Prognosis after cerebral ischaemia of arterial origin: clinical characteristics and genetic information

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Prognosis after cerebral ischaemia of arterial origin: clinical characteristics and genetic information

Prognose na cerebrale ischemie van arteriële origine:
klinische kenmerken en genetische informatie
(met een samenvatting in het Nederlands)

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"Gaat het niet zoals het moet, dan moet het maar zoals het gaat"

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1

Introduction and outline

General Introduction

Introduction, disease severity

Cardiovascular diseases are the world's largest killers, claiming 17.3 million lives a year, mainly due to myocardial infarction and stroke.[1] With increasing age, gain of bodyweight and the high incidence of risk factors for vascular disease like hypertension and hyperlipidaemia, this burden of cardiovascular disease will remain a large health care problem over the coming years.

Atherosclerosis and prognosis

Patients with coronary artery disease (CAD), cerebrovascular disease (CVD), or peripheral arterial obstructive disease (PAOD) are thought to represent different clinical expressions of a single underlying disease: atherothrombosis.[2] Plaque disruption, thrombosis, and thromboembolism can affect different arterial beds and, depending on arterial size and severity of the distal occlusion, may lead to different clinical manifestations. The prognosis of patients who have had atherothrombotic disease depends on several factors. Probably, the most important factor is the site where the disease first becomes clinically evident. After ischaemic stroke a recurrent event most often is a new ischaemic stroke, whereas after myocardial ischaemia the next vascular event is another episode of ischaemia in the coronary circulation in two thirds of patients.[3] Patients with PAOD often fear amputation. However; the 10-year rate is less than 10%.[4] These patients have a slightly higher risk of myocardial infarction than of ischaemic stroke, and these events are more often fatal.[3,5] Other factors that determine prognosis of patients with atherosclerotic disease are the presence and severity of classical vascular risk factors and the measures taken for secondary prevention.[3] To optimize this secondary prevention for the individual patient it is important to to foresee potential future problems.

Prognosis after cerebral ischaemia

Patients with a recent stroke have an increased risk of new cardiovascular events. Vascular events will occurr in half of the patients in the 10 years following a TIA or minor ischaemic stroke.[6] Assessment of this risk is important to identify patients at (early) high risk of recurrent events and to guide rapid secondary prevention therapy.

Patients with TIA or minor ischaemic stroke are very heterogeneous in

terms of symptoms, risk factors, underlying pathology and also prognosis. The risk factors for cardiovascular diseases and not the characteristics of the events itself were shown to be independent predictors of long-term stroke risk.[7] To predict short-term outcome a number of risk scores has been developed, of which the ABCD^2-score is most widely used. Patients are allocated points for five factors: age 60 years or older (1 point); blood pressure elevation on first assessment after TIA (1 point; systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg); clinical features of TIA (unilateral weakness, 2 points; or speech impairment without weakness, 1 point); duration of TIA (≥ 60 minutes, 2 points; or 10-59 minutes, 1 point); and diabetes (1 point).[8] In a recent systematic review this score was validated and shown to have an area under the Receiver Operating Characteristic (ROC) curve of 0.70 (0.66-0.73) for 7-day prediction and 0.69 (0.66-0.72) for 90-day prediction of recurrent strokes.[9] For the long-term no good prediction models are available.[10] A recent validation study showed that all models available nowadays have similar performance with an area under the ROC curve of around 0.64 for the prediction of major vascular events.[11] The stroke prognosis instrument (SPI)-II and the ABCD^2-score prediction model were the most adequate.[11] The SPI-II predicts the more long-term outcome (>2 years) compared with ABCD^2-score (90 days outcome). In the SPI-II score patients scored 3 points for congestive heart failure, diabetes, and prior stroke each; Age> 70 years and previous stroke were each assigned 2 points; and severe hypertension and coronary artery disease were each counted for 1 point. It takes some more additional examinations to score the SPI-II score for the individual patient than for the ABCD^2-score. Addition of stronger predictors, like tissue based criteria or implementation of continuous variables might improve these models further.[12] Neither of the models described included genetic information.

Genetics and Stroke

Associations between genetic polymorphisms and new vascular events after cerebral ischaemia have received little attention. A strong link between classical risk factors for ischaemic stroke, such as hypertension, diabetes mellitus, hypercholesterolaemia, and genetic factors in the prediction of stroke recurrence has been suggested.[13] Several studies have been performed with a candidate gene approach to find an association between polymorphisms and first ischaemic strokes, often with conflicting results. Only a few single nucleotide polymorphisms (SNP) could be identified that accounted for

a moderately increased risk of ischaemic stroke. Probably genetic changes account only for a small increase in risk of complex diseases, such as stroke. To find this small risk large sample sizes are needed. Furthermore, multiple gene-environment interactions with cardiovascular risk factors and gene-gene interactions can mask the small true risk accounted for by one single SNP. In genetic studies it is of great importance to properly phenotype the patients.[14] In patients with atrial fibrillation two loci were found to be associated with cardioembolic stroke risk.[15,16] Also for large vessel stroke two loci, one on chromosome 9 and one in the HDAC9 gene, were suggested to be associated.[17,18] The mechanism by which these variants gives rise to the increased risk is not completely clear yet. Further research to find more genetic variants associated with stroke subtypes is warranted. The fact that different stroke phenotypes seem to have different associations with different genetic variants, suggests different pathogeneses between these subtypes. Differences between small and large vessel ischemic stroke are still a matter of debate, but the latest genetic findings strongly suggest that the underlying pathology is different between these two subtypes.[17,19,20]

Research questions

In part 1 of this thesis we explore differences between atherosclerotic diseases concerning baseline risk factor differences and prognosis. Moreover, we try to properly phenotype subtypes of cerebral ischaemia.

In part 2 we explore genetics and stroke. We hypothesised that addition of genetic information to a prognostic model only containing classical risk factors would improve the performance of this model in patients who suffered from cerebral ischaemia of arterial origin.

Outline of the Thesis

Part 1: prognosis after atherosclerotic disease

Chapter 2 outlines the differences in risk factor profile at baseline and cardiovascular prognosis for patients with different atherothrombotic diseases. *Chapter 3* describes in more detail the major difference in risk for haemorrhagic events between patients with cerebrovascular or peripheral artery disease and patients with coronary artery disease. In *Chapter 4* the performance of the Rose angina questionnaire in prognostic research was assessed against subsets of questions concerning chest pain. *Chapter 5* describes the comparison of prognosis of patients with small and large vessel stroke of arterial origin.

Part 2: prognosis of stroke and genetics

Chapter 6 is the design article written at the start of this doctorate thesis and outlines the aims of the study. In its addendum we explain how the methods changed over time. *Chapter 7* describes the main results of this thesis, the additional effect of genetic information in the prediction of the risk of ischaemic events for patients after cerebral ischaemia of arterial origin. In *Chapter 8* we did a replication study of the finding of an association between the HDAC9 genetic variant and large vessel disease stroke. *Chapter 9* is the general discussion.

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2

Patients with coronary, cerebrovascular or peripheral arterial obstructive disease differ in risk for new vascular events and mortality. The SMART study

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Abstract

Aims: Atherosclerosis causes coronary artery disease (CAD), cerebrovascular disease (CVD) or peripheral arterial obstructive disease (PAOD). The risk of new vascular events and mortality is high. Direct comparisons of vascular event rates are scarce.

Methods and results: Vascular risk factors and outcome events of 3563 patients from one university hospital presenting with non-disabling CAD, CVD or PAOD were compared with regression analyses, adjusted for age and sex (median follow-up 3.9 years). Primary outcome was the composite of myocardial infarction, stroke and vascular death. The risk between the three groups of outcomes was compared with Cox regression. At baseline CAD patients were most obese; PAOD patients smoked most and had more often hypertension and hyperlipidaemia. Average rate of vascular events was 2.5%/year; HR CVD/CAD 1.7 (95%CI 1.3-2.2) and PAOD/CAD 1.8 (95% CI 1.5-2.0). PAOD patients had a higher risk for coronary events than CAD (HR 1.6; 95%CI 1.2-2.1). Patients with CVD or PAOD had a higher risk for major bleeding than CAD patients (HR 2.1; 95%CI 1.4-3.2).

Conclusion: Patients with a recent CVD or PAOD have almost twice the risk for future vascular events than those with CAD.

Introduction

Patients with coronary artery disease (CAD), cerebrovascular disease (CVD), or peripheral arterial obstructive disease (PAOD) are thought to represent different clinical expressions of a single underlying disease: atherothrombosis.[1] Plaque disruption, thrombosis, and thromboembolism can affect different arterial beds and, depending on arterial size and severity of the distal occlusion, may lead to different clinical manifestations. The prognosis of patients who have had atherothrombotic disease depends on several factors. One important factor is the site where the disease first becomes clinically evident. After ischaemic stroke a recurrent event most often is a new ischaemic stroke,[2] whereas after myocardial ischaemia the next vascular event is another episode of ischaemia in the coronary circulation in two thirds of patients.[2] Patients with PAOD often fear amputation. However; the 10-year rate is less than 10%.[3] These patients have a slightly higher risk of myocardial infarction than of ischaemic stroke, and these events are more often fatal.[2,4] Other factors that determine prognosis of patients with atherosclerotic disease are the presence and severity of classical vascular risk factors and the measures taken for secondary prevention.[2]

Although cardiologists, neurologists and surgeons are aware of the risk for subsequent vascular events in other organ systems, inception cohorts have been necessarily limited to a single medical discipline. There are no large, prospective cohort studies, with uniform definitions of endpoints that compare baseline characteristics and the risk for subsequent vascular events in patients with CAD, CVD, or PAOD with regard to baseline risk profile and events during follow-up. Therefore we studied differences in baseline risk profiles and the occurrence of new events between patients with CAD, CVD and PAOD.

