Chapter 9

Summary and general discussion

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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

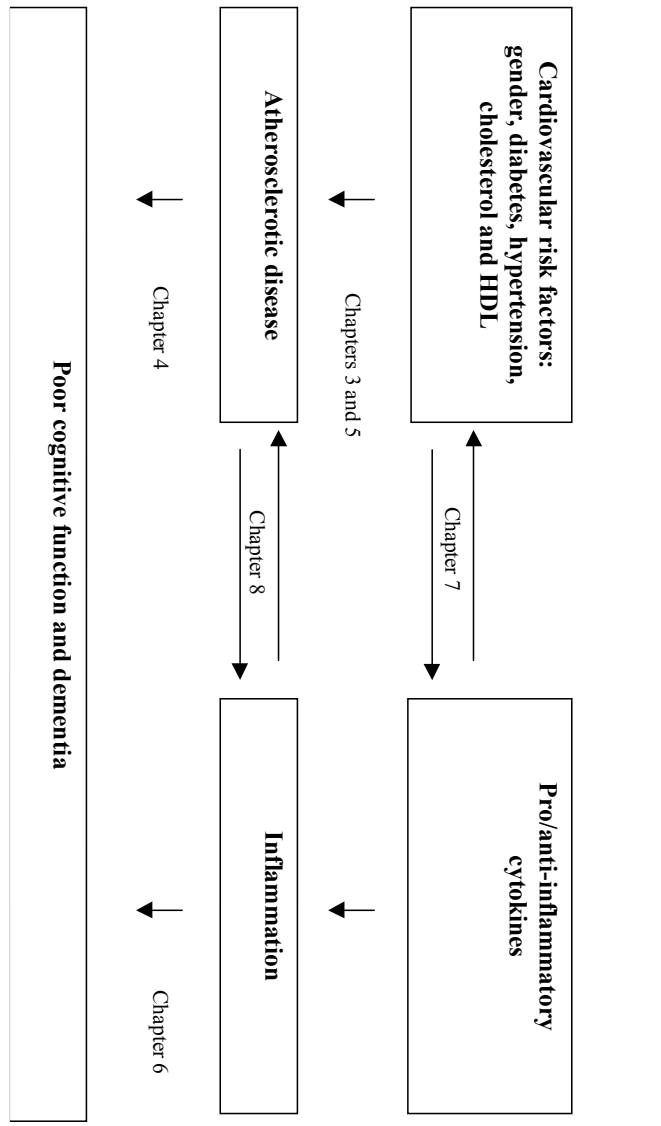

Figure 1 *Atherosclerosis, inflammation and cognitive function*

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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