trend in each stratum >0.1). Similar trends as shown in figure 1 were obtained fortotal-total-cholesterol, HDL-cholesterol, LDL-cholesterol and glucose (data not shown).

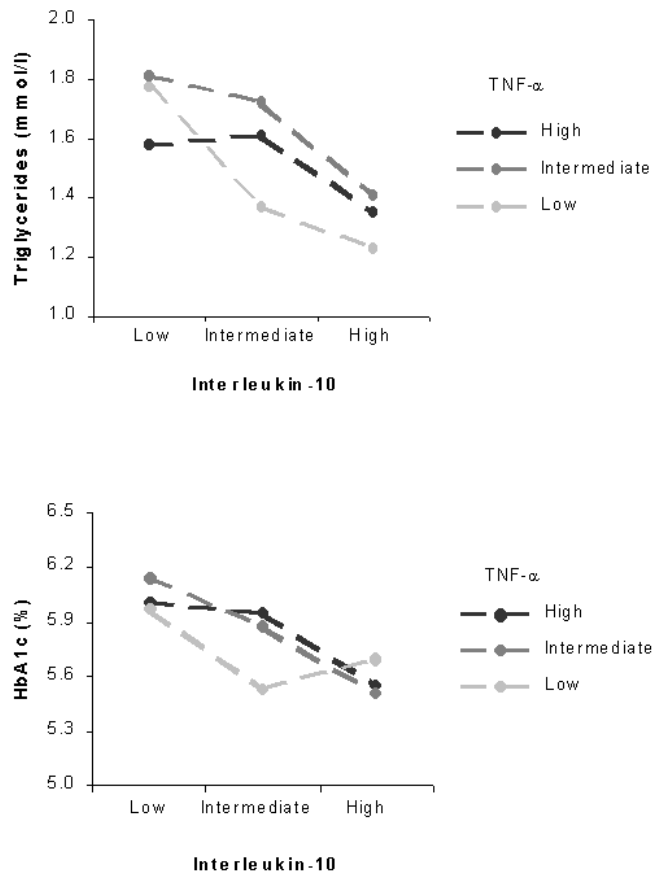

Figure 1 *Mean concentrations of triglycerides and HbA1c in strata of IL-10 and TNF-α. Trends of triglycerides and HbA1c over strata of IL-10 production, p for trend in each stratum <0.05. Trends over strata of TNF-α production, p for trend in each stratum >0.1.*

Interleukin-10 and type-2 diabetes

The proportion of subjects with type-2 diabetes gradually decreased over the strata representing a higher IL-10 production capacity (p for trend =0.001, Table 3). The odds ratio for type-2 diabetes increased to 2.7 (CI 95% 1.5-4.9) when subjects with the highest IL-10 production capacity were compared to those with the lowest IL-10 production capacity. The odds ratio for type-2 diabetes was slightly higher after adjustment for TNF-α. In an additional analysis we excluded the newly diagnosed subjects with a plasma glucose of 11.1 mmol/l or higher (n=10), since it was unknown whether these subjects had symptoms of diabetes and therefore did not fulfill all the criteria of the American Diabetes Association to diagnose diabetes mellitus[22] . The results as shown in table 3 were unaffected.

Table 3 *Odds ratios for type-2 diabetes in relation to IL-10 production*

	IL-10 production			
	Low	Intermediate	High	p for trend
Type-2 diabetes				
Present	42 (23%)	29 (16%)	18 (10%)	0.001
Absent	142 (77%)	156 (84%)	166 (90%)	
Odds ratio				
Crude	2.7 (1.5-4.9)	1.7 (0.9-3.2)	1*	0.001
Adjusted	3.4 (1.6-7.1)	1.9 (1.0-3.6)	1*	0.001

** Reference category. Adjustments for TNF-α production using logistic regression.*

Discussion

This analysis of the Leiden 85-plus Study shows that low IL-10 production capacity, i.e. a pro-inflammatory cytokine response, is associated with high plasma glucose, high HbA1c, type-2 diabetes and dyslipidemia. When production capacity of IL-10 is taken into account, production capacity of TNF-α only adds little to these metabolic parameters.

Possible mechanisms

Pro-inflammatory cytokines have earlier been associated with the development of the metabolic syndrome and type-2 diabetes. Experimental studies in humans and animals show that treatment with pro-inflammatory cytokines induces hypertriglyceridemia and insulin resistance [9,12]. TNF-α down-regulates the tyrosine kinase activity of the insulin receptor, thereby increasing insulin resistance [5,12,13]. Serum IL-6 and C-reactive protein concentrations are higher in subjects with the metabolic syndrome or type-2 diabetes compared to controls[10] . Pro-inflammatory cytokines also contribute to dyslipidemia by increasing lipolysis[7,9]. We feel that IL-10 at least partly represents the effect of an anti-inflammatory response on the metabolic syndrome and type-2 diabetes, since studies on the innate immune system suggest that IL-10 is a key regulator and a powerful suppressor of the immune response[15] . We hypothesize that high IL-10 prevents the development of the metabolic syndrome and type-2 diabetes, by limiting the effects of the inflammatory response, i.e. counter regulating the effects of pro-

inflammatory cytokines such as TNF-α and IL-6. This hypothesis is partly derived from our findings, which suggest that when production capacity of IL-10 is taken into account, production capacity of TNF -α only adds little to markers of the metabolic syndrome, such as triglycerides and HbA1c. High levels of IL-10 should theoretically cause an upregulation of tyrosine kinase activity of the insulin receptor and decrease lipolysis, by counter regulating the effects of TNF-α and IL-6[5,7,9,12,13] . Therefore, a high IL-10 production capacity could confer protection against the metabolic syndrome and type-2 diabetes, while a low IL-10 production capacity would predispose to the metabolic syndrome and type-2 diabetes.

It has been questioned why the characteristics of the metabolic syndrome are so prevalent in humans as these have such deleterious effects later in life. Evolutionary theories on aging can explain for this paradox. Critical in this understanding is that the force of selection decreases with age[23] . Therefore, pleiotropic genes that have beneficial effects early in life are favored by selection even if these genes have deleterious effects later in life[24] . Selection for genes encoding for the metabolic syndrome fit within these theories. Subjects with a pro-inflammatory cytokine response, i.e. a low IL-10 production capacity[18] and hypercholesterolemia[25,26] are relatively protected against infection early in life. In times when infant mortality from infectious disease was high, survivors are likely to have had an innate pro-inflammatory host response. The trade off for this survival benefit is the pro-inflammatory host response predisposing for the metabolic syndrome, type-2 diabetes and the development of atherosclerosis at late age.

Limitations

The cross-sectional nature of the relation between low IL-10 production capacity and the lipid and glucose metabolism is a limitation of our study. It is tempting to speculate that the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes is causal, since we have previously shown in family studies of first-degree relatives and twins that as much of 75 percent of the variance in IL-10 production capacity in humans derives from genetic factors[18]. Causality between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, however, can only be determined when these associations can be confirmed in, healthy, first degree relatives of subjects with the metabolic syndrome or type-2 diabetes, or using a prospective design in younger subjects, with a long-term follow-up.

A second limitation could be that the blood samples were collected under non-fasting conditions. It is likely that we have thus underestimated the effect of IL-10 on glucose and triglyceride, since it could be argued that the relation between IL-10 production capacity, glucose and triglycerides was diluted by non-differential misclassification, i.e. misclassification of glucose and triglycerides independent on IL-10 production capacity. We found, however, that low IL-10 production capacity was associated with both high glucose and high HbA1c, suggesting that the association between IL-10 production capacity and glucose metabolism is real, since post-prandial changes in HbA1c are absent. Finally, we argue that it is a necessity to determine fasting triglycerides when absolute levels of triglycerides are assessed for

clinical purposes, i.e. to diagnose hypertriglyceridemia in individuals. However, this premise can be somewhat relaxed when we tried to elucidate the relation between inflammation, the metabolic syndrome and type-2 diabetes, in the population at large.