Methods

Study design and patient population

Patients aged 18 to 79 years, newly referred to the University Medical Center Utrecht, The Netherlands, with traditional risk factors for arterial disease (hypertension, hyperlipidaemia, diabetes mellitus) or with symptomatic arterial disease (coronary heart disease, cerebrovascular disease, abdominal aortic aneurysm, or peripheral arterial disease) were included in the Second

Manifestations of ARTerial disease (SMART) study. A detailed description of the study was published previously.[5] Briefly, patients who gave their written informed consent underwent a standardised vascular screening programme, including a health questionnaire, laboratory assessment, and ultrasonography to investigate the prevalence of additional vascular diseases. The Ethics Committee of the hospital approved the study.

For the current study, the data of 3563 consecutive patients presenting with transient or non-disabling manifestations of coronary (1882), cerebrovascular (881) or peripheral arterial obstructive disease (800) were available. These patients were included between September 1996 and February 2006 and were followed until March 2007 or death.

Definitions and follow up

In patients with PAOD, which were defined as a resting ankle brachial index (ABI) of 0.90 or lower or ABI post exercise decreasing 20% or more in at least one leg,[6] we recorded the severity of the vascular disease at baseline with the Fontaine scale.[7] Stage 1 (pain-free walking distance >200 meters), and stage 2 (pain-free walking distance <200 meters) were defined as mild or moderate ischaemia, whereas stage 3 (rest pain) and stage 4 (ulceration or gangrene), were defined as severe ischaemia. In patients with CAD, disease severity was rated according to the number of coronary arteries with marked atherosclerosis (>70% stenosis or fractional flow reserve <0.80, or treatment of the vessel). One-vessel, two-vessel, three-vessel, left main disease with or without right coronary artery involvement was rated in all patients with CAD on the basis of coronary angiography, percutaneous coronary intervention (PCI) or CABG reports. For patients with CVD the severity of the disease was classified with a handicap scale, the modified Rankin Score (mRS).[8]

Outcome events were defined as the first occurrence of ischaemic coronary event, ischaemic stroke or vascular death (Webtable 1). In addition vascular interventions during follow-up were recorded: amputation of foot or leg, vascular surgery (carotid endarterectomy or coronary artery bypass grafting [CABG]) or percutaneous transluminal angioplasty (PTA), with or without stenting). For potential outcome events reported by the patient we retrieved hospital discharge letters and the results of relevant laboratory and radiology examinations. Three members of the SMART Endpoint Committee independently audited all events on basis of available information. This committee consisted of physicians from different departments. In case of

disagreement, consensus was reached by consulting other members of the Endpoint Committee.

Statistical methods

Baseline data of the three groups of patients were compared pairwise with linear regression analysis for continuous variables and with logistic regression for dichotomous variables. The incidence of recurrent events was compared with Cox regression analysis. All regression analyses generated 95% confidence intervals (95%CI) to describe the precision of prevalence differences, odds ratios or hazard ratios. All effect estimates were adjusted for age and sex.

We also performed a sensitivity analysis on a subgroup of patients presented in this article, with the same methods as described above. These subgroups included patients with CAD with angina pectoris, patients with CVD with a modified Rankin grade of 0 or 1 and patients with PAOD with Fontaine grades 1 or 2.

To generate cumulative event curves (one minus survival curves) we used Cox regression analyses adjusted for age and sex.

Results

Baseline

Baseline characteristics and severity of disease of the three cohorts of patients are summarised in Table 1. The age- and sex-adjusted differences and odds ratios are presented in Table 2. The majority of patients were males with a mean age of 60 years. In the CAD group 95% were included because of angina pectoris, and only 5% because of a recent myocardial infarction. Of the patients with CAD 51% had single coronary vessel disease, 30% had two-vessel disease; and 14% had triple vessel disease. Less than one percent had involvement of the left main coronary artery. The majority of patients with PAOD suffered from intermittent claudication Fontaine grade 2 (92%). Forty-six percent of patients with CVD had had a transient ischaemic attack. About two thirds of patients with CVD recovered without any residual deficit or symptom (modified Rankin grade 0; 64%).

Table 1. Baseline characteristics

	with data	*Cerebrovascular disease (n=881)*	*Peripheral artery disease (n=800)*	*Coronary artery disease (n=1882)*
Age (years) (mean, SD)	3563	61.1 (10.7)	59.1 (10.7)	59.2 (9.5)
Male sex	3563	625 (71%)	526 (66%)	1535 (82%)
Qualifying diagnosis	3563			
TIA		402 (46%)		
Stroke		346 (39%)		
Transient Blindness		107 (12%)		
Retinal infarction		26 (3%)		
Myocardial infarction				82 (4%)
Angina pectoris				1622 (96%)
Claudication Fontaine 2			737 (92%)	
Claudication Fontaine 3-4			63 (8%)	
Severity of the disease				
CVD Modified Rankin 0		549 (62%)		
Modified Rankin 1		247 (28%)		
Modified Rankin 2		69 (8%)		
Modified Rankin 3		16 (2%)		
CAD 1 vessel disease				965 (51%)
2 vessel disease				554 (30%)
3 vessel disease				268 (14%)
History				
Stroke		360 (41%)	45 (6%)	45 (2%)
Carotid surgery		42 (5%)	23 (3%)	11 (1%)
Myocardial infarction		105 (12%)	122 (15%)	851 (45%)
Coronary surgery		74 (8%)	81 (10%)	1055 (56%)
Vascular surgery legs		52 (6%)	131 (16%)	63 (3%)
Cigarette smoking	3545			
Never smoked		156 (18%)	74 (9%)	428 (23%)
Packyears (mean, SD)		28 (20)	31 (19)	25 (19)
Known with hypertension	1446	421 (48%)	292 (37%)	733 (39%)
Hypertension at inclusion		161 (38%)	107 (37%)	223 (30%)
Not known with hypertension	2087	454 (52%)	492 (63%)	1138 (61%)
Hypertension at inclusion		94 (21%)	123 (25%)	119 (10%)

	with data	*Cerebrovascular disease (n=881)*	*Peripheral artery disease (n=800)*	*Coronary artery disease (n=1882)*
Known with hyperlipidaemia	1750	374 (44%)	269 (35%)	1107 (60%)
Hyperlipidaemia at inclusion		170 (45%)	146 (54%)	359 (32%)
Not known with hyperlipidaemia	1700	484 (56%)	500 (65%)	734 (40%)
Hyperlipidaemia at inclusion		318 (66%)	396 (79%)	331 (45%)
Known with diabetes	542	138 (16%)	135 (18%)	269 (15%)
Normal glucose (<6.0mmol/L)		20 (14%)	14 (10%)	34 (13%)
Impaired glucose (>6.0 mmol/L)		118 (86%)	121 (90%)	235 (87%)
Not known with diabetes	2862	709 (84%)	613 (82%)	1540 (85%)
Normal glucose (< 6.0mmol/L)		556 (78%)	440 (72%)	1130 (73%)
Impaired fasting (6.1-6.9mmol/L)		123 (17%)	123 (20%)	313 (20%)
New diabetes (>7.0mmol/L)		30 (4%)	50 (8%)	97 (6%)
BMI (kg/m2) (mean, SD)	3561	26.3 (3.9)	26.0 (4.1)	27.3 (3.6)
BMI > 30	3561	121 (14%)	114 (14%)	384 (20%)
ABI < 0.90	3530	157 (18%)	640 (82%)	126 (7%)
IMT (mm) (mean, SD)	3478	1.00 (0.33)	0.96 (0.32)	0.90 (0.27)
Carotid stenosis > 50%	3478	417 (49%)	113 (14%)	102 (6%)
Abdominal aorta > 3 cm	3512	35 (4%)	33 (4%)	41 (2%)
Kidney length <9.5 cm	3497	246 (29%)	208 (26%)	439 (24%)
Creatinin clearance (Cockroft)	3538			
Normal		309 (35%)	350 (44%)	889 (47%)
Mild impairment		489 (56%)	349 (44%)	889 (47%)
Severe impairment		77 (9%)	90 (11%)	96 (6%)
Hyperhomocysteinaemia	3545	121 (17%)	99 (15%)	152 (8%)
Use of antithrombotics	3563			
Antiplatelets		661 (75%)	343 (43%)	1450 (77%)
Oral anticoagulants		79 (9%)	82 (10%)	171 (9%)

Smoking was observed more often in patients with PAOD in comparison with the other two groups. History of hypertension was more present in patients with CVD, but patients with PAOD were found to have the most hypertension

at inclusion. Hyperlipidaemia was known most in patient with CAD, at inclusion it was found more often in patients with CVD and PAOD. Patients with PAOD were most known with diabetes and also had more impaired glucose levels. Obesity was more present in patients with CAD. The use of antithrombotic medication was not different between the three groups.

