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General discussion

The work described in this thesis was based on studies that have been performed in the Rotterdam Study, a large population-based cohort study that started in 1990 and included 7,983 persons aged 55 years and older of whom 7,721 had not experienced a previous stroke. The cohort was continuously monitored for incident stroke by linkage of the Rotterdam Study database with medical records from general practitioners and by regular visits to the general practitioners who were not included in the automated database (15%). Until January 1, 1999, including 46,011 person-years of follow up, we identified 432 first-ever stroke cases. We investigated risk factors for stroke. The main focus was on atherosclerosis in relation to stroke. Besides, we studied putative risk factors such as sex hormones and genetic factors, namely the Angiotensin Converting Enzyme (ACE) polymorphism and mutations in the genes for hemochromatosis (HFE genes). In the following we will discuss some methodological and conceptual issues that relate to the studies described in this thesis, summarize our findings and comment on the clinical relevance of our findings. Further, we will make recommendations for future research.

METHODOLOGICAL CONSIDERATIONS

Atherosclerosis

Atherosclerosis is the main risk factor for stroke and is involved in more than 70% of all strokes. To the extent that non-invasive measures of subclinical atherosclerosis can predict stroke risk, they can be used to identify high-risk persons who may benefit most from preventive interventions.

A number of issues merit attention if we consider measures of atherosclerosis in relation to stroke risk. Atherosclerosis in general can be related to stroke in several ways. First, it can be causally related to stroke, either directly, e.g. by occlusion of vessels or release of thrombo-emboli, or indirectly, e.g. by contributing to an unfavorable risk profile that causes the stroke. For example, atherosclerosis can be associated with cardiac disorders like atrial fibrillation, leading to emboli or hypoperfusion of the brain. But apart from playing a role in the etiology of stroke, atherosclerosis can also predict the risk of stroke because it marks the presence of other, causally related, factors. Moreover, different non-invasive measures of atherosclerosis exist and a specific measure of atherosclerosis can be related to the risk of stroke as a reflection of atherosclerosis elsewhere.

An other issue to consider is that atherosclerosis can be divided into two distinct pathophysiological entities, namely atherosis and sclerosis.[1] Atherosis refers to the structural changes that occur during the atherosclerotic process, whereas sclerosis causes functional changes in the vessel bed such as an increased arterial stiffness. These different entities may have distinct pathophysiological mechanisms in relation to stroke.

Stroke classification

Our follow-up was virtually complete which helped to reduce misclassification of stroke cases. We classified strokes as cerebral infarction, intracerebral hemorrhage or unspecified. In the majority of our studies we classified stroke subtypes based on CT or MRI scan, which was available for approximately 60% of the cases. Subtyping of cerebral infarctions was done based on size (lacunar infarctions in small perforating vessels or larger, non-lacunar infarctions) and location (anterior and posterior circulation). Investigation of the relation between atherosclerosis and subtypes of cerebral infarction may give more insight in the underlying mechanisms. For example, in order to investigate the causal role of carotid atherosclerosis in stroke, we analyzed infarctions anterior and posterior circulation of the brain separately. Blood flowing through the carotid arteries mainly supplies the anterior circulation of the brain. Therefore, a causal relation between carotid atherosclerosis and stroke would result in a stronger relation with anterior as compared to posterior circulation infarction. The fact that a considerable proportion of strokes remained unspecified reduced the power in our study. To the extent that the lack of additional diagnostic information was related to the determinants under study, it may have biased our findings.

In several clinical studies the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification is used, which classifies cerebral infarctions according to etiology.[2] We did not use this classification because it is doubtful whether such a classification is useful in etiologic research since it would induce a circular argument. For example, atherosclerosis would by definition be related to infarctions that are caused by large vessel disease. Another drawback of the TOAST classification is that it so too simplistic. For example, it is doubtful whether all strokes related to atrial fibrillation are cardioembolic as the TOAST criteria dictate. It has been reported that hypoperfusion may also play a role in

strokes that occur in persons with atrial fibrillation, as may other co-existing vascular pathology.[3]

MAIN FINDINGS

Incidence of stroke in the elderly

In the last decades, a decreasing trend in stroke mortality has been observed.[4-9] Yet, stroke is still the third leading cause of death in many countries. Decreasing trends for stroke morbidity are less clear. Cerebrovascular diseases put a large burden on health care budgets. Limited data existed on the incidence and lifetime risk of stroke in the elderly. We found that the incidence rate of stroke increased with age. Further, the incidence rate was higher in men than in women over the entire age range. However, as a net result of shorter survival and higher incidence rates of stroke in men as compared to women, lifetime risks were similar for both sexes (21% for men and women aged 55 years). This study suggests that depletion of susceptibles for stroke with age does not occur.