A final question, which could arise, is whether 85 years is a rather late age to study the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, since subjects aged 85 years are long-term survivors from a far larger birth cohort. This does not alter the validity of the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, as found in the oldest old. However, as with all studies, it would be valuable to demonstrate the same association in different populations, using different study designs and using younger subjects.

Conclusion

We found an association between low IL-10 production capacity, i.e. a pro-inflammatory host response, and high serum glucose, high HbA1c, type-2 diabetes and dyslipidemia. We are not aware of other studies reporting on the effect of IL-10, a strong anti-inflammatory cytokine, on these metabolic parameters. It is a challenge performing further studies that can confirm the hypothesis that low IL-10 production capacity, i.e. a pro-inflammatory response, predisposes to the metabolic syndrome and type-2 diabetes.

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Chapter 8

Inflammation and Stroke

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ABSTRACT

Background Experimental evidence indicates that IL-10 deficiency is associated with the development of cardiovascular and cerebrovascular disease. We analyzed the relation between low Interleukin-10 (IL-10) production levels, a history of stroke and incident fatal stroke.

Methods All 85-year-old inhabitants of Leiden (n=599) were visited at their place of residence (response rate 87%). Production levels of the anti-inflammatory cytokine IL-10 were assessed in a whole blood assay whereby lipopolysaccharide was used as a stimulus. Plasma concentrations of C-reactive protein (CRP) were also used as a marker of inflammation. A history of stroke was obtained at baseline (prevalence 10%). The number of fatal strokes were prospectively obtained for a median follow-up of 2.6 years (incidence 1.82 per 100 person-years at risk).

Results Subjects with a history of stroke had significant lower median IL-10 production levels at baseline than subjects without stroke (558 pg/ml versus 764 pg/ml, $p<0.05$). They also had significant higher median CRP concentrations (6 mg/l versus 3 mg/l, $p<0.05$). The odds ratio for a history of stroke increased to 2.30 (95 % CI 1.12-4.72) over strata representing decreasing production levels of IL-10. The relative risk for incident fatal stroke was 2.94 (95 % CI 1.01-8.53) when we compared both subjects with low or intermediate baseline IL-10 production levels to those with high production levels of IL-10.

Conclusion Our data support the hypothesis that subjects with low IL-10 production levels have an increased risk of stroke.

Introduction

Accumulating evidence suggests that inflammation plays an important role in the development of cardiovascular and cerebrovascular disease[1-3] . Markers of inflammation, such as C-reactive protein (CRP)[1,2] , and pro-inflammatory cytokines are associated with stroke [4].

Interleukin-10 (IL-10) is a centrally operating anti-inflammatory cytokine, which plays a crucial role in the regulation of the innate immune system. It has strong de-activating properties on the inflammatory host response, and potently inhibits the production of pro-inflammatory cytokines [5]. Animal models investigating the protective role of IL-10 in atherosclerosis show that IL-10 deficient mice have a high susceptibility to atherosclerosis[6] . Moreover, IL-10 deficient mice have an increased stroke lesion size after ligation of the mid-cerebral artery [7], whereas rats treated with IL-10 have a decreased stroke lesion size [8].

Here, we tested the hypothesis that a pro-inflammatory cytokine response predisposes to stroke. We therefore analyzed the association between low IL-10 production levels and a history of stroke. We also determined the association between low IL-10 production levels measured at baseline and incident fatal stroke.

Methods

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 Leiden 85-plus Study. There were no selection criteria on health or demographic characteristics. The Medical Ethical Committee of the Leiden University Medical Center approved the study. Fourteen inhabitants died before they could be enrolled. The response rate was 87%, a total of 599 subjects (397 women, 202 men) participated. There were no significant differences for various demographic characteristics between the 599 respondents and the source population.

The WHO definition of stroke "rapidly developing clinical signs of focal (at times global) disturbance of cerebral functioning lasting > 24 hours" was used to identify subjects with a history of stroke. All subject's general practitioners or the individual subject's treating (nursing home) physician were interviewed to obtain a complete medical history, including a history of stroke. The advantage of using general practitioners to obtain a history of stroke was that subjects with a history of stroke, who were not hospitalized, were also included in the study. All subjects were followed up for mortality until 1st September 2001. The primary and secondary causes of death were obtained from subjects' general practitioners or treating physicians. Fatal stroke was classified according to the ICD-10 codes I60-I69.

IL-10 production in lipopolysaccharide stimulated whole blood samples varies between individuals. This interindividual variation has a strong genetic basis. Family studies of first-degree relatives and analysis of twins indicate that as much of 75% of the differences in quantitative IL-10 production in humans derive from heritable factors[9,10] . The innate IL-10 production was assessed with an *ex vivo* whole-blood

assay[11] . The full methods by which whole-blood samples were simulated with 10 ng/ml of lipopolysaccharide have been described elsewhere[9] . Unstimulated baseline samples were obtained to serve as a control for contamination. Subjects with detectable Tumor Necrosis Factor-α concentrations (TNF-α) under unstimulated conditions (TNF-α >100 pg/ml) were therefore excluded from further analysis[10,11] . Plasma levels of CRP were measured using a fully automated Hitachi 911.

Subjects were classified as having diabetes when they met at least one of the following criteria: (1) history of diabetes, obtained from the subject's general practitioner, or treating physician, (2) use of sulphonylureas, biguanides or insulin, obtained from the subject's pharmacist, or (3) non fasting glucose concentrations of 11.1 mmol/l or higher. Subjects were classified as having hypertension when they met at least one of the following criteria: (1) history of hypertension, (2) use of β–blockers, ACE-inhibitors, thiazide diuretics or calcium antagonists, obtained from the subject's pharmacist, or (3) a diastolic blood pressure of 95 mm Hg or higher or a systolic blood pressure of 180 mm Hg or higher. Subjects were classified as having cardiovascular disease, when they met at least one of the following criteria: (1) history of myocardial infarction, angina pectoris, arterial surgery, or intermittent claudication, (2) signs of myocardial infarction or myocardial ischaemia, recorded on the electrocardiogram, which was obtained in all subject's. Finally, use of non steroidal anti-inflammatory drugs, including aspirin, was obtained from the subject's pharmacist.

Data analysis

Data are presented as medians with corresponding 95% confidence intervals for the median[12], representing the range of values, which includes the "true" median. The non-parametric Mann-Whitney test was used, because IL-10 production levels and CRP concentrations were not normally distributed and skewed to the right. The production levels of IL-10 were grouped in three equal strata representing decreasing IL-10 production levels. This was done separately for women and men, since women had a lower IL-10 production than men. CRP concentrations were grouped using a similar approach. Multivariate odds ratios and 95% confidence intervals were obtained by logistic regression analysis, to determine the risk of a history of stroke depending on IL-10 production levels and plasma CRP concentrations at baseline (age 85). The p-values for trend over strata of IL-10 production and stroke and strata of CRP concentrations and stroke were determined using the log-likelihood statistic with one degree of freedom. Mortality risks were determined using multivariate Cox regression. To prevent that low IL-10 production levels were markers for imminent death, we excluded in an additional analysis those subjects, who died during the first half-year of follow-up.

Results

Data on the history of stroke were incomplete in two out of the 599 subjects. IL-10 production levels upon stimulation with endotoxin (lipopolysaccharide) in whole blood could not be obtained in 46 subjects. Since seven subjects died before a blood sample could be drawn, 30 subjects refused to give a blood sample and, nine subjects had detectable TNF-α concentrations (TNF-α >100 pg/ml) underunstimulated conditions and were therefore excluded from all analyses [10,11]. Complete data on stroke and IL-10 production levels were therefore available for 551 subjects.

Cross-sectional study

Table 1 shows clinical and inflammatory characteristics of the subjects at baseline. Subjects with a history of stroke had lower median IL-10 production levels and higher median CRP concentrations than subjects without stroke (IL-10 production levels 558 pg/ml versus 764 pg/ml, p=0.047 and CRP 6 mg/l versus 3 mg/l, p=0.004). We found a dose-response relationship between IL-10 production levels and subjects without a history of stroke, subjects with one stroke (n=45) and 10 subjects with two or more strokes (IL-10 production levels 764 pg/ml, 597 pg/ml and 542 pg/ml respectively, p for trend =0.06). In an additional analysis, we excluded subjects who used non-steroidal anti-inflammatory drugs (n=153). The IL-10 production levels and plasma CRP concentrations in subjects with a history of stroke compared to those without stroke remained similar (IL-10 production levels 545 pg/ml versus 756 pg/ml, p=0.12 and CRP 8 mg/l versus 3 mg/l, p=0.003).