Table 2. Age and sex adjusted differences and odds ratios in baseline characteristics

	CVD vs CAD	*CVD vs PAOD*	*PAOD vs CAD*
Vascular history			
Stroke	* 29 (21-40)	* 12 (8-16)	* 2.6 (1.7-3.9)
Carotid artery surgery	* 7.5 (3.8-14.8)	* 1.6 (0.9-2.7)	* 4.8 (2.3-9.9)
Myocardial infarction	* 0.17 (0.14-0.21)	* 0.67 (0.50-0.89)	* 0.23 (0.19-0.29)
Coronary artery surgery	* 0.06 (0.05-0.08)	* 0.72 (0.52-1.01)	* 0.08 (0.07-0.11)
Vascular surgery peripheral arteries/amputation	* 1.8 (1.2-2.6)	* 0.32 (0.23-0.45)	* 5.7 (4.2-7.9)
Cigarette smoking			
Current	* 2.5 (2.1-3.1) §	*0.43 (0.35-0.53)§	* 5.9 (4.8-1.2)§
Pack-years (mean, SD)	3.4 (1.7-5.1)	-3.2 (-5.2 to -1.2)	6.6 (4.8-8.3)
Known with hypertension			
Hypertension at inclusion	* 1.3 (1.0-1.7)	* 1.01 (0.74-1.39)	* 1.28 (0.95-1.71)
Not known with hypertension			
Hypertension at inclusion	* 2.0 (1.5-2.7)	* 0.68 (0.50-0.93)	* 2.9 (2.2-2.9)
Known with hyperlipidaemia			
Hyperlipidaemia at inclusion	* 1.7 (1.4-2.2)	* 0.68 (0.50-0.94)	* 2.4 (1.8-3.1)
Not known hyperlipidaemia			
Hyperlipidaemia at inclusion	* 2.3 (1.8-3.0)	* 0.49 (0.36-0.65)	* 4.6 (3.5-6.0)
Not known with diabetes			
Normal glucose			
Impaired fasting	*0.76 (0.61-0.94)†	*0.62 (0.48-0.80) †	*1.22 (0.99-1.50)†
New diabetes			
BMI (kg/m2) (mean, SD)	-1.0 (-1.3 to -0.7)	0.3 (-0.1-0.7)	-1.3 (-1.7 to -1.0)
BMI >30	* 0.59 (0.47-0.74)	* 1.01 (0.76-1.33)	* 0.57 (0.45-0.72)
ABI <0.90	* 2.8 (2.1-3.6)	* 0.04 (0.03-0.06)	* 68 (52-90)
IMT (mm) (mean, SD)	0.10 (0.08-0.12)	0.02 (-0.01-0.05)	0.07 (0.05-0.10)
Carotid stenosis > 50%	* 17 (13-21)	* 5.6 (4.4-7.2)	* 2.9 (2.2-3.9)

	CVD vs CAD	*CVD vs PAOD*	*PAOD vs CAD*
Abdominal aorta > 3 cm	* 1.7 (1.1-2.8)	* 0.8 (0.5-1.3)	* 2.1 (1.3-3.4)
Kidney length <9.5 cm	* 1.2 (1.0-1.5)	* 1.1 (0.7-1.4)	* 1.1 (0.9-1.4)
Creatinin clearance (Cockroft)			
Normal			
Mild impairment	* 1.5 (1.2-1.8)‡	* 1.2 (0.9-1.5)‡	* 1.3 (1.0-1.6)‡
Severe impairment			
Hyperhomocysteinemia	* 2.1 (1.6-2.7)	* 1.0 (0.8-1.4)	* 2.1 (1.6-2.7)

ABI, Ankle Brachial Index, IMT, Intima Media Thickness.
* Odds ratio, § current + recent smoking versus past or no smoking, † impaired fasting glucose and new diabetic vs. normal, ‡ mild or severe impairment vs. normal

Follow-up

Median follow-up time in the CVD cohort was 4.4 years, in the CAD cohort 3.2 years, and in the PAOD cohort 4.2 years. Follow-up was complete in 98.1% of the patients. The annual risk of vascular events was 3.1% in the CVD cohort (132 events), 3.2% in the PAOD cohort (120 events) and 1.8% in the CAD cohort (124 events) (Tables 3 and 4).

In Webtables 2 and 3 presented on the internet you find the annual risks for vascular interventions in these groups. Figure 1 shows age- and sex-adjusted time-to-event curves for the three disease categories.

Table 3. Outcome events

	Cerebrovascular disease (n = 881)	*Peripheral arterial obstructive disease (n= 800)*	*Coronary disease (n = 1882)*
Person years	*4246*	*3760*	*6863*
Death	121 (2.8%)	140 (3.7%)	88 (1.3%)
Ischaemic coronary event	50 (1.2%)	77 (2.0%)	96 (1.4%)
Ischaemic stroke	68 (1.6%)	25 (0.7%)	19 (0.3%)
Major bleeding complication	44 (1.0%)	37 (1.0%)	32 (0.5%)
Any vascular event	132 (3.1%)	120 (3.2%)	124 (1.8%)

annual risks between parentheses

Cerebrovascular vs. coronary artery disease

Patients with CVD were more prone to vascular events than patients

with CAD, (Hazard ratio [HR] 1.7; 95%CI 1.3-2.2). Patients with CVD had more major bleeding complications (HR 2.2; 95%CI 1.4-3.6) than patients with CAD.

Table 4. Outcome hazard ratios, age- and sex adjusted

	Cerebral versus coronary disease	*Cerebral versus peripheral disease*	*Peripheral versus coronary disease*
Death	1.6 (1.2-2.1)	0.6 (0.5-0.8)	2.6 (2.0-3.5)
Ischaemic coronary event	0.79 (0.55-1.12)	0.49 (0.34-0.71)	1.55 (1.15-2.11)
Ischaemic stroke	6.1 (3.6-10.2)	2.3 (1.5-3.7)	2.5 (1.3-4.5)
Major bleeding complication	2.2 (1.4-3.6)	0.92 (0.59-1.44)	2.1 (1.3-3.5)
Any vascular event	1.7 (1.3-2.2)	0.87 (0.68-1.12)	1.8 (1.5-2.0)

Peripheral arterial obstructive vs. coronary artery disease
Patients with PAOD had a higher risk of all outcome events than patients with CAD (HR 1.8; 95%CI 1.5-2.0).

Cerebrovascular vs. peripheral arterial obstructive disease
Vascular events were equally frequent on follow-up in patients with CVD and in those with PAOD (HR 0.87; 95%CI 0.68-1.12). No differences were found with regard to major bleeding complications. Fewer patients with CVD had a vascular death (HR 0.6; 95%CI 0.5-0.8) than patients with PAOD.

Discussion

Principal findings

In this cohort study with long-term follow-up of patients with non-disabling but clinically manifest vascular disease, those who presented with CVD or PAOD more closely resembled each other than those with CAD, both at baseline and during follow up. More cardiovascular risk factors were found in patients with CVD and PAOD than in those with CAD (i.e. hypertension, hyperlipidaemia and cigarette smoking). Accordingly but remarkably, during follow up patients with PAOD had both a higher death rate and a higher event

rate of ischaemic coronary events than patients with CAD. Another striking finding was the relatively high bleeding risk in patients with CVD or PAOD, compared with patients with CAD. On the whole, patients with CVD or CAD tended to remain "true to type" with respect to the type of follow up event, whereas patients with PAOD did not.

In the context of previous studies

The initiators of the REduction of Atherothrombosis for Continued Health (REACH) study, a worldwide population-based study designed to compare differences in vascular risk factors and to record subsequent events in outpatients with CAD, CVD, and PAOD, have presented baseline data and one year follow-up.[9,10] Diagnostic workup occurred in many different facilities, whereas follow-up and assessment of outcome events were not performed according to a similarly strict and standardised approach as in SMART. In our study the baseline results are comparable with REACH; patients with CAD were more obese and had more often hypercholesterolemia, and PAOD patients smoked more often. In REACH one out of six patients with CAD, CVD, or PAOD had symptomatic involvement of one or two other arterial beds. From these results the investigators concluded that atherothrombosis is best treated as systemic disease. We agree with this position, but would like to add that patients with CVD and PAOD have other risk profiles and more, if not other, subsequent events than patients with CAD. During follow-up the REACH investigators made a comparison between subjects with cardiovascular risk factors alone and patients with established cardiovascular disease, irrespective of its location, whereas in our study we distinguished patients according to the initial manifestation of arterial disease.[10] In accordance with REACH we found that mortality was highest in the group of patients with PAOD, and the rate of nonfatal stroke in patients with CVD.

Apart from the REACH study, two other studies also followed patients with different types of atherosclerotic disease, from different sources.[2,4,10] The main conclusions were that disease in multiple locations increases the risk of new events,[4;10] and that in patients with CAD and CVD these subsequent events are mostly 'true to type',[4;10] as found in our population and, earlier on, in many single-specialty cohorts. That patients with PAOD relatively often develop vascular disease at other locations in the vascular tree and vascular death has been reported before.[11-13] Although patients with PAOD often fear amputation, coronary and cerebrovascular events threaten even survival, with

an up to four times higher risk of vascular death than in patients with CVD or CAD, depending on the severity of PAOD.[12] In our study we found a lower bleeding risk in the patients with CAD than in patients with PAOD and CVD. This cannot be explained by a difference in the use of anticoagulant or antiplatelet drugs taken by the different patient groups (Table 1). Known risk factors for bleeding, such as age and female sex,[14-16 17] did not differ importantly between the three study groups, hence we can only speculate about other explanations.

According to baseline risk factors, our study matches with a study performed in France. Patients with PAOD smoked more often, patients with CAD were more obese and patients with CVD had more hypertension. Also less treatment for atherosclerotic risk factors was given in patients with PAOD and CVD.[18]

Strengths and Limitations

Strengths of the present study include the large sample size, with three groups of vascular patients being treated in the same hospital and evaluated and treated according to standardised protocols both at presentation and during follow-up. The hospital-based origin of our series can limit the generalisability of our study findings. However, we believe that the comparison between the three disease categories with respect to baseline profile and subsequent events has not been distorted by this selection. Moreover, the follow-up results are in harmony with those from the REACH study, which is a population based study. More patients with coronary disease were included in the SMART study than patients with peripheral arterial obstructive or cerebrovascular disease, because of the many referrals to the cardiology department for elective PCI. The large number of statistical tests that we performed can be seen as a limitation of this study. However, virtually all relationships shown in Table 4 (and Webtable 3) had p-values below 0.005, rendering the likelihood of chance findings low.

One may argue whether the three different categories of disease are of the same severity. Because of the non-disabling character of the disease (as is an inclusion criteria for the SMART study), valid comparisons can be made between these patients. When restricting the analyses to a subgroup of patients with CAD with angina pectoris, patients with CVD with a modified Rankin grade of 0 or 1 and patients with PAOD with Fontaine grades 1 or 2 we found comparable results.

Another limitation is the secondary preventive treatment given to the patients. We assume all patients were treated according to guidelines and received the treatment they needed. In this study only baseline medication use was available, changes during follow up were not noted.