Markers of structural vessel wall change and generalized atherosclerosis

Ankle-arm index

Ankle arm index is a simple measure of peripheral arterial disease and atherosclerosis. It has been suggested that ankle arm index can be used to select persons at high risk of cardiovascular disease.[10] The Atherosclerosis Risk In Communities Study has shown that the risk associated with a low ankle arm index diminished after adjustment for cardiovascular risk factors.[11] In line with their observation we found that ankle arm index lost the additional value for the prediction of stroke if compared to a model including cardiovascular risk factors. When we compared the predictive value of several measures of atherosclerosis, we found similar results in the sense of little value of adding ankle arm index. These results suggest that a low ankle arm index acts as a marker of an unfavorable cardiovascular risk profile but does not directly reflect etiologically relevant pathology.

Carotid intima-media thickness

Carotid intima-media thickness has been widely studied as a marker of generalized atherosclerosis. It has been confirmed that an increased intima-media thickness is related to the risk of stroke and cerebral infarction, independently of traditional cardiovascular risk factors.[12-14] However, the relation between stroke subtypes, and in particular cerebral hemorrhages was not yet clear. We have shown that an increased intima-media thickness is related to all subtypes of stroke, including intracerebral hemorrhages and lacunar infarction. This opens the possibility that intima-media thickness is also reflecting small vessel disease, since both lacunar infarction and intracerebral hemorrhage are reported to be related to arteriolosclerosis of small intracerebral vessels.[15-17]

Compared to other measures of atherosclerosis, intima-media thickness was most strongly related to stroke, even when a history of cardiovascular disease was taken into account. Most likely, an increased intima-media thickness is a marker of generalized atherosclerosis, possibly including small vessel pathology. An increased intima-media thickness may be used to select persons at high risk for stroke, irrespective of other cardiovascular risk factors.

Carotid plaques

The significance of carotid plaques in relation to stroke in persons without previous stroke or TIA was not clear. Knowledge on the relation with subtypes of cerebral infarction and on the importance of plaque location was limited. Furthermore, it had not been established whether carotid plaques are merely sources of emboli, or markers of generalized atherosclerosis. Our study on the relation between carotid plaques at six locations in the carotid arteries and subtypes of cerebral infarction yielded several new insights. First, plaques increased the risk of stroke and cerebral infarction, irrespective of plaque location. We could not confirm that plaques at locations with a lot of turbulence like the bifurcation carried a higher risk than plaques elsewhere. Secondly, plaques were related to anterior but not to posterior circulation infarctions, which suggests that plaques may just be sources of thrombo-emboli. Thirdly, carotid plaques were related to the risk of lacunar infarction, which suggests that carotid plaques are also related to small vessel disease. Another explanation might be that part of the lacunar infarctions are related to emboli. In the last

decade, more evidence is accumulating that lacunar infarctions may be caused by emboli.[18-22] Our conclusion is that carotid plaques are both markers of generalized atherosclerosis and sources of thrombo-emboli.

When compared to other markers of atherosclerosis, carotid plaques were not the strongest predictor of stroke. Increased carotid intima-media thickness for example was a stronger predictor. Just counting number of plaques, as we did, may be too crude a measure. Possibly, measurement of the thickness of all plaques and measurement of plaque characteristics like echolucency, ulceration and intraplaque hemorrhage may help to more precisely measure plaques.

Calcifications in the vessel wall

In this thesis we studied two measures of calcification, namely aortic calcifications and coronary calcifications. We showed that presence of aortic calcifications is related to an increased risk of stroke and cerebral infarction, irrespective of cardiovascular risk factors. Furthermore, we showed that presence of coronary calcifications as detected by electron-beam tomography is related to a history of stroke. The most likely explanation for these results is that calcifications in the vessel wall reflect the extent of atherosclerotic lesions elsewhere. Alternatively, calcifications in the vessel wall may also functionally be related to increased arterial stiffness, leading to an increased pulse pressure and risk of stroke. A third explanation is that aortic and coronary calcifications reflect calcifications in the aortic arch from which emboli can arise. Unfortunately, we did not have data on atherosclerosis in the aortic arch, so we were not able to investigate this further.

When we compared the predictive value of between several measures of atherosclerosis, we found that aortic calcifications were among the best predictors for stroke and were related to stroke, independently of cardiovascular risk factors and intima-media thickness.