Table 1 *Clinical and inflammatory characteristics in relation to a history of stroke*

	Stroke		
	Absent (n=496)	Present (n=55)	p value
Clinical characteristics			
Age (years)	85	85	
Women	332 (67%)	36 (65%)	0.83
Type 2 diabetes	78 (16%)	11 (20%)	0.41
Hypertension	277 (56%)	38 (69%)	0.06
Cardiovascular disease	285 (57%)	36 (65%)	0.25
Use of NSAID*	125 (25%)	28 (51%)	<0.001
Inflammatory characteristics †			
Interleukin-10 (pg/ml)	764 (727-803)	558 (465-817)	0.047
C-reactive protein (mg/l)	3 (3-4)	6 (3-9)	0.004

** NSAID, including use of aspirin. † Data are presented as medians and corresponding 95% confidence intervals.*

The odds ratio for a history of stroke, adjusted for type 2 diabetes, hypertension, use of non-steroidal anti-inflammatory drugs, and cardiovascular disease increased to 2.30 (95 % CI 1.12-4.72) over strata representing decreasing production levels of IL-10 (figure 1, p for trend =0.018). The adjusted odds ratio for a history of stroke increased to 2.11 (95 % CI 1.00-4.40) over strata representing increasing CRP concentrations (p for trend =0.031). Each 500-pg/ml increase of IL-10 production corresponded to a 26% lower risk of having a history of stroke, odds ratio 0.74 (95 % CI 0.52–1.00). The results remained similar after adjustment for gender, type 2 diabetes, hypertension, use of non-steroidal anti-inflammatory drugs, and cardiovascular disease, odds ratio 0.70 (95 % CI 0.52–1.00).

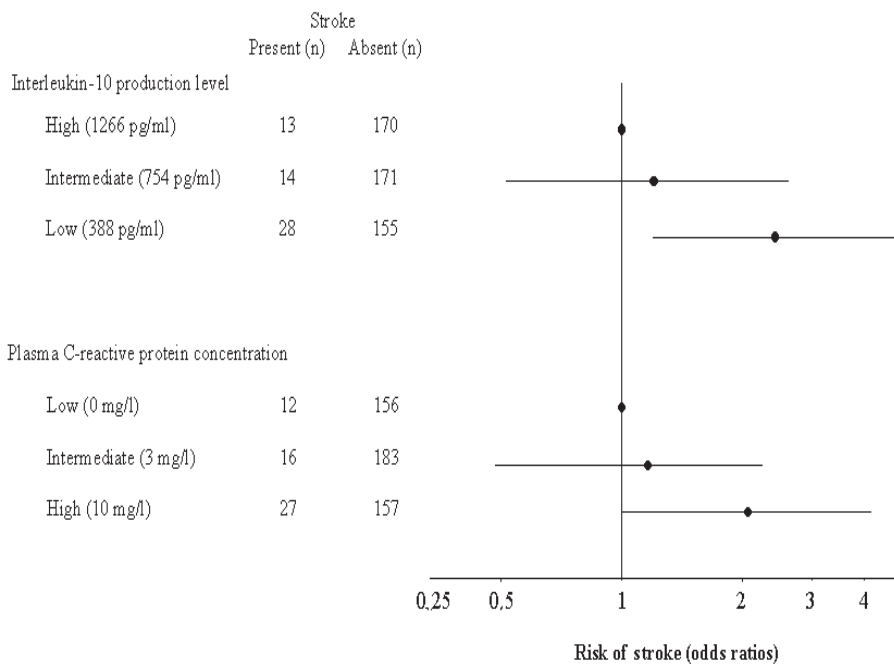
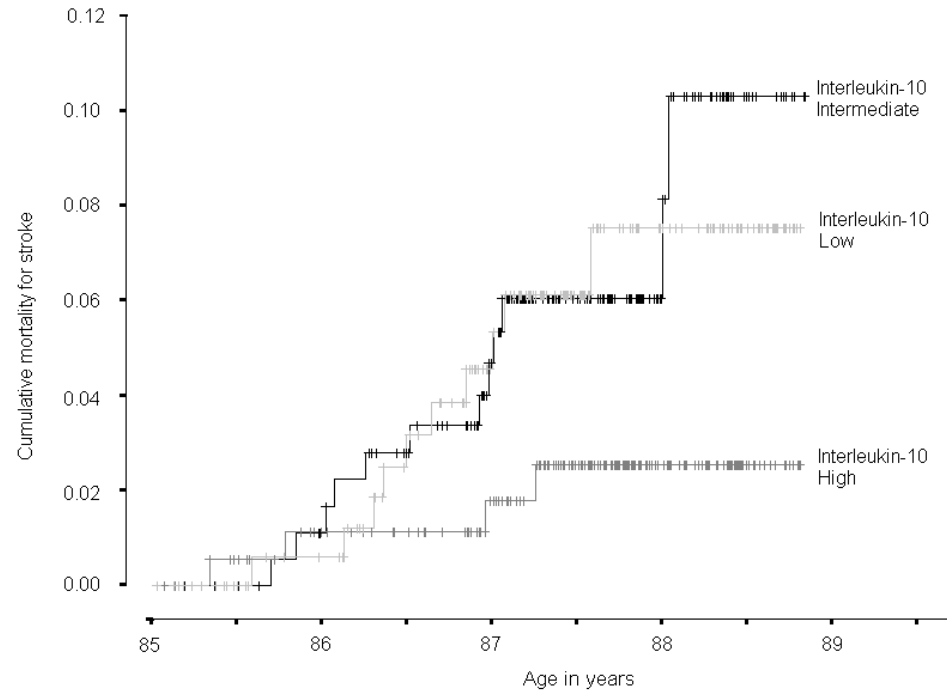


Figure 1 *Risk of stroke in relation to Interleukin-10 production and C-reactive protein*
The odds ratios represented here are adjusted for diabetes, hypertension, use of non-steroidal anti-inflammatory drugs and cardiovascular disease. Bars represent 95% confidence intervals. Production level of IL-10 and plasma CRP concentrations are presented as medians.

Follow-up study

In total, 147 subjects died during a median follow-up of 2.6 years (incidence 1.82 per 100 person years at risk; 95 % CI 1.12-2.53). Twenty-six of them suffered a fatal stroke. Eight out of the 26 subjects with fatal stroke had a history of stroke at baseline. Only 4 out of the 183 subjects (2.1%) with high IL-10 production levels suffered a fatal stroke, whereas 12 subjects out of the 185 subjects (6.5%) with intermediate IL-10 production levels and 10 out of the 183 (5.5%) with low IL-10 production levels suffered a fatal stroke. Figure 2 shows the cumulative mortality for stroke, of the 551 participating subjects, over strata of IL-10 production and strata of CRP. The highest cumulative mortality for stroke was present for those with low or intermediate IL-10 production levels (p=0.06, Cox regression), and those with high or intermediate CRP (p=0.095, Cox regression).

The crude relative risk for incident fatal stroke was 2.94 (95 % CI 1.01-8.53) when we compared both subjects with low or intermediate production levels of IL-10 measured at baseline to those with high IL-10 production levels. Each 500-pg/ml increase of IL-10 production corresponded to a 36% decrease in mortality due to stroke, risk ratio 0.64 (95 % CI 0.39–1.00). The results remained similar after adjustment for gender, type 2 diabetes, hypertension, use of non-steroidal anti-inflammatory drugs, and cardiovascular disease, risk ratio 0.67 (95 % CI 0.41–1.00). The median IL-10 production level, measured at baseline, was lower in those with a fatal stroke (n=26) compared to those without a fatal stroke (n=525), 764 pg/ml vs. 715 pg/ml, p=0.07. The median CRP concentration, measured at baseline, was higher in those with a fatal stroke compared to those without a fatal stroke, 5mg/l vs. 3mg/l, p=0.14. To prevent that low IL-10 production levels were markers for intercurrent fatal disease we excluded those subjects who died during the first half-year of follow-up (n=11), leaving 540 subjects in the analysis. Adjustments were made for diabetes, hypertension, use of non-steroidal anti-inflammatory drugs, history of stroke and presence of cardiovascular disease at baseline, using Cox regression. After the age of 85.5 years the adjusted relative risk for incident fatal stroke (n=25) was 3.63 (95 % CI 1.08-12.21) when we compared both subjects with low or intermediate production levels of IL-10 measured at baseline to those with high IL-10 production levels.