Implications

Although the underlying disease is largely similar, the present findings demonstrate that patients with CAD, PAOD or CVD have different risk profiles and different risks for future vascular events. Previous studies already emphasised the concept of a unitary underlying disease, namely atherothrombosis. In the past, treatment strategies for secondary prevention were often extrapolated between these different disease entities. Already in 1996 the CAPRIE trial showed dissimilar effects of aspirin and clopidogrel in patients with CAD, CVD or PAOD.[19] Nowadays, recommendations for antithrombotic treatment are often different for CVD, CAD, PAOD or combinations of these.[20]

Among patients with PAOD many had newly found hypercholesterolemia (79%), and of the patients treated for this condition still 54% had impaired lipid values. This definitely is an important target for prevention. The same is true for smoking habits; since the smallest proportion of never-smokers is found in the PAOD group. Intensive supervision is possibly very effective for secondary prevention in this group, given the high event rate and vascular death rate in follow up.[21] Treatment of risk factors in combination with extra care of a nurse practitioner is beneficial to the cardiovascular risk profile of high-risk patients.[22]

Even for patients with CVD it is striking that hyperlipidaemia is still undertreated, as is the proportion of patients with hypertension in despite of treatment (39%). For patients with CAD obesity is a relatively large problem; optimal prevention and treatment are warranted. The possible differences between the three groups in intensity of risk factor control may contribute to the occurrence of future events.

A lot of established risk factors for atherosclerosis are represented in the different groups. Aggressive treatment of these risk factors is still warranted, especially for PAOD patients, for optimal prevention of future events.

Conclusions

Among patients with clinical evident vascular diseases, patients with

cerebrovascular artery disease and patients with peripheral arterial obstructive disease are at the highest risk for future vascular events. Risk factor profile at baseline differs between groups of vascular patients.

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Web appendix

Webtable 1. Definitions of fatal and nonfatal events

Event	*Definition*
Death	All cause death
Ischaemic stroke	Relevant clinical features that caused an increase in impairment of at least one grade on the modified Rankin scale,[hier nog referentie 7 invoegen] associates with a relevant infarction on a repeat brain scan
Ischaemic coronary event	Myocardial infarction: at least two of the following criteria 1. Chest pain >20 min, not disappearing after administration of nitrates 2. ST elevation >1 mm in two following leads or a left bundle branch block 3. CK elevation of atleast two times its normal value and an MB fraction >5% of total CK Sudden death: unexpected coronary death occurring within 1 h after onset of symptoms or within 24 h given convincing circumstantial evidence. Terminal heart failure
Major bleeding complication	Intracranial bleeding: intraventricular, intracerebral, epidural, subdural or subarachnoidal bleeding seen on brain imaging. Retinal bleeding: typical complaints, changes with fundoscopy and impaired sight. Rupture of an abdominal aneurysm Severe extracranial haemorrhage: causing death or requiring intervention or admission to the hospital
Vascular event	Fatal or non fatal stroke, ischaemic cardiac event; other causes of vascular death including fatal rupture of abdominal aneurysm
Vascular event or intervention	Vascular events plus interventions; amputation of part of leg because of ischaemic cause, all CABG, PCI, CEA, PTA or stenting of intra- or extracranial vessels
Cerebrovascular event or intervention	Ischaemic stroke, PTA or surgery of the carotids
Coronary event or intervention	Ischaemic coronary event, CABG or PCI
Aorta related event or intervention	Fatal or non fatal rupture of abdominal aneurysm confirmed by echography, CT scan or seen during laparotomy. Surgery or PTA of abdominal aneurysm
Kidney related event or intervention	Renal impairment; serum creatinine >120 μmol/l or ratio of microprotein/ creatinine >20.0 mg/mmol Terminal renal impairment with the need for dialysis or kidney transplantation. Kidney transplantation or other kidney surgery including vessel surgery or PTA

Event	*Definition*
Iliaca related event or intervention	Surgery or PTA of iliacal vessels
Leg related event or intervention	Surgery of PTA of vessels in the legs or amputation of leg because of ischaemic disease
Iliaca or leg related event or intervention	Combination of iliaca and leg related event or intervention

CT, Computed tomography; ECG, electrocardiogram; CK, creatinine kinase; MB, myocardial band; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention; CEA, carotid endarterectomy; PTA, percutaneous transluminal angiography

Webtable 2. Intervention outcome events in patients with cerebrovascular, coronary or peripheral arterial obstructive disease

	Cerebrovascular disease (n = 881)	*Peripheral arterial obstructive disease (n= 800)*	*Coronary disease (n = 1882)*
Person years	4246	3760	6863
Any vascular event or intervention	247 (5.8%)	338 (9.0%)	409 (5.9%)
Cerebral event or intervention	101 (2.4%)	43 (1.1%)	29 (0.4%)
Coronary event or intervention	104 (2.5%)	123 (3.3%)	343 (4.9%)
Aorta related event or intervention	13 (0.3%)	47 (1.3%)	13 (0.2%)
Kidney related event or intervention	7 (0.2%)	11 (0.3%)	1 (0.01%)
Iliac artery related event or intervention	18 (0.4%)	78 (2.1%)	22 (0.3%)
Leg related event or intervention	33 (0.8%)	141 (3.8%)	24 (0.3%)
Iliac or leg related event or intervention	46 (1.1%)	202 (5.4%)	40 (0.6%)

Between parentheses annual risks are mentioned

Webtable 3. Intervention outcome hazard ratios, age- and sex adjusted

	CVD vs CAD	*CVD vs PAOD*	*PAOD vs CAD*
Any vascular event/intervention	0.95 (0.81-1.12)	0.53 (0.45-0.63)	1.75 (1.51-2.02)
Cerebral event/intervention	6.2 (4.1-9.4)	2.0 (1.4-2.9)	2.8 (1.8-4.6)
Coronary event/intervention	0.44 (0.35-0.56)	0.66 (0.51-0.86)	0.64 (0.51-0.78)
Aorta related event/intervention	1.6 (0.7-3.5)	0.25 (0.14-0.47)	7.1 (3.8-13.1)
Kidney related event/intervention	9.7 (1.2-80.6)	0.50 (0.19-1.30)	19.6 (2.5-154.2)
Iliac artery event/intervention	1.6 (0.8-2.9)	0.22 (0.13-0.37)	6.9 (4.2-11.1)
Leg related event/intervention	2.0 (1.1-3.4)	0.17 (0.12-0.25)	12.8 (8.3-19.8)
Iliac or leg event/intervention	1.9 (1.2-2.9)	0.17 (0.13-0.24)	11.4 (8.1-16.1)

CVD = cerebrovascular disease, CAD = coronary artery disease, PAOD = peripheral artery obstructive disease

3

Differential propensity of major haemorrhagic events in patients with different types of arterial diseases

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Abstract

Aims: Atherosclerosis is the most frequent cause of coronary artery disease (CAD), cerebrovascular disease (CVD) or peripheral arterial obstructive disease (PAD). We previously found that patients with CVD or PAD had a two times higher risk of major haemorrhagic complications than patients with CAD. We investigated whether this difference was attributable to baseline risk factors or genetic variants involved in haemostasis.

Methods and results: We included 2622 consecutive patients from a single university hospital who presented with non-disabling CAD, CVD or PAD. All patients were followed for the occurrence of major haemorrhagic complications during a mean of 6.6 years. Major haemorrhagic events included intracranial haemorrhagic, fatal haemorrhagic event and any haemorrhagic complication requiring hospitalisation, irrespective of interventions. Major haemorrhagic complications occurred in 122 patients (annual event rate 0.77%). Patients with CVD or PAD had more haemorrhagic complications than patients with CAD (HR: 2.05 (95%CI: 1.39-3.01). Hypertension, diabetes, renal failure and use of oral anticoagulants or antiplatelet therapy did not explain the difference (HR adjusted for all characteristics 1.74; 95%CI 1.14-2.61). Additional adjustment for genetic variants did not further change the HR.

Conclusion: Patients with CVD or PAD are at higher risk for major haemorrhagic events than patients with CAD. This difference could not be explained by known risk factors, use of antithrombotic agents or genetic variants involved in haemostasis. Further research to find the reason for this difference and possible differences in pathogenesis is warranted.

Introduction

Patients with coronary artery disease (CAD), cerebrovascular disease (CVD), or peripheral arterial obstructive disease (PAD) are thought to represent different clinical expressions of atherosclerosis. Atherosclerotic plaque rupture, arterial thrombosis, and thromboembolism can affect different arterial beds and, depending on arterial size and severity of the distal occlusion, may lead to different clinical manifestations. Patients with atherosclerotic vascular diseases are at risk for new ischaemic events as well as haemorrhagic events.[1] To prevent future cardiovascular ischaemic events treatment with antithrombotic agents, including anticoagulants or antiplatelet therapy is recommended.[2-4]

In a previous study we found that patients with CVD of presumed atherosclerotic origin or with PAD had an increased risk of major haemorrhagic events compared with patients with CAD.[5] This differential bleeding propensity may be attributable to a different distribution of risk factors for haemorrhage among the three presentations of vascular disease. Moreover, genetic variants involved in haemostasis could be distributed inequally between the three groups. Finally preventive antithrombotic treatments might be different. Hence we investigated whether this difference in haemorrhagic risk was associated with baseline risk factors, use of antithrombotic agents or genetic variants involved in haemostasis.

Methods

Study design and patient population

Patients aged 18 to 79 years, newly referred to the University Medical Center Utrecht, The Netherlands, with classical risk factors for arterial disease (hypertension, hyperlipidaemia, diabetes mellitus) or with symptomatic arterial disease (CAD, CVD, PAD or abdominal aortic aneurysm) were included in the Second Manifestations of ARTerial disease (SMART) study (for definitions see Web table 1). A detailed description of the study was published previously.[6] Briefly, patients who gave their written informed consent underwent a standardised vascular screening programme, including a health questionnaire, laboratory assessment, and ultrasonography to investigate the prevalence of additional vascular diseases. The Ethics Committee of the hospital approved the study.

For the current study we used data only from patients with CAD, CVD or PAD and we prespecified the comparison of CVD and PAD patients versus CAD patients.