Functional changes in the vessel wall

Arterial stiffness

Stiffening of the arterial tree is considered the main cause of an increased pulse pressure. We investigated two measures of arterial stiffness in relation to stroke. The pulse-wave velocity in the aorta is calculated as the ratio between the transit

time of the foot of the pulse wave to travel along the arterial tree and the distance of the arterial segment. Another measure of arterial stiffness is the distensibility, i.e. change in arterial diameter due to change in arterial pressure over the cardiac cycle. We found that aortic stiffness is not clearly related to a previous stroke. However, carotid stiffness was related to a previous stroke. The relation remained after adjustment for carotid plaques. One other but small case control study investigated the relation between pulse-wave velocity and stroke. They reported a relation between pulse-wave velocity and stroke.[23] Recently, it was reported that aortic stiffness as assessed with transoesophageal echocardiography is related to ischemic stroke in an elderly population, independently of thickness of aortic plaques.[24] These results suggest that assessing aortic stiffness, or sclerosis, may add prognostic information in assessing the risk of stroke in the elderly.

A limitation of our studies on coronary calcifications and arterial stiffness is the cross-sectional design. This may have induced misclassification of exposure. More importantly, only survivors of stroke were analyzed. This prevalence-incidence bias may have influenced our results.

Endogenous sex hormones

Sex hormones may play a role in the difference between men and women in the occurrence of atherosclerosis and cardiovascular disease. Estrogen is considered to have a protective effect in women, since the frequency of cardiovascular disease increases after menopause. However, recent clinical trials evaluating the effect of hormone replacement therapy in relation to stroke reported negative results.[25,26] We found no relation between endogenous estrogen levels and stroke risk, neither in men nor in women. A low testosterone level was recently reported as a potential risk factor for cardiovascular disease in men.[27,28] However, prospective studies on the relation between testosterone and stroke were lacking. We showed that a low testosterone level was related to an increased risk of stroke in male non-smokers. Adjustment for atherosclerosis yielded similar results, suggesting that other mechanisms than atherosclerosis may underlie this relation. Levels of testosterone in men gradually decrease with age. One proposed mechanism is that a low testosterone level is related to a bad general health and comorbidity.[29] Other possible pathways are that low testosterone levels are related to oxidative stress, vasoconstriction or arterial

stiffness.[30-32] At present, use of testosterone replacement therapy in men is not yet warranted as a measure to prevent stroke.

Genetic factors

Genetic factors may be related to stroke, most likely by modulating the effects of, or predisposing to risk factors for stroke such as atherosclerosis and hypertension. We evaluated two possible candidate genes in relation to stroke. Mutations in the hemochromatosis gene (HFE), namely C282Y and H63D were related to both stroke and carotid atherosclerosis in persons who smoked or had hypertension. Possible mechanisms underlying these relations are an increased iron level, leading to increased blood viscosity, or increased oxidative stress and subsequent vascular damage.[33,34] For the Angiotensin Converting Enzyme (ACE) gene our results were less clear. The D-allele is hypothesized to influence atherosclerosis and to have other influences on the vascular system, namely by inducing, hypertension, influencing endothelial function or regulation of smooth muscle tone. [35-37] We found that overall, the D-allele was not related to the risk of stroke or cerebral infarction. However, there was a significant interaction between presence of the D-allele and hypertension in relation to cerebral infarction. The results remained similar after adjustment for carotid atherosclerosis. Possibly hypertension has different effects in persons with and without the D-allele.

CLINICAL RELEVANCE

Cardiovascular disease has long time been considered a disease of men. Our study on the incidence of stroke confirmed that incidence rates were higher in men than in women. However, since men have poorer survival, lifetime risks of stroke were similar for men and women. Women have their stroke at a higher age than men. These results illustrate that stroke prevention in the elderly, also in women, is a very important issue, especially since populations are rapidly growing older. In coming decades, stroke will put a larger burden on the health care budget. Treatment possibilities have improved in recent years and implementation of stroke units and stroke services has become widespread. Nevertheless, prevention of stroke is still the best therapy. What is needed for prevention to be effective is identification of modifiable causal risk factors and identification of people at high risk who may benefit from the intervention.

We analyzed different measures of atherosclerosis and we showed that measurement of ankle arm index has no additional value to classical cardiovascular risk factors in the prediction of stroke. Carotid intima-media thickness and aortic calcifications were the best predictors for stroke, independent of cardiovascular risk factors. Adding information on calcification to carotid intima-media thickness may thus be a promising method to select high risk groups. Still, clinical application should be preceded by further research e.g. on cost-effectiveness. Another measure of atherosclerosis related to stroke was carotid plaques. Just counting the number of plaques may provide a useful and easy tool to select persons at high risk for stroke, cerebral infarction and lacunar infarction. However, the prognostic value for stroke was less if compared to intima-media thickness and aortic calcifications. We further showed that coronary calcifications as detected by electron beam tomography may not only predict the risk of myocardial infarction, but also the risk of stroke. Still, this relation first needs to be evaluated prospectively before clinical management of persons with coronary calcifications should be altered.