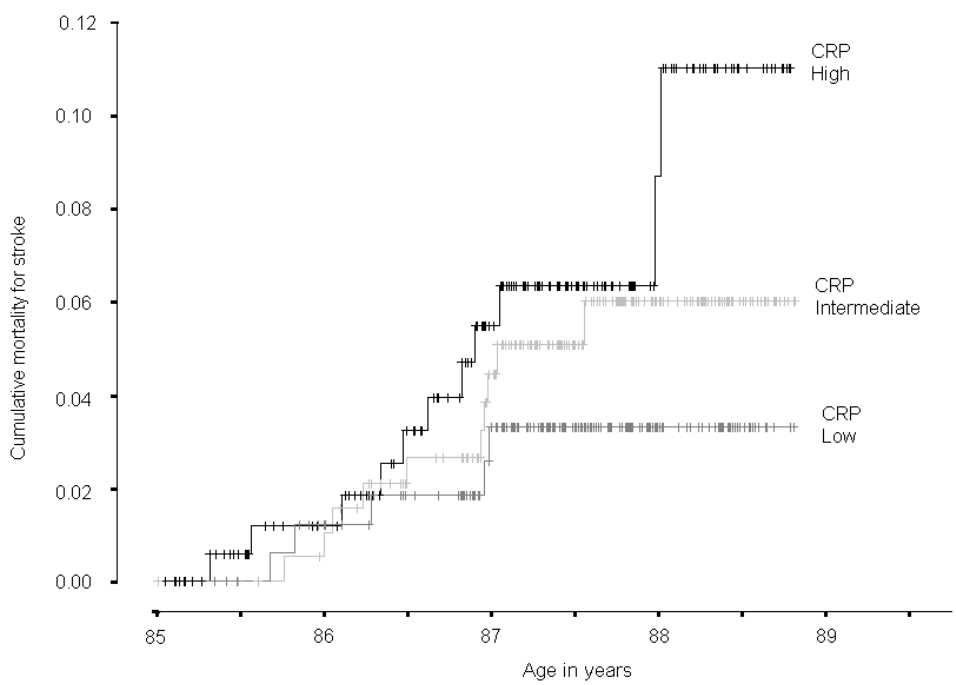


Figure 2 *Cumulative mortality for stroke*

Discussion

This analysis of the Leiden 85-plus Study shows that low IL-10 production levels are associated with both a history of stroke, in the cross-sectional study, and increased mortality due to stroke, as obtained in the prospective follow-up study. These associations persisted after adjustment for known risk factors for stroke. In line with earlier clinical studies we also showed that high CRP was associated with stroke[1,2] . Our findings extend data from animal models, which showed that IL-10 deficiency predisposes to atherosclerosis [6] and increases stroke lesion size[7,8] .

Since both our cross-sectional and longitudinal data showed an association between low IL-10 production levels and an increased risk of stroke, it is tempting to speculate that the association between low IL-10 production levels and stroke is causal. Furthermore we have previously shown associations between innate IL-10 production and multiple sclerosis[10] and used a family design in which the cytokine response of the patient was estimated in first-degree relatives[9-11] . Also, the genetic basis of IL-10 production favors a causal interpretation of the association. Finally, other findings suggest that the cytokine response in whole blood induces the same effects in the brain across the blood-brain barrier[13] .

In our study both high CRP and low IL-10 production are associated with stroke. We feel that CRP and IL-10 at least partly represent the effect of an inflammatory response on stroke. Studies on cerebral ischaemia emphasize the relevance of an inflammatory response on lesion size, in which IL-10 is a key regulator[5,7,8] . IL-10 is a powerful suppressor of the immune response, produced by T cells, B cells, monocytes, macrophages and microglia [5,14]. It inhibits pro-inflammatory cytokines such as TNF-α and IL-6[5] . It could also inhibit CRP, since it has been suggested that IL-6 partly regulates CRP production[15] . Moreover, IL-10 limits the size of ischaemic brain damage, occurring after occlusion of cerebral arteries[8] . IL-10 could therefore represent a potential therapeutic agent for inflammatory diseases such as atherosclerosis and stroke.

In summary, low IL-10 production levels and high plasma CRP concentrations are associated with an increased risk of stroke, obtained at baseline and during follow-up. These findings support the hypothesis that a pro-inflammatory response predisposes to stroke.

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Chapter 9

Summary and general discussion

Summary and general discussion

This thesis on the impact of atherosclerosis and inflammation on cognitive function had two aims. The first aim was to determine whether atherosclerosis may be the *initiating event* that leads to ischaemia in the brain and subsequently to neuro-inflammation, followed by neurodegenerative processes that ultimately result in cognitive impairment and dementia. The second aim was to investigate the association between the inflammatory response and atherosclerosis. These aims have been based on findings of experimental animal studies. These studies show that the development of atherosclerotic disease and the size of stroke lesions are dependent on an innate pro-inflammatory cytokine response.

In this final chapter the findings will be summarised and generalised in order to answer the two aims of this thesis. Furthermore, some methodological considerations will be addressed and the implications for future research will be discussed.

Findings

In this thesis two different routes have been studied to answer the two aims. Figure 1 depicts a schematic representation of these two routes. The "classic" route shows the associations between cardiovascular risk factors, atherosclerotic disease, and a poor cognitive function. The associations between gender and cognitive functioning, between cardiovascular disease and cognitive impairment, and between HDL-cholesterol and cognitive impairment have been described in chapters 3, 4 and 5. The "alternative" route concerns the first aim of this thesis, and shows the effect of atherosclerosis and a pro-inflammatory response on cognitive function (chapter 6). The second aim of this thesis, i.e. to investigate the association between the inflammatory response and atherosclerosis, is depicted in Figure 1 by two junctions between the "classic" and the "alternative" route. The first junction concerns the association between the metabolic syndrome – i.e. a clustering of cardiovascular risk factors, such as insulin resistance, dyslipidemia and hypertension – and interleukin-10, a strong anti-inflammatory cytokine (chapter 7). The second junction concerns the relation between atherosclerosis – i.e. cerebrovascular disease – and inflammation (chapter 8).

"Classic" route

In **Chapter 3** the idea has been tested that gender differences in cognitive functioning can be explained by differences in the level of formal education. Since, in general, older women have received less formal education than older men, this idea may provide an explanation for the finding that the prevalence and incidence of dementia are higher in women than in men[1,2] . Contrary to expectations the findings of this study show that women, despite a lower level of education, have a better cognitive function than men. After adjustment for depressive symptoms and level of education women had an approximately twofold better cognitive speed and memory than men. These findings support the idea that biological differences between women and men contribute to the gender differences in cognitive function. Since women have a lesser atherosclerotic burden than men of the same age [3], this biological difference may explain the better performance of women.

In **Chapter 4** the hypothesis that atherosclerotic burden contributes to the gender difference in cognitive function in old age has been tested. As expected, more men than women have a history of

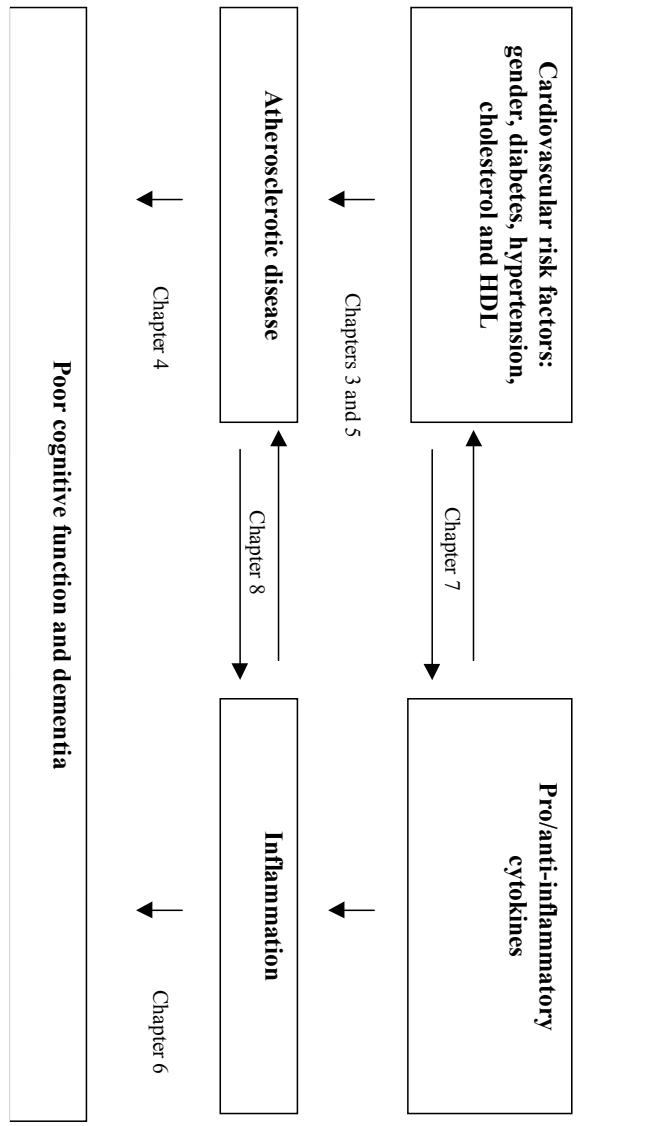

Figure 1 *Atherosclerosis, inflammation and cognitive function*

cardiovascular disease (67% compared to 59%). The median score on the Mini-Mental State Examination (MMSE) – a test to measure global cognitive functioning – is 26 points in both men and women without cardiovascular disease and decreases to 25 points for men and women with two or more cardiovascular pathologies. A similar dose-response relation has been found for cognitive speed, but not for memory. These data suggest that generalised atherosclerosis in old age significantly contributes to cognitive impairment, which develops at an earlier age in men than in women.

In **Chapter 5** the effects of total and fractionated cholesterol on cognitive functioning have been reported. It has been explored whether the observed associations are dependent or not dependent on atherosclerotic disease. Median scores on MMSE are significantly lower in subjects with low HDL-cholesterol (25 *vs.* 27 points). For other lipids and lipoproteins no differences in scores on MMSE have been found. Compared to subjects with high HDL-cholesterol subjects with low HDL-cholesterol run a twofold increased risk of having dementia. Subjects without cardiovascular disease or stroke and with low HDL-cholesterol run a threefold increased risk of having dementia. This study shows that low HDL-cholesterol is associated with cognitive impairment and dementia. The data suggest that at least part of the association between HDL-cholesterol and cognitive function is independent of atherosclerotic disease, since subjects without cardiovascular disease or stroke and with low HDL-cholesterol also run an increased risk of having dementia.

"Alternative" route

The findings in **Chapter 6** suggest that the combination of atherosclerosis and a pro-inflammatory response predisposes to a poor cognitive function and dementia in the population at large. Experimental evidence indicates that the size of ischaemic lesions and the neurological outcome are dependent on a pro-inflammatory cytokine response[4,5] . Production of the pro-inflammatory cytokine tumour-necrosis factor-a (TNF-a) and the anti-inflammatory cytokine interleukin-10 (IL-10) has been assessed to classify subjects as having an innate pro- or anti-inflammatory response. The risk of poor cognitive speed, poor memory and dementia gradually increases two- to threefold over the strata representing an increasing pro-inflammatory response. Stratification for atherosclerosis shows that only subjects with atherosclerosis have increasing odds ratios for poor cognitive function over the strata representing an increasing pro-inflammatory response.

Junctions between the "classic" and the "alternative" route

The findings in **Chapter 7** show an association between low IL-10 production (a pro-inflammatory response), the metabolic syndrome (a clustering of cardiovascular risk factors) and type-2 diabetes. Earlier studies have shown that pro-inflammatory cytokines, such as TNF-a, are involved in the development of both the metabolic syndrome and type-2 diabetes[6,7]. Serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, glucose and HbA1c gradually decrease over strata representing higher IL-10 production, whereas the concentration of HDL-cholesterol gradually increases. Compared to subjects with high IL-10 production subjects with low IL-10 production run an

almost threefold increased risk to develop type-2 diabetes. These findings support the hypothesis that a low IL-10 production capacity, i.e. an innate pro-inflammatory cytokine response, predisposes to the metabolic syndrome, a clustering of cardiovascular risk factors.

In **Chapter 8** the relation between inflammation and atherosclerotic disease, i.e. stroke, has been assessed, since "stroke" is associated with the development of dementia. Similar to the studies described in the previous chapters, low IL-10 is associated with an increased risk of developing a stroke. Compared to subjects with high IL-10 production subjects with low IL-10 production run a twofold increased risk of having a stroke, and a threefold increased risk of developing a fatal incident stroke. These findings are in line with experimental animal studies, which show that IL-10 deficiency increases the risk of developing atherosclerosis[8] and stroke lesions[5] . Again this supports the hypothesis that a pro-inflammatory response predisposes to having a stroke, a poor cognitive function and dementia in the population at large.

Methodological issues

Selection bias

In **Chapter 2** it has been shown that the effect of selection bias in the Leiden 85-plus Study is virtually absent, since demographic characteristics, such as gender, marital status and socio-economical status, and clinical characteristics, such as cumulative mortality, are similar in the 599 participating subjects and the 705 subjects from the source population.

Cross-sectional data

Due to the cross-sectional nature of the data the most important limitation of the studies described in this thesis is that temporal relations between variables cannot be determined. In the longitudinal part of the Leiden 85-plus Study this problem will be overcome. On the other hand, it is unlikely that the longitudinal part of the Leiden 85-plus Study will find other relations when the effects of gender and atherosclerosis on cognitive functioning are being studied, since it is unlikely that a poor cognitive function leads to atherosclerosis or a change in gender (chapters 3 and 4). However, the relations found between HDL (chapter 5), pro-inflammatory cytokine response (chapter 6) and cognitive function may in part be explained by a poor cognitive function. After all, a poor cognitive function is associated with poor health, and poor health can affect the concentration of HDL-cholesterol and the inflammatory response. The same is true for the relation between low IL-10, the metabolic syndrome (chapter 7) and stroke (chapter 8).

Several analyses have been carried out to explore whether the reported relations may be explained by the cognitive function altering the determinants that have been studied.

First of all, cytokine levels have been measured in lipopolysaccharide-(LPS-)stimulated whole-blood samples. The reason why this has been done is that cytokine levels determined after LPS stimulation vary in individuals. This interindividual variation has a strong genetic basis. Family studies indicate that as much as 75% of the differences in quantitative IL-10 production in humans derive from heritable genetic factors[9-12] . The results presented in this thesis therefore suggest that the innate cytokine response precedes a poor cognitive function, the metabolic syndrome and stroke.

In two studies data from the ongoing follow-up have been used in addition to the cross-sectional design. As a result of the determined association between HDL-cholesterol and cognitive function a restricted analysis has been carried out, i.e. subjects who died within the first year of follow-up were excluded to prevent that subjects with low HDL-cholesterol as a marker of intercurrent fatal disease are taken into account. The results remained unaltered. Follow-up data have also been used as a result of the determined association between low IL-10 production and stroke. Compared to subjects with high IL-10 production subjects with low IL-10 production at baseline run an increased risk to suffer from a fatal incident stroke.

Non-differential and differential misclassification

The Leiden 85-plus Study studies the majority of the 85-year-old inhabitants of Leiden. Of course, random errors and non-differential misclassification, i.e. misclassification independent of the values of other variables, occur when collecting data. These effects weaken the results, hence they have no major consequence when statistically significant relations are found.

However, the possible effects of differential misclassification, i.e. measurement errors of the determinants on which the outcome is dependent, are far more important.

Since all neuropsychological tests have been administrated by the same trained research nurse, it is very unlikely that differential misclassification of cognitive tests has occurred. Furthermore, she has reported on her evaluation of the tests, in particular whether the test scores reflected the subject's ability to perform the test at that time.

Two classifications have been used as approximation to determine the presence of atherosclerosis. For the first approximation electrocardiograms have been recorded and analysed by using automated Minnesota coding[13,14] . A subject was classified as having atherosclerosis when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia. For the second approximation the classification of the Second Manifestations of ARTerials disease (SMART) study has been used[15] . The SMART classification is partly based on the subject's medical history. In this study the subject's history of cardiovascular disease has been obtained from the general practitioner or the physician in attendance, i.e. the nursing home physician. A subject was classified as having atherosclerosis when a positive history of myocardial infarction, angina pectoris, arterial surgery, stroke, or intermittent claudication was present, or when the electrocardiogram revealed signs of myocardial infarction or myocardial ischaemia. The latter classification, as it partly has been based on medical histories obtained from general practitioners and physicians in attendance, could suffer from differential misclassification, since it is possible that the presence of atherosclerosis is underreported in subjects with a poor cognitive function. This could lead to an underestimation of the association between atherosclerosis and cognitive function. However, the use of a classification either solely based on electrocardiograms or on both electrocardiograms and medical histories of cardiovascular disease gave similar results when studying the effects of atherosclerosis and inflammation on cognitive functioning. This outcome suggests that differential misclassification of atherosclerosis is negligible.

Implications and future directions

"Classic" route

The finding that cardiovascular risk factors, in particular low HDL-cholesterol, and cardiovascular disease, i.e. atherosclerosis, may be causal factors in the development of a poor cognitive function should be studied in trials investigating the effect of secondary prevention of atherosclerosis and its effect on cognitive functioning. The longitudinal part of the Leiden 85-plus Study can produce supporting evidence for the idea that atherosclerosis leads to a poor cognitive function in case it will be found that cardiovascular disease precedes dementia. Moreover, in case it will be found that the incidence of cardiovascular and cerebrovascular disease becomes higher in women than in men, this study can explain the higher incidence of dementia in women. Finally, the focus of attention should be on longitudinal studies investigating the effect of HDL-cholesterol on cognitive functioning and on trials investigating drugs that increase HDL-cholesterol, contrary to the current practice emphasizing cholesterol lowering drugs.

"Alternative" route

The finding that the biological interaction between atherosclerosis and inflammation is associated with a poor cognitive function and dementia offers new possibilities for prevention of dementia, but first observational longitudinal studies have to confirm the findings as described in this thesis. It is essential to understand that the interaction between atherosclerosis and inflammation can lead to a poor cognitive function and dementia, since people who are "at risk" to develop dementia may benefit from the results of clinical prevention trials studying the effect of anti-inflammatory drugs on cognitive functioning. However, randomised clinical trials studying the effect of anti-inflammatory drugs on cognitive functioning in subjects with dementia did not show beneficial effects[16,17] . These results suggest that anti-inflammatory drugs are only effective in the primary prevention of dementia and cognitive impairment. Trials should therefore be focussed on "healthy" middle-aged subjects with a pro-inflammatory response.

Junctions between the "classic" and the "alternative" route

A pro-inflammatory response not only affects cognitive functioning. It is also associated with a clustering of cardiovascular risk factors, which is found in the metabolic syndrome, and type-2 diabetes. Furthermore, it predisposes to cardiovascular and cerebrovascular disease, as is shown in this thesis.

It could be questioned why a pro-inflammatory response has such deleterious effects later in life. This question could be answered by evolutionary theories addressing the reasons for ageing. Critical in understanding why humans do age is that the force of selection decreases with age[18-20] . In the wild survival has declined to such small numbers that the force of selection is too weak to oppose the accumulation of germ-line mutations with late-acting deleterious effects[21] . In the same line is the view that pleiotropic genes with beneficial effects early in life are favoured by selection, even if these genes have deleterious effects later in life[9,19] . Selection for genes encoding for a pro-inflammatory response fit

within these theories. An innate pro-inflammatory response is associated with a better survival of patients with sepsis[9] . In times that infant mortality due to infectious diseases was high, it is likely that survivors had an innate pro-inflammatory response. The trade-off of this survival benefit could be that an innate pro-inflammatory response predisposes to an increase in lipids and lipoproteins, which have been suggested to protect against infection[22,23] , and at the same time predisposes to the development of cardiovascular disease, cerebrovascular disease, cognitive impairment and dementia.

It would be interesting to focus on the deleterious effects of a pro-inflammatory response later in life. Brain imaging techniques and clinical studies should be done to determine whether there is a difference in stroke lesion size and neurological outcome between subjects with a pro- and an anti-inflammatory cytokine response. The longitudinal part of the Leiden-85 plus study may determine the direction of the temporal relations between atherosclerosis, inflammation and cognitive function, i.e. could determine wheter atherosclerosis and a pro-inflammatory response leads to cognitive impairment and dementia. More important, also the concept that a pro-inflammatory cytokine response leads to more tissue damage than an anti-inflammatory cytokine response could be implemented when studying late-onset depression, which is thought to be caused by atherosclerosis [124].

Finally, the finding that low IL-10 production is associated with low HDL-cholesterol points to the junction between the "classic" and the "alternative" route. Since the data presented in this thesis suggest that IL-10 production is associated with HDL-cholesterol concentrations (chapter 7), and both low IL-10 production and low HDL-cholesterol are associated with cognitive impairment and dementia (chapters 5 and 6).

Trials investigating the use of drugs with anti-inflammatory properties, such as fibrates and statins [25,26], which also increase HDL-cholesterol could elucidate whether changes in HDL-cholesterol are associated with changes in IL-10 production. These trials could confirm the relation between HDL-cholesterol and IL-10 as suggested in this thesis. Moreover trials investigating the effect of fibrates and statins, could determine whether these anti-inflammatory drugs could decrease the risk of cardiovascular disease[27,28] and cerebrovascular disease[28,29] and could prevent the development of dementia[30,31] in subjects with an innate pro-inflammatory response.

Conclusions

1. A high response is not required to prevent selection bias (chapter 2).
2. 85- year old women have a better cognitive function than 85 year old men (chapter 3).
3. Atherosclerosis is associated with a poor cognitive function (chapter 4) .
4. The association between low HDL-cholesterol and a poor cognitive function is only partly explained by the presence of atherosclerosis (chapter 5).
5. Atherosclerosis may trigger an inflammatory response that leads to neurodegeneration, i.e. cognitive impairment and dementia (chapter 6).

Subjects with a pro-inflammatory cytokine response are at risk to develop atherosclerosis, since:

6. Low IL-10 production, i.e. a pro-inflammatory cytokine response, leads to detrimental changes in lipid and glucose metabolism, which could contribute to the development of the metabolic syndrome and type-2 diabetes (chapter 7).
7. Low IL-10 production is associated with an increased risk of stroke (chapter 8).

These findings from the Leiden 85-plus Study suggest that an innate pro-inflammatory response contributes to the development of cardiovascular risk factors and cerebrovascular disease. Which ultimately could lead to cognitive impairment and dementia.

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Chapter 10

Samenvatting

Zijn atherosclerose en ontsteking oorzaken van cognitieve achteruitgang bij oudste ouderen?

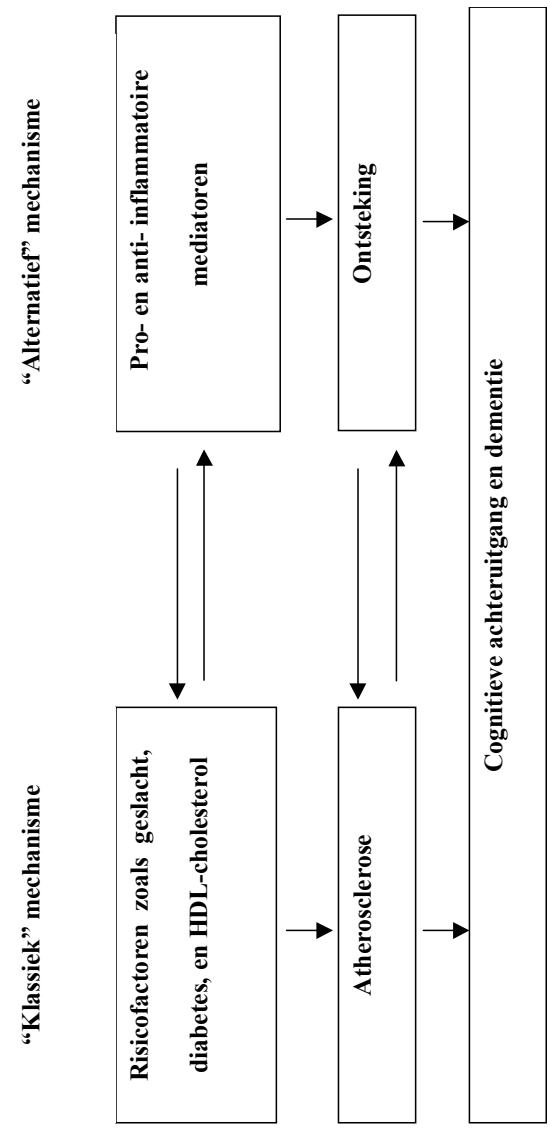
Inleiding

Een achteruitgang van verstandelijke vermogens, oftewel cognitieve achteruitgang, komt veelvuldig voor op hoge leeftijd. Een ernstige cognitieve achteruitgang wordt veelal veroorzaakt door dementie. Hoewel dementie vanaf middelbare leeftijd voorkomt, is het overgrote deel van de patiënten 70 jaar of ouder. Vanaf die leeftijd neemt de kans op dementie proportioneel toe om pas op de hoogste leeftijd weer af te vlakken. De klinische diagnose dementie wordt gesteld indien er een aantoonbare stoornis is van het korte- en lange termijn geheugen met daarnaast ten minste één stoornis in het abstract denken, in het oordeelsvermogen, specifieke andere hersenfuncties, of, het optreden van persoonlijkheidsveranderingen. Daarnaast is het voor het stellen van de diagnose essentieel dat de cognitieve achteruitgang een belangrijke negatieve invloed heeft op het dagelijks functioneren, sociale activiteiten en/of relaties van de patiënt.
Het doel van de studies in dit proefschrift is om de oorzaken van de achteruitgang in cognitief functioneren bij de oudste ouderen te bestuderen. Hiertoe werden twee vraagstellingen geformuleerd:

1. Zijn atherosclerose en ontsteking geassocieerd met stoornissen in het cognitief functioneren bij de oudste ouderen?
2. Wat is de directe relatie tussen atherosclerose en ontsteking bij de oudste ouderen?

Deze klinische vraagstellingen zijn tot stand gekomen op geleide van recent dier-experimenteel onderzoek. Deze experimenten tonen aan dat dieren met een erfelijke aanleg voor heftige ontstekingsreacties méér atherosclerose hebben dan dieren die deze aanleg niet hebben. Bovendien laten deze studies zien dat, wanneer de bloedtoevoer naar de hersenen wordt belemmerd, de dieren met heftige ontstekingsreacties grotere herseninfarcten hebben dan de dieren die deze aanleg niet hebben.

De uitwerking van de twee vraagstellingen in de Leiden 85-plus Studie wordt weergegeven in de figuur. Deze figuur beschrijft twee mechanismes die het ontstaan van cognitieve achteruitgang en dementie op hoge leeftijd kunnen verklaren. De algemeen aanvaarde pathofysiologie van atherosclerose en cognitieve achteruitgang wordt aangeduid als het “klassieke mechanisme”. Deze laat zien dat atherosclerose zelf, zowel als risicofactoren voor atherosclerose, zoals diabetes en hoog cholesterol geassocieerd zijn met cognitieve achteruitgang. Deze zijn aanleiding tot doorbloedingsstoornissen van de hersenen, hetgeen kan leiden tot hersenschade en uiteindelijk in stoornissen van het cognitief functioneren. In het “alternatieve mechanisme” wordt gepostuleerd dat ontsteking belangrijk bijdraagt aan het ontstaan van cognitieve achteruitgang. Ontstekingsreacties kunnen direct of indirect via het ontstaan van atherosclerose bijdragen aan cognitieve stoornissen op hoge leeftijd. Daarnaast zijn de pro- en anti-inflammatoire mediatoren die de ontstekingsreactie in het lichaam sturen ook van invloed op risicofactoren van atherosclerose zoals diabetes.


"Klassiek" mechanisme
"Alternatief" mechanisme
Risicofactoren zoals geslacht, diabetes, en HDL-cholesterol
Atherosclerose
Pro- en anti- inflammatoire mediatoren
Ontsteking
Cognitieve achteruitgang en dementie

Leiden 85-plus Studie

Allereerst wordt in het proefschrift de opzet van de studie beschreven waarin alle deelonderzoeken zoals beschreven in dit proefschrift werden uitgevoerd (hoofdstuk 2).
De Leiden 85-plus Studie is een bevolkingsonderzoek, waarvoor alle ouderen uit Leiden die 85 jaar werden tussen 1 september 1997 en 1 september 1999, werden uitgenodigd.
Omstandig wordt ingegaan op de benadering van de 85-jarigen. De ouderen die aanvankelijk aarzelden deel te nemen aan de studie, werden door een verpleegkundige aan huis bezocht. Door deze intensieve benadering werd de respons verhoogd van 74% tot 87%. Dat wil zeggen, van de 691 ouderen die gevraagd werden deel te nemen, hebben in de eerste ronde 511 (74%) ouderen hun medewerking toegezegd en dit aantal kon worden verhoogd tot 599 (87%) in de tweede ronde. Het kon aannemelijk worden gemaakt dat deze 599 deelnemers representatief zijn voor de 691 ouderen van 85 jaar, aangezien er geen verschillen waren in demografische en klinische kenmerken tussen de twee groepen. Mede als gevolg van deze ongewoon hoge respons is de onderzoekspopulatie een goede afspiegeling van alle 85-jarigen in Leiden. Omdat er geen redenen zijn om aan te nemen dat de Leidse 85-jarigen biologisch anders zijn dan 85-jarigen elders, zijn de resultaten van de studies in dit proefschrift extrapoleerbaar naar de oudste ouderen in het algemeen.

Op 85-jarige leeftijd hebben vrouwen een betere cognitie dan mannen

Allereerst werd het idee getoetst dat op hoge leeftijd het verschil in cognitief functioneren tussen vrouwen en mannen verklaard kan worden door een verschillend opleidingsniveau (hoofdstuk 3). Eerder uitgevoerde studies laten zien dat de prevalentie en incidentie van dementie hoger zijn bij vrouwen dan bij mannen. Omdat vrouwen in het algemeen, en vrouwen uit dit geboorte cohort in het bijzonder, een lager opleidingsniveau hebben dan mannen zou dit de bevinding kunnen verklaren. Het cognitief functioneren werd gemeten met vier neuropsychologische tests, die onder meer aandacht, verwerkingssnelheid, en geheugen meten. De neuropsychologische tests laten zien dat vrouwen in vergelijking tot mannen zowel een hogere cognitieve snelheid hebben alsook een beter geheugen. Deze bevindingen suggereren dat verschillen in cognitief functioneren tussen vrouwen en mannen eerder verklaard kunnen worden door biologische verschillen dan door verschillen in opleidingsniveau. Een mogelijke verklaring voor het gevonden betere cognitief functioneren van vrouwen ten opzichte van mannen is dat op deze leeftijd de atherosclerose minder uitgesproken is bij vrouwen dan bij mannen. Immers, hart- en vaatziekten treden gemiddeld 10 jaar later op bij vrouwen dan bij mannen. Dit verschil wordt echter kleiner op de hoogste leeftijd.

Hart- en vaatziekten en cognitief functioneren

Aansluitend werd onderzocht of het verminderd voorkomen van hart- en vaatziekten de verschillen in cognitief functioneren tussen vrouwen en mannen kan verklaren (hoofdstuk 4). Voor dit onderzoek werd ook gebruik gemaakt van de Mini-Mental State Examination (MMSE), een veelgebruikte screeningstest om het cognitief functioneren in kaart te brengen. Zoals verwacht hadden vrouwen minder hart- en vaatziekten dan mannen. De score op de MMSE bij vrouwen en mannen zónder hart- en vaatziekten was gelijk; beide hadden een score van 26 punten. Zowel bij vrouwen als bij mannen

daalde de MMSE score naar 25 punten in de groep deelnemers met twee of meer hart- en vaatziekten. Een verslechtering in het cognitief functioneren bij een toenemend aantal hart- en vaatziekten werd ook gevonden voor cognitieve snelheid. Gebaseerd op deze gegevens concluderen wij dat aanwezigheid van hart- en vaatzieken, als maat voor gegeneraliseerde atherosclerose, geassocieerd is met een verminderd cognitief functioneren en dat het verminderd voorkomen daarvan bij vrouwen het betere cognitief functioneren op oude leeftijd kan verklaren.

Risicofactoren voor atherosclerose en cognitief functioneren

Een hoog plasmacholesterol is een bekende risicofactor voor het ontstaan van atherosclerose. Daarnaast is atherosclerose geassocieerd met een verminderd cognitief functioneren op hoge leeftijd. Wij onderzochten daarom of een hoog cholesterol gehalte vaker voorkwam bij mensen met een verminderd cognitief functioneren (hoofdstuk 5). Omdat uit experimenteel onderzoek in cellen en proefdieren blijkt dat het cholesterol metabolisme direct van invloed is op het neerslaan van beta-amyloïd, het eiwit dat aanleiding geeft tot de ziekte van Alzheimer, onderzochten we of dit verband uitsluitend kan wordne verklaard door de aanwezigheid van atherosclerose. Mogelijk is er ook een direct verband tussen de hoogte van het cholesterolgehalte en het cognitief functioneren. Deelnemers met een laag gehalte van high-density lipoprotein-cholesterol (HDL-cholesterol), het 'goede' cholesterol, hadden een slechtere cognitie dan deelnemers met een hoog HDL-cholesterol gehalte. Er waren geen verschillen in cognitief functioneren wanneer deelnemers met lage en hoge concentraties van de andere cholesterolfracties, en triglyceriden in het bloed, werden vergeleken. Het verband tussen laag HDL-cholesterol en verminderd cognitief functioneren was het sterkst bij de deelnemers die nooit een beroerte of andere hart- en vaatziekten hadden doorgemaakt. Deze gegevens suggereren dat een verlaagd gehalte van HDL-cholesterol op hoge leeftijd sterk bijdraagt aan een verminderd cognitief functioneren via atherosclerose én via een atherosclerose onafhankelijk mechanisme.

Atherosclerose, ontsteking en cognitief functioneren

De hypothese is dat ouderen met atherosclerose én een aanleg tot heftige ontstekingsreacties een grotere kans hebben op cognitieve functiestoornissen dan ouderen met minder heftige ontstekingsreacties. De gedachte hierachter is dat een doorbloedingsstoornis door atherosclerose meer hersenschade geeft bij personen met een heftige ontstekingsreactie. In lijn met deze gedachtevorming konden wij aantonen dat deelnemers met een aanleg tot heftige onstekingsreacties een twee- tot driemaal grotere kans hebben op een verminderde cognitieve snelheid, een slecht geheugen, én dementie (hoofdstuk 6). Wanneer dit verband werd geanalyseerd enkel bij deelnemers zonder tekenen van hart- en vaatziekten, bleek de relatie tussen ontsteking en cognitief functioneren afwezig. Conform onze hypothese, hadden deelnemers mét tekenen van hart- en vaatziekten én een heftige ontstekingreactie een vijf tot tienmaal verhoogde kans op cognitieve functiestoornissen in vergelijking tot deelnemers met géén van deze eigenschappen.

Ontsteking en diabetes

Ook wordt in dit proefschrift de relatie beschreven tussen interleukine-10 en risicofactoren voor hart en vaatziekten, zoals diabetes mellitus type 2 en het metabole syndroom (hoofdstuk 7). Interleukine-10 is een onstekingsmediator die onstekingsreacties in het lichaam remt. Het metabole syndroom is een clustering van risicofactoren voor hart- en vaatziekten waaronder insuline resistentie die op termijn diabetes type-2 kan veroorzaken. De ziekte diabetes type-2 zelf is een belangrijke risicofactor voor het ontstaan van atherosclerose. De plasmaconcentraties van totaal cholesterol, LDL-cholesterol, triglyceriden, glucose en HbA1c, allen uitingen van het metabole syndroom, daalden wanneer de aanleg van deelnemers om interleukine-10 te produceren toenam. De plasmaconcentratie van het 'goede' HDL-cholesterol nam toe bij een toename van de productie van interleukine-10. Onder de deelnemers met een lage interleukine-10 productie was het risico op diabetes type-2 aanzienlijk verhoogd. Deze bevindingen ondersteunen het idee dat een aanleg tot een lage productie van interleukine-10 geassocieerd is met risicofactoren voor hart- en vaatziekten.

Ontsteking en beroerte

Ten slotte is de relatie onderzocht tussen ontsteking en het optreden van een beroerte (hoofdstuk 8). In onze studie hadden deelnemers met een lage interleukine-10 productie, dus neiging tot een heftige ontstekingsreactie, een vergroot risico op een beroerte dan deelnemers met een hoge interleukine-10 productie. De kans om te overlijden aan een beroerte was drie keer groter in deelnemers met een lage interleukine-10 productie in vergelijking tot deelnemers met een hoge interleukine-10 productie. Deze bevindingen zijn identiek aan de waarnemingen zoals deze in dierexperimenteel onderzoek zijn verkregen en ondersteunen het idee dat ontstekingsreacties belangrijk bijdragen aan het ontstaan van atherosclerose en hart- en vaatziekten.

Implicaties

De belangrijkste implicatie van de bevindingen uit dit proefschrift is dat preventie van risicofactoren voor atherosclerose en preventie van hart- en vaatziekten gepaard zal gaan met een reductie van het aantal ouderen met cognitieve stoornissen en dementie. Eén van de nieuwe mogelijkheden is het gebruik van HDL-cholesterol verhogende geneesmiddelen ter preventie van cognitieve achteruitgang. Dit is anders dan het gebruik van cholesterol verlagende geneesmiddelen, zoals statines, die wél het LDL-cholesterol verlagen maar relatief minder invloed hebben op het HDL-cholesterol. Een tweede implicatie van dit onderzoek is dat de zoektocht naar geneesmiddelen met een ontstekingsremmende werking moet worden voortgezet. Dit ondanks de uitkomsten van klinische trials met NSAID's, prednison en andere ontstekingsremmende middelen die op heden niet succesvol zijn geweest in het voorkomen van dementie op oude leeftijd. Onze gegevens duiden echter onmiskenbaar op een sterke relatie tussen ontstekingsreacties en hersenschade.

Nawoord

Wetenschap betekent samenwerking.
De Leiden 85-plus Studie is een schoolvoorbeeld van een gezamenlijke onderneming. Dit proefschrift kon niet tot stand komen zonder de hulp van alle leden van deze onderzoeksgroep en zonder de bijdragen van alle Leidse 85-jarigen, de deelnemende (verpleeg)huisartsen, de deelnemende apothekers, en de gemeente Leiden.

Mijn dank gaat uit naar hen allen.

Curriculum vitae

Curriculum vitae van de auteur.

1970	Geboren te Amsterdam, op 27 mei
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2002	Start opleiding tot psychiater, GGZBA, locatie Valeriusplein, Amsterdam