Genetic variant selection and genotyping

Twenty-two variants in 14 genes based on previous associations with ischaemic stroke or myocardial infarction or functionality studies were selected in an earlier study and determined at that time because of involvement in atherothrombotic diseases.[7] We hypothesized in accordance with literature that without these genetic variants the risk of haemorrhagic events might be higher.[8]

DNA was isolated from blood samples and amplified with polymerase chain reaction (PCR). Genotyping was performed with the 5'nuclease/TaqMan assay. PCRs with fluorescent allele-specific oligonucleotide probes (Assay-by-Design/Assay-on-Demand: Applied Biosystems, Foster City, CA, USA) were performed on a PTC-225 thermal cyler (Biozym, Hessisch Oldendorf Germany), and fluorescence end point reading for allelic discrimination was performed on an ABI 7900 HT (Applied Biosystems). All genotypes were determined without knowledge about patient characteristics and outcome.

Outcome

Primary outcome was defined as the first occurrence of a fatal or non-fatal haemorrhagic event. This included any intracranial haemorrhagic, fatal haemorrhagic and any haemorrhagic complication requiring hospitalisation, irrespective of interventions. For potential outcome events reported by the patient we retrieved hospital discharge letters and the results of relevant laboratory and radiology examinations. Three members of the SMART Outcome Event Committee independently audited all events on basis of available information. This committee consisted of physicians from different departments. In case of disagreement, consensus was reached by consulting other members of the Outcome Event Committee.

Data analyses

The incidence of major haemorrhagic complications in patients with PAD, CVD or CAD was compared with Cox regression analysis, which generated hazard ratios with accompanying 95% confidence intervals (95%CI). Patients with incomplete follow-up were censored at the last time of observation. We used Kaplan-Meier curves for graphical display of the data.

We compared patients with PAD or CVD together with patients with CAD since there were no differences in haemorrhagic risk between patients with CAD and PAD. We first calculated crude hazard ratios and then assessed the influence of risk factors and genetic variants in bi-variable analyses, yielding adjusted hazard ratios. Variables changing the crude hazard ratio by more than 5% were included in a multivariable model. The influence of genetic variables was assessed in a similar way. For the analysis of genetic variants we used a dominant model of inheritance: variant allele heterozygotes and homozygotes were compared with wildtype homozygotes.

In addition we performed a sensitivity analysis for which we selected only patients without clinically manifest disease in other vascular beds than the one with which the patient enrolled into SMART. For example, patients included with symptomatic coronary vessel disease with a history of peripheral obstructive arterial or cerebrovascular disease were excluded from these analyses.

Results

Baseline

For the current study, the data of 2622 consecutive patients presenting with transient or non-disabling manifestations of coronary (1237), cerebrovascular (711) or peripheral arterial obstructive disease (674) with information on genetic variants were available. These patients were included between September 1996 and April 2005 and were followed until March 2009 or death.

Baseline characteristics on risk factors for haemorrhagic complications are summarised in Table 1. The majority of patients was male and mean age was 60 years. Male sex, renal failure, hypertension and diabetes were more common in patients with CVD or PAD compared with patients with CAD. At baseline the use of oral anticoagulant drugs was not different between the three patient groups and platelet inhibitors and statins were less used by patients with PAD compared with the other patients.

Follow up and occurrence of haemorrhagic events

Median follow-up time in the CVD cohort was 6.8 years, in the CAD cohort 6.6 years, and 6.4 years in the PAD cohort. Follow-up was complete in

98.1% of the patients. The annual risk of haemorrhagic events was 0.91 % in the CVD cohort (44 events), 0.94 % in the PAD cohort (41 events) and 0.45 % in the CAD cohort (37 events) (Table 1). Figure 1 shows the unadjusted time-to-haemorrhage curves for the three disease categories.

Patients with CVD and PAD had more haemorrhagic events than patients with CAD (HR 2.05 95%CI; 1.39-3.01). Age, sex, hypertension and renal failure had the biggest influence on this risk difference (Table 2). Simultaneous adjustment for these four risk factors lowered the HR to 1.65 (95%CI 1.10-2.48). Bleeding complications in patients with PAD and CVD were more often intracranial haemorrhages than in patients with CAD (16 events out of a total of 85 (19%) haemorrhages versus 4 events out of 37 (11%) haemorrhages). Most of the haemorrhagic events occurred in the gastro-intestinal tract (Table 1). Patients with CVD or PAD with a haemorrhagic event used less often platelet inhibitors at the time of the event than patients with CAD (59% vs 75% RR 0.78; 95%CI 0.60-1.00). Three patients with CAD and five patients with PAD or CVD used combined platelet inhibitors at the time of the haemorrhagic event. There was no difference in the use of oral anticoagulants at that time.

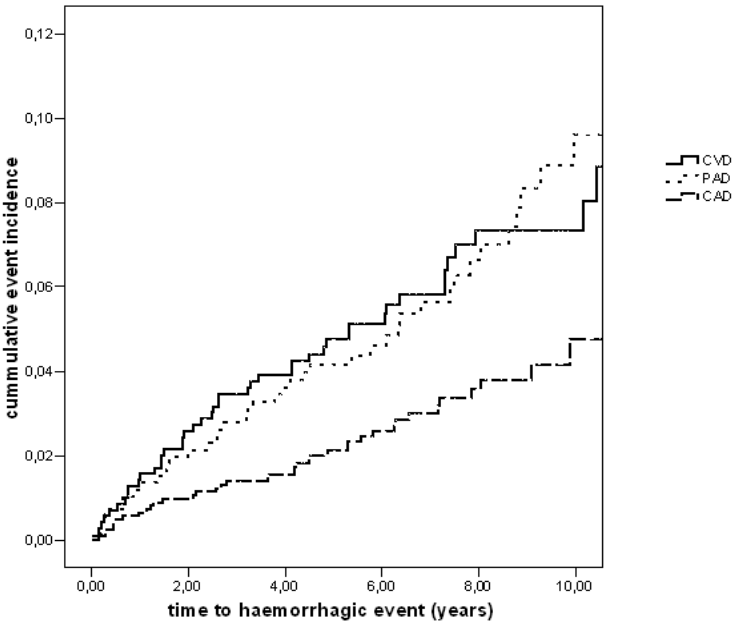

Figure 1. Kaplan Meier Curves for three atherosclerotic disease categories in regard to haemorrhagic events.

Table 1. Baseline characteristics of patients with CVD, PAD and CAD

	CVD (n=711)	*PAD (n=674)*	*CAD (n=1237)*
Age mean (SD)	61.7 (10.4)	59.1 (10.7)	58.4 (9.4)
Male sex	524 (73.7%)	448 (66.5%)	1013 (81.9%)
History stroke	277 (38.9%)	40 (5.9%)	21 (1.7%)
Hypertension*	220 (30.9%)	197 (29.2%)	198 (16.0%)
Diabetes	116 (16.7%)	122 (18.7%)	152 (12.6%)
Renal failure	63 (9.5%)	84 (13.3%)	50 (4.3%)
Platelet inhibitors*	525 (73.8%)	274 (40.7%)	869 (70.3%)
Oral anticoagulants*	60 (8.4%)	66 (9.8%)	94 (7.6%)
Statins*	159 (22.4%)	89 (13.2%)	430 (34.8%)
Number of haemorrhagic events (follow-up)	44 (6.2%)	41 (6.1%)	37 (3.0%)
Person years	4859	4326	8206
Annual risk of haemorrhagic events	0.91%	0.94%	0.45%
Type of bleeding			
Intracranial haemorrhage non-fatal	5 (11.4%)	4 (9.8%)	3 (8.1%)
Intracranial haemorrhage fatal	4 (9.1%)	3 (7.3%)	1 (2.7%)
Aortic aneurysm non-fatal	2 (4.6%)	0	0
Aortic aneurysm fatal	2 (4.5%)	1 (2.4%)	0
Extra cranial haemorrhage: admission **	19 (43.2%)	21 (51.2%)	26 (70.3%)
Extra cranial haemorrhage: intervention	12 (27.3%)	12 (29.3%)	7 (18.9%)

* At time of inclusion ** Mostly haemorrhage in the gastro-intestinal tract

Influence of prothrombotic genetic variants on the occurrence of haemorrhagic event

The overall call rate for all 22 genetic variants was 98.9% (range 95.8–99.7). Genotype specific risks and hazard ratios of major haemorrhagic events are presented in Table 3. Not one of the 22 variants was found to be associated with the risk of major haemorrhagic events. Additional adjustment of the HR adjusted for the four vascular risk factors with the three genetic characteristics most strongly related to the incidence of major haemorrhages did not change the HR further (Table 2).

Sensitivity analysis

If we restricted our analyses to the 2234 patients without other clinically manifest arterial disease apart from the referral diagnosis the results

changed slightly: HR PAD versus CVD from 0.90 (0.58-1.41) to 0.71 (0.40-1.24) and HR PAD and CVD versus CAD from 1.64 (1.09-2.46) to 1.42 (0.91-2.21).

Table 2. Adjusted hazard ratios for haemorrhagic events according to different vascular diseases

	Peripheral artery vs cerebrovascular disease	*Peripheral and cerebrovascular vs coronary artery disease*
Total	1385	2622
Haemorrhagic event	85	122
Unadjusted	0.99 (0.65-1.52)	2.05 (1.39-3.01)
Adjusted for		
Age (years)	0.85 (0.56-1.31)	1.88 (1.27-2.77)
Sex	0.96 (0.62-1.46)	2.11 (1.43-3.11)
Hypertension	0.99 (0.65-1.51)	1.86 (1.26-2.75)
Diabetes	0.99 (0.65-1.51)	2.13 (1.44-3.15)
Renal failure	1.07 (0.69-1.66)	1.83 (1.23-2.72)
Platelet inhibitors*	1.00 (0.63-1.59)	2.07 (1.40-3.06)
Oral anticoagulants*	0.99 (0.65-1.52)	2.05 (1.39-3.02)
Multiple**	0.91 (0.59-1.43)	1.65 (1.10-2.48)
GP1BA Thr145Met	0.99 (0.65-1.51)	2.05 (1.39-3.01)
F3 A(-603)G	1.00 (0.65-1.53)	2.05 (1.39-3.01)
F5 Arg506Gln	0.99 (0.65-1.51)	2.05 (1.39-3.01)
All three genetic variants	1.00 (0.65-1.52)	2.04 (1.39-3.00)
Multiple***	0.90 (0.58-1.41)	1.64 (1.09-2.46)

GP1BA, glycoprotein 1 b-alpha; F3, tissue factor; F5, coagulation factor V. Data are numbers (%) or hazard ratio (95%CI).
*Use at time of inclusion **Adjustment for most influential variables; age, sex, hypertension and renal failure
***Adjustment for age, sex, hypertension, renal failure and four genetic variants

Table 3. Genetic variants and haemorrhagic risk

		Risk of new haemorrhagic event per genotype			*Hazard ratio (95% CI)*
Gene symbol	Variant	AA*	AB*	BB*	
F2	G20210A	2414/121	57/1	1/0	0.33 (0.05-2.36)
F3	A(–603)G	743/27	1221/74	523/21	1.49 (0.97-2.28)
F5	Arg506Gln	2326/118	145/4	1/0	0.52 (0.20-1.40)

		Risk of new haemorrhagic event per genotype			*Hazard ratio (95% CI)*
Gene symbol	Variant	AA*	AB*	BB*	
F13A1	Val34Leu	146/10	910/46	1390/66	0.73 (0.38-1.39)
F13A1	Tyr204Phe	2337/113	143/8	4/1	1.27 (0.65-2.51)
F13A1	Pro564Leu	1544/73	833/36	107/13	1.09 (0.76-1.56)
F13B	His95Arg	2064/100	410/18	15/3	1.00 (0.63-1.60)
FGA	Thr312Ala	1264/64	1017/45	206/12	0.90 (0.63-1.29)
FGB	G(–854)A	1747/86	681/31	59/5	1.00 (0.68-1.47)
FGB	G(–455)A	1591/73	785/44	91/2	1.15 (0.79-1.66)
FGG	G7874A	1292/68	990/45	197/8	0.84 (0.58-1.20)
FGG	T9340C	1213/63	1028/47	244/13	0.91 (0.64-1.30)
FGG	G5836A	2305/111	175/9	3/0	1.08 (0.55-2.13)
TPA	C7351T	1061/55	1141/53	283/13	0.88 (0.62-1.26)
VKORC-1	T2255C	998/48	1108/55	380/18	1.03 (0.72-1.48)
VWF	Thr789Ala	1147/58	1016/49	273/15	0.98 (0.69-1.39)
GP1BA	T(–5)C	1875/86	562/33	49/3	1.24 (0.84-1.83)
GP1BA	Thr145Met	2142/99	331/22	15/1	1.46 (0.93-2.30)
GP6	Ser219Pro	1731/88	674/32	73/1	0.89 (0.60-1.33)
GP6	Thr249Ala	1700/85	697/53	90/2	0.96 (0.65-1.41)
GP6	Gln317Leu	1696/84	699/36	89/2	0.99 (0.68-1.46)
GP6	His322Asn	1775/87	65/33	63/2	1.01 (0.70-1.51)

Description of gene symbols: F2, coagulation factor II; F3, tissue factor; F5, coagulation factor V; F13A1, coagulation factor XIII subunit A; F13B, coagulation factor XIII subunit B; FGA, fibrinogen alpha; FGB, fibrinogen beta; FGG, fibrinogen gamma; TPA, tissue plasminogen activator; VKORC1, vitamin K epoxide reductase complex-1; VWF, von Willebrand factor; GP1BA, glycoprotein 1 b-alpha; GP6, glycoprotein VI. Odds: Wild type vs. heterozygote and homozygote variant genotype. * Numbers represent patients without major haemorrhagic event/patients with major haemorrhagic event.

Discussion

Patients with PAD and CVD are more prone to major haemorrhagic events than patients with CAD. This risk difference was not explained by vascular risk factors; a selection of prothrombotic genetic variants did neither explain the difference.

To the best of our knowledge this is the first study describing differences in haemorrhagic risk in patients with different manifestations of atherosclerotic vascular diseases. Many studies focus on reduction of new

thrombotic events rather than on haemorrhagic risk.[9-11] A meta-analysis showed that treatment with antithrombotics, mainly aspirin, was safe and useful to prevent new events in patients with cardiac, cerebrovascular and peripheral arterial obstructive disease.[12] The absolute benefit of the treatment in terms of vascular event reduction or mortality substantially outweighed the absolute risk of major extracranial haemorrhage. No analyses were shown on potential differences in bleeding risk between patients with different locations of atherosclerotic vascular diseases. Data on individual studies included in this meta-analysis were published in a web-appendix and allowed us to make such comparisons ourselves. We found that in the population with previous myocardial infarction three patients suffered from a haemorrhagic event in 23098 person-years (annual event risk 0.01%); patients with a previous stroke or transient ischaemic attack had 80 haemorrhagic events in 27872 person-years (annual event risk 0.29%); patients with intermittent claudication had 60 haemorrhagic events in 4492 person-years (annual event risk 0.35%) [12]. The comparison between PAD/CVD and CAD patients gives an incidence rate ratio of 23 (95%CI 7-72). These findings support our own results.
With restriction of our analysis to patients with clinical manifestation in only one vascular bed the HR for PAD and CVD versus CAD lost its statistical significance; 1.42 (0.91-2.21). From this analysis we inferred, however, that the main result remained essentially the same, even though the hazard ratio decreased somewhat. The loss of statistical significance in the sensitivity analysis should be attributed mainly to the smaller number of patients in this analysis.

Strengths of the present study include the large sample size and direct comparison of three groups of patients with different locations of vascular disease being treated in the same hospital and evaluated and treated according to standardised protocols both at presentation and during follow-up. The university hospital-based origin of our series can limit the generalizability of our study findings. Compared with patients with atherosclerotic disease from the general population our patients are for example slightly younger. However, we believe that the comparison between the three disease categories with respect to major haemorrhagic events in follow up has not been distorted by this selection. Moreover, the follow-up results are in harmony with those found in a major meta-analysis.[12] The aim was to include consecutively, however, we have no register about patients missed for inclusion, or patients excluded for some reason or those who did not give informed consent thus we cannot be completely certain whether our series was completely consecutive.

A limitation of our study is that we were only informed about the use of antithrombotic drugs and statins at baseline of the study. Classes of medications like platelet inhibitors were noted, no detailed information on which precise type of drug the patient used was recorded. During follow up changes in medication usage were also not recorded. To determine if major changes occurred in drug regime over the years we re-assessed the information (mainly discharge letters) from the patients with major haemorrhagic events. At the time of the haemorrhagic event, no difference was found in the use of oral anticoagulant therapy between PAD/CVD and CAD patients (RR 1.41 95%CI 0.71-2.83), but PAD/CVD patients used less platelet inhibitors compared with CAD patients (RR 0.78 95%CI 0.60-1.00). Thus lower occurrence of major haemorrhagic events in the CAD group was associated with more use of platelet inhibitors.

Statins were prescribed as protection against new ischaemic events, but may lead to a higher risk of haemorrhagic intracerebral events.[13] Patients with the lowest haemorrhagic risk, CAD patients, used the most statins at baseline (35% versus 19% of patients with PAD/CVD), hence it is unlikely that statin use may explain the findings of our study. Genetic variants tested in our patients did not explain the differences in haemorrhagic events. A few reports discuss single polymorphisms as possible risk factors for major haemorrhage with mostly conflicting results.[14,15] Gathering of different variants with genome wide association studies might lead to more results in this complex disease.[15]

The reason for the difference in haemorrhagic risk between patients with PAD/CVD and CAD is not clear. We found no explanation for this difference in the literature and thus can only speculate about the origin of our finding. Possibly patients with PAD and CVD have more fragile vessels, which together with atherosclerotic disease of these vessels rupture easily causing haemorrhagic events.

In past publications all atherothrombotic diseases were often thought to arise from the same mechanism namely atherosclerosis.[16] In a previous study we showed differences in baseline risk factors and follow up between CAD, CVD and PAD patients.[5] The difference in haemorrhagic risk shown in the current analyses should be taken into account in future research, since it might point to different underlying mechanisms to different manifestations of what seems to be a single disease. Treatment options might not be extrapolated from one atherosclerotic disease to another when this difference in haemorrhagic risk is taken into account.

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Webappendix

Webtable 1. Definitions of enrollement diagnosis

Enrolment diagnosis	*Definition*
Angina pectoris	Chest pain with or without documented ischaemia on the ECG and with documented stenosis on the angiography (In practical terms: patients with indication for percutaneous transluminal coronary angioplasty).
Myocardial infarction	At least two of the following: 1. Chest pain for at least 20 minutes, not disappearing after administration of nitrates. 2. ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK.
Peripheral artery disease	Resting ABPI <0.90 or post exercise ABPI decreasing 20% or more in at least one leg, with signs of intermittent claudication, rest pain or gangrene/ulcers
Transient ischaemic attack or minor ischaemic stroke	According to the criteria established by the neurologist[1]

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4

Risk of vascular events after non-disabling small and large vessel cerebral ischemia

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Abstract

Background. Small and large vessel disease (SVD and LVD) might have a different pathogenesis and prognosis but the long-term risk of death and recurrent stroke appears to be similar in previous studies. In this study we investigated the long-term cardiovascular prognosis of patients with LVD and SVD in a large cohort of well documented patients.
Methods. We included 971 patients with transient ischemic attack or non-disabling ischemic stroke of atherosclerotic origin referred to a university hospital in The Netherlands between 1994 and 2005 and followed them for the occurrence of vascular events or death. Primary outcome was a composite of fatal or non-fatal ischemic events, whichever happened first. Classification of SVD/LVD was primarily based on brain imaging. We used regression analyses to generate hazard ratios with 95% confidence intervals (HR;95%CI). Sensitivity analyses were performed in subsets of the population; patients with subtype classification based on imaging, excluding TIA patients, first ever stroke patients and LVD patients without a symptomatic carotid stenosis.
Results. During a mean follow-up of 6.3 years new vascular events occurred in 56 of 312 SVD patients (3.3%/year) and in 128 of 659 LVD patients (2.9%/year). These were ischemic strokes in 33 of the 56 events in SVD patients (2.0%/year) and 54 of 128 events in LVD patients (1.2%/year). The corresponding age and sex adjusted HR for all new vascular events for LVD versus SVD was 0.76; 95%CI 0.56-1.05 When this risk was split for early risk (<1 year) and late risk (>1 year) it was not significantly different for 1-year risk of vascular events (HR 1.04; 95%CI 0.57-1.91), however, after 1 year of follow-up LVD patients had less outcome events compared with SVD patients (HR 0.66; 95% CI 0.46-0.96). For ischemic strokes the overall HR was 0.60; 95%CI 0.39-0.94. As with the primary outcome also here the 1-year risk was not significantly different as compared with the over 1-year risk (resp. HR 1.31; 95%CI 0.62-2.81 and HR 0.36; 95%CI 0.21-0.63). The sensitivity analyses showed virtually the same results.
Conclusion. In patients with non-disabling cerebrovascular disease we found, despite no differences at baseline in vascular risk factors, a better long-term prognosis for patients with LVD for all vascular events, especially for recurrent strokes. Our observations support a different pathogenesis in SVD and LVD patients and optimal prevention is indicated for patients with formerly thought 'benign' SVD stroke.

Introduction

Ischemic stroke can have multiple causes. Among the most frequent causes are large-vessel disease (LVD) and small-vessel disease (SVD) [1]. Short-term prognosis on functional outcome after SVD stroke tends to be better than in LVD patients [2]. This is thought to be mainly the result of a smaller infarction size in SVD strokes, and possibly by later recurrence of new strokes. Recurrent strokes in patients with SVD tend to be true to type, e.g. recurrent strokes were more likely to be lacunar if the index event was lacunar, whereas recurrent strokes in patients with large vessel atherosclerotic disease can be caused by either SVD of LVD [3]. Long-term prognosis after lacunar stroke is worse than within the general population in terms of death and recurrent stroke but findings about possible differences in the long-term prognosis between patients with lacunar and non-lacunar strokes are conflicting [4-7]. In this study we investigated the long-term cardiovascular prognosis of patients with non-disabling LVD and SVD in a large cohort of well documented patients.

Methods

Study design and patient population

We collected data on patients with non-disabling cerebral ischemia of arterial origin who were referred to the University Medical Center Utrecht and were consecutively included in the SMART (Second Manifestations of Arterial disease) study, or the Utrecht Stroke Database (USDB). Patients with a potential source of embolism in the heart were excluded as were patients with a modified Rankin score of more than three. A detailed description of the SMART study was published previously [8]. Briefly, patients who gave their written informed consent underwent a standardized vascular screening program, including a health questionnaire, laboratory assessment, and ultrasonography to investigate the prevalence of additional vascular diseases. All patients received optimal medical and, if necessary, surgical treatment, according to guidelines. Patients were followed up with bi-annual questionnaires. In the USDB extensive baseline data have been prospectively collected for consecutive patients with transient ischemic attack (TIA) or ischemic stroke since 1991, from 1994 onwards also blood samples were

collected from these patients. Follow-up data were collected by contacting these patients or their general practitioners. The Ethics Committee of the hospital approved both studies.

For the current study, the data of 971 consecutive patients were available. These patients were included between April 1994 and March 2005 and were followed until March 2009 or death.

Definitions and outcome

TIA patients were included if either imaging or clinical symptoms were indicative (ischemic lesions on imaging, or acute focal neurological deficits resolving within 24 hours), patients were prescribed secondary preventive medication and had no other diagnosis during follow-up explaining there symptoms. Subtype classification was primarily based on imaging. SVD was classified in case of infarcts of < 15 mm in diameter localized in the deep regions of the brain or in the brain stem. All other infarcts were classified as LVD. The differentiation between SVD and LVD was based on clinical features if imaging showed no visible relevant lesion or unavailable. Motor or sensory deficit of only the face, arm or leg was classified LVD. Motor or sensory deficits of two or three areas of face, arm or leg without cognitive function disorder were classified as SVD as were patients with an ataxic hemiparesis or a dysarthria-clumsy hand syndrome. Cerebellar syndromes were classified LVD and brainstem syndromes as SVD. Retinal ischemia was classified LVD [9]. Patients with symptomatic ipsilateral carotid stenosis (>70% stenosis) were classified as LVD independent of their clinical features. 20 patients with a carotid stenosis were classified as SVD because of lacunar stroke visible on imaging or clinical signs of SVD.

The primary outcome event was defined as a composite of the first occurrence of coronary event, ischemic stroke or vascular death. Secondary outcome events were defined as overall mortality, recurrent ischemic stroke, cardiac ischemia or major hemorrhage (Web-Table 1). For potential outcome events reported by the patient on the biannually send questionnaire, we retrieved hospital discharge letters and the results of relevant laboratory and radiology examinations. Three medical physicians independently audited all events on basis of available information. In case of disagreement, consensus was reached by consulting of other physicians. Patients with incomplete follow-up were censored at the last time of observation.

Statistical methods

The incidence of recurrent events in the two different groups was compared with Cox regression analysis. The regression analyses generated hazard ratios (HR) with accompanying 95% confidence intervals (95%CI). HRs were adjusted for age and sex except when indicated otherwise. To compare the short-term and long-term prognosis we calculated HRs for 1-year and over-1-year follow-up time.

We performed the following sensitivity analyses in subsets of our population: (1) patients with classification of stroke based on imaging data only, (2) excluding TIA and ocular ischemic patients, (3) patients with first ever stroke, and (4) excluding patients with LVD with symptomatic carotid stenosis.

Results

Baseline results

Of the 971 patients with non-disabling cerebral ischemia included in this study 312 (32%) patients had SVD and 659 (68%) patients LVD. This classification was based on visible lesions on the imaging (mainly CT imaging) in 404 patients. The rest of patients were classified as described in the methods section. Baseline characteristics are given in Table 1.

There was no difference in age and sex. In patients with SVD the index event was more often a non-disabling stroke than a TIA. Further baseline characteristics were not different.

Table 1. Baseline characteristics

	Large vessel disease (N=659)	*Small vessel disease (N=312)*
Mean age in years (range)	63 (26-89)	61 (23-90)
Male sex	456 (69%)	208 (67%)
Index event		
Ischemic stroke	279 (42%)	216 (69%)
TIA	254 (39%)	96 (31%)
Retinal infarction	18 (3%)	0
Transiant Monocular blindness	108 (16%)	0
Symptomatic carotid stenosis	391 (59%)	20 (6 %)

	Large vessel disease (N=659)	*Small vessel disease (N=312)*
Current smoking	125 (19%)	90 (29%)
History		
Hypertension	315 (48%)	157 (51%)
Diabetes	99 (15%)	44 (14%)
Hyperlipidemia	248 (38%)	97 (31%)
Cerebral ischemia	150 (23%)	53 (17%)
Myocardial infarction	86 (13%)	23 (7%)
Angina pectoris	51 (8%)	16 (5%)
Intermittend claudication	60 (9%)	14 (5%)
Cardiovascular disease†	276 (42%)	88 (28%)

† Composite of history of cerebral ischemia, myocardial infarction, angina pectoris and intermittent claudication.

Follow-up

Mean follow-up time was 6.3 years (6087 person-years). For 96.9% of patients follow-up was known till death or March 2009. The annual risk for the primary outcome event was 3.3% in the SVD cohort (56 events) and 2.9% in the LVD cohort (128 events) (Table 2).

Figure 1 shows the time-to-event curves for the primary outcome. Patients with LVD showed a trend to fewer new ischemic cardiovascular events than those with SVD; HR 0.76 (95%CI 0.56-1.05; Table 3). Recurrent cerebral ischemia was less frequent in patients with LVD (HR 0.60; 95%CI 0.39-0.94). No differences in mortality, major hemorrhagic events and cardiac events were observed. The sensitivity analyses showed virtually the same results (Table 3).

Table 2. Number of different outcome events and annual risks in patients with large and small vessel disease

	Large vessel disease (N=659)	*Small vessel disease (N=312)*
Person years	4403	1683
Any ischemic vascular event	128 (2.9%)	56 (3.3%)
Death	143 (3.2%)	45 (2.7%)
Ischemic stroke	54 (1.2%)	33 (2.0%)
Ischemic myocardial infarction	57 (1.3%)	15 (0.9%)
Major hemorrhagic event	39 (0.9%)	13 (0.8%)

Table 3. Sensitivity analyses in subsets of patients: Hazard ratios (95%CI) for primary and secondary outcome events

LVD vs. SVD	*All patients*	*Based on positive scan*	*Excluding TIA and ocular ischemia*	*First ever stroke*	*LVD without SCS*
N	971	404	495	768	580
Primary outcome*	0.76 (0.56-1.05)	0.68 (0.43-1.06)	0.70 (045-1.08)	0.67 (0.46-0.96)	0.52 (0.33-0.81)
Overall mortality	0.96 (0.69-1.35)	0.99 (0.62-1.58)	0.89 (0.59-1.35)	0.89 (0.60-1.32)	0.85 (0.55-1.30)
Cerebral ischemia†	0.60 (0.39-0.94)	0.54 (0.29-1.00)	0.74 (0.41-1.35)	0.51 (0.31-0.84)	0.42 (0.22-0.79)
Cardiac ischemia†	1.18 (0.66-2.09)	0.93 (0.41-2.08)	0.82 (0.39-1.74)	1.23 (0.63-2.41)	0.81 (0.38-1.72)
Major hemorrhage†	1.08 (0.57-2.03)	1.34 (0.54-3.34)	0.80 (0.37-1.75)	0.93 (0.44-1.96)	1.10 (0.52-2.35)

Table shows age and sex-adjusted hazard ratios with 95% CIs
*Composite of fatal and non-fatal ischemic events, whichever happened first, †Fatal and non-fatal events
LVD = large vessel disease, SVD= small vessel disease, SCS = symptomatic carotid stenosis

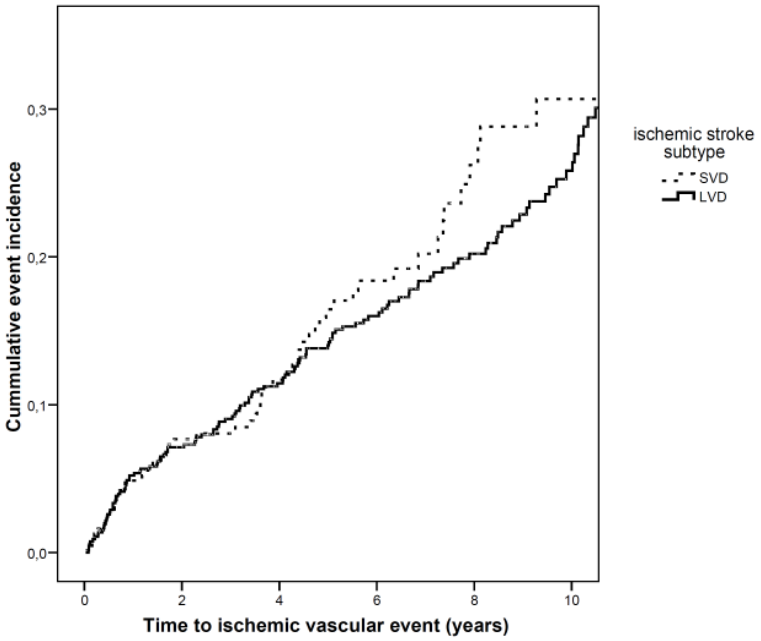

Figure 1. Time to primary outcome curve for patients with small (SVD) and large (LVD) vessel disease. The event curve shown in this figure is based on crude data.

As shown in Table 4, the short-term prognosis (≤1 year) was not different comparing LVD and SVD concerning the primary outcome. After the first year the ischemic stroke patients with LVD had less events than patients with SVD (HR 0.66; 95%CI 0.46-0.96). The biggest component of this difference was explained by a higher risk of recurrent ischemic stroke. After the first year the risk of recurrent stroke was lower in patients with LVD than in patients with SVD (HR 0.36; 95%CI 0.21-0.63). The annual risk of cardiac events tended to rise after one year of follow-up in patients with LVD (1.4%) with no rise in patients with SVD (0.9%; HR 1.20; 95%CI 0.64-2.28).

Forty-three of 54 LVD patients with recurrent stroke had another large vessel stroke (80%). Twenty of 33 SVD patients with recurrent stroke had a new SVD event (61%).

Discussion

In this cohort study with long-term follow-up of patients with non-disabling cerebral ischemia of arterial origin, those presenting with SVD at baseline tended to have a trend to a higher rate of serious vascular events, especially with respect to ischemic stroke after one year. Furthermore, recurrent cerebral ischemic events tended to remain true to type.

We found no differences in baseline characteristics as diabetes and hypertension between patients with SVD and patients with LVD. Previously this has been thought to be different and the subtyping of stroke was partly based on these differences [10]. However, more recently the presumed difference in risk factor profile was discussed thoroughly and found to be non-existent [11]. In a meta-analysis, 27 studies were reviewed on differences in death rates, recurrent ischemic strokes and myocardial infarction in patients with lacunar or non-lacunar strokes [6]. The populations in the studies discussed were heterogeneous. Risk of death within one month of stroke onset was more frequent in patients with non-lacunar stroke than those with a lacunar stroke. After one month the risk difference attenuated, suggesting that the early excess was related to infarct size or early recurrent stroke[12].

Table 4. 1-year and over-1-year risk of primary and secondary outcome events

	LVD events/total (*)	*SVD events/total (*)*	*Crude HR (95%CI) LVD vs. SVD*	*Adjusted for age and sex HR (95%CI) LVD vs. SVD*
1-year risk of primary outcome†	34/659 (5.2)	15/312 (4.8)	1.07 (0.58-1.96)	1.04 (0.57-1.91)
>1 year risk of primary outcome†	94/611 (2.7)	41/284 (3.3)	0.78 (0.54-1.13)	0.66 (0.46-0.96)
1-year risk of death	17/659 (2.6)	5/312 (1.6)	1.61 (0.59-4.37)	1.54 (0.57-4.18)
>1 year risk of death	126/631 (3.5)	40/295 (3.1)	1.10 (0.77-4.37)	0.90 (0.63-1.29)
1-year risk of recurrent stroke	25/659 (3.8)	9/312 (2.9)	1.32 (0.61-2.82)	1.31 (0.62-2.81)
>1 year risk of recurrent stroke	29/613 (0.8)	24/286 (1.9)	0.42 (0.24-0.72)	0.36 (0.21-0.63)
1-year risk of cardiac event	7/659 (1.1)	3/312 (1.0)	1.10 (0.29-4.26)	1.05 (0.27-4.06)
>1 year risk of cardiac event	50/630 (1.4)	12/293 (0.9)	1.43 (0.76-2.70)	1.20 (0.64-2.28)
1-year risk of bleeding	7/659 (1.1)	1/312 (0.3)	3.33 (0.41-27.03)	3.19 (0.39-26.08)
>1 year risk of bleeding	32/625 (0.9)	12/295 (0.9)	1.02 (0.52-1.99)	0.92 (0.47-1.80)

LVD= large vessel disease, SVD= small vessel disease
* Annual risks
† Composite outcome event of fatal and non-fatal ischemic events, whichever happened first

We did not observe an early excess risk with LVD, which is probably attributed to our inclusion of only patients with non-cardiac and non-disabling cerebral ischemia. A meta-analysis of prognosis after lacunar stroke describes that lacunar strokes might not be as benign as formally thought, but be a predictor of unfavorable cardiovascular outcome [2]. Also pre-existing SVD may predict poor outcome in stroke patients with a symptomatic intracranial large artery atherosclerosis.[13]

One possible explanation for the better long-term prognosis of patients with LVD in our study may be the large proportion of patients with a symptomatic carotid stenosis since carotid endarterectomy reduces the risk of recurrent stroke importantly. However, with restriction of our analyses to patients with LVD without symptomatic carotid stenosis, the benefit for LVD patients tended to be even larger (HR 0.52; 95%CI 0.33-0.81). Another explanation could be the inclusion of patients with ocular events, who tend to have a better prognosis, however, the results of the sensitivity analysis excluding all patients with (transient) monocular blindness are virtually the same (Table 3). We are not sure about the pathophysiology underlying this difference in recurrent stroke risk.

Strengths of our study include the large sample size, and the patients with well documented SVD and LVD being treated in the same hospital, evaluated and treated according to standardized protocols both at presentation and during follow-up. The hospital-based origin of our series can limit the generalisability of our results. However, we believe that this comparison has not been distorted by this selection, since nowadays most patients with TIA and stroke are referred to a hospital for further evaluation. Furthermore the inclusion of only non-disabling stroke patients may limit the generalisability. We think, however, that prognosis after stroke is important especially for this non- disabled group of patients given their much better prospects than after disabling stroke. The inclusion of non-disabling stroke patients only was prespecified and chosen because of above mentioned reasons. The classification method to define subtype used in this study is not a validated one, but is known to perform superior to classification based on risk factors [1;14]. When imaging was available our classification is comparable to the TOAST classification. We performed subset analysis in patients with classification based on imaging only, since approximately 20% of patients can be misclassified when subtype was only based on clinical symptoms.[15] As shown in Table 3, this subset of patients performs equally as compared with the whole group.

Unfortunately one major shortcoming of this study is the lack in information about treatment regimes and adherence to it in our patients. Since the single center design of our study we assumed all patients received treatment according to local protocol and it should not differ between groups. It would, however, for future studies be interesting to see whether SVD and LVD patients indeed retain in the same amount to the recommended secondary prevention medication.

In conclusion, while at baseline no differences in risk factors for cardiovascular disease could be established between patients with non-disabling cerebral ischemia on basis of SVD or LVD, the differences in long-term prognosis of cardiovascular risk, especially with respect to recurrent stroke, indicate that optimal prevention is indicated for patients with formerly thought 'benign' SVD stroke.

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Webappendix

Webtable1. Definitions of outcome events

Event	*Definition*
Death	All cause death
Ischemic stroke	Relevant clinical features that caused an increase in impairment of at least one grade on the modified Rankin scale1 associated with a relevant infarction on a repeat brain scan
Ischemic coronary event	Myocardial infarction: at least two of the following criteria: 1. Chest pain >20 min, not disappearing after administration of nitrates 2. ST elevation >1 mm in two following leads or a left bundle branch block 3. CK elevation of at least two times its normal value and an MB fraction >5% of total CK Sudden death: unexpected coronary death occurring within 1 h after onset of symptoms or within 24 h given convincing circumstantial evidence Terminal heart failure
Major hemorrhagic event	Intracranial bleeding: intraventricular, intracerebral, epidural, subdural or subarachnoidal bleeding seen on brain imaging. Retinal bleeding: typical complaints, changes with fundoscopy and impaired sight Rupture of an aneurysm of the abdominal aorta Severe extracranial hemorrhage: causing death or requiring intervention or admission to the hospital

The primary outcome event was defined as the composite of all fatal and nonfatal ischemic events, whichever happened first.
Secondary outcome events were ischemic stroke, ischemic coronary event, all cause death, and major hemorrhagic event seperatly.

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5

Prognostic value of the Rose questionnaire; a validation with future coronary events in the SMART study

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Abstract

Aim. The Rose questionnaire was developed in epidemiological studies to obtain a reproducible diagnosis of angina pectoris. We studied the prognostic value of this questionnaire with respect to the occurrence of future coronary events.

Methods and Results. We studied 7