In several studies we investigated the relation between atherosclerosis and lacunar infarctions. Atherosclerosis is not commonly considered an important risk factor for lacunar infarctions. However, our studies have shown that presence of carotid atherosclerosis increases the risk of lacunar infarctions, irrespective of cardiovascular risk factors like diabetes and hypertension. These results question the traditional distinction between large artery atherosclerosis and small vessel disease as different disease processes. Boiten et al. identified two types of lacunar infarctions. First, singe lacunes related to traditional vascular risk factors and microatheromata and secondly, multiple (silent) lacunar infarctions related to hypertension and leukaraiosis.[15] The latter type is considered to be related to lipohyalinosis or arteriolosclerosis, and has a worse prognosis.[16] In our study, we could not distinguish between the two groups of lacunar infarctions. However, it is conceivable that most of the lacunes in our study were single, related to traditional risk factors and microatheromata. In this light, carotid atherosclerosis as measure of large vessel disease can also act as an indicator of small vessel atheromata. Another possibility is that lacunar infarctions can be caused by micro-emboli, resulting from large artery disease.[18,38] Our results and the recent studies point out that the concept that lacunar infarctions are only related to small vessel disease does not hold.

Hormone therapy in postmenopausal women is a major issue of concern in cardiovascular disease prevention. Recently, evidence has become available to suggest that estrogen replacement therapy in women has no, or even and adverse effect on stroke risk.[25,26,39] Our study showed that endogenous estradiol is not related to stroke in postmenopausal women. However, we cannot generalize these results to exogenous estradiol. Therefore, we cannot infer that estrogen replacement therapy has no effect on stroke in women. In recent years, increasing attention has been payed to testosterone replacement therapy in men. It is considered to have beneficial effects on mood disorders, libido, well being and possibly cardiovascular disease.[40] Adversely, testosterone therapy may also increase the risk of prostate cancer.[40] We found that a low testosterone level in men increases the risk of stroke. These finding needs further confirmation. Further, the precise mechanism underlying this relationship needs to be explored. At present, testosterone suppletion in men in order to prevent stroke can not seriously be considered.

Finally, we showed that the ACE genotype, and mutations in the gene for hemochromatosis (HFE) were related to stroke in certain subgroups. Research on genetic factors in stroke is still in the beginning. As more knowledge on genetic factors in stroke will accumulate, intensified preventive therapy in susceptible persons may become possible.

FUTURE RESEARCH

We examined several measures of atherosclerosis in relation to stroke. Further epidemiological research can help unravel what underlies the relation between atherosclerosis and stroke. Measures like coronary calcifications and arterial stiffness need prospective evaluation in relation to stroke. Furthermore, these measures need comparison with other measures of atherosclerosis in relation to stroke. We identified carotid intima-media thickness and aortic calcifications as strong and independent predictors for stroke. Research is needed to evaluate the cost-effectivity of applying both measures to select high risk persons. A topic related to the latter is the development of risk scores in populations, including measures of atherosclerosis.

Another field of interest is the identification of factors that trigger the occurrence and progression of atherosclerosis. For example, measuring plaque characteristics like echolucency and surface regularity in population-based studies may help the identification of persons at higher risk of embolic stroke. It

is reported that these characteristics are related to unstable plaques and subsequent release of thrombo-emboli.[41-43] The influence of genetic and thrombotic factors on atherosclerosis and stroke also needs further investigation. Another factor that needs further investigation is medication use. The role of aspirin has become more clear in last decades. In a recent clinical trial it was found that use of statins decreases overall mortality and stroke risk in persons with any cardiovascular risk factor.[44] The effect of statins on atherosclerosis, either by reducing cholesterol or by antioxidant or other effects needs further study to explain the preventive mechanism.

We identified a low testosterone level as a risk factor for stroke in men. Further experimental and observational research is needed to investigate whether testosterone is related to vascular disease, or just a measure of bad health. If testosterone turns out to be directly related to the vascular system, then clinical trials on the effect of testosterone-replacement therapy on cardiovascular disease in men at risk for stroke should be conducted.

It is obvious that the field of genetics in stroke is just beginning to develop. We found a weak relation between the ACE D-allele and risk of stroke. The possible underlying mechanism needs further study. Studies on the effect of ACE inhibitors may help in this.

There is a need for an improved identification of stroke subtypes in population-based cohort studies. One approach is a more widespread use of neuro-imaging techniques to assess more subtypes, especially in the elderly who less often get a scan and in whom the burden of stroke is largest. Another approach is the use of more advanced neuro-imaging techniques such as perfusion and diffusion weighted imaging. This may help a more precise subtyping of cerebral infarctions. More detailed information on stroke subtypes and their prognosis may help to understand underlying pathophysiological mechanisms and to optimalize preventive strategies. Moreover, the classification of stroke subtypes may need revision.

Any future stroke investigator should realize that the effects of risk factors can be manifold and that stroke is a heterogeneous disease.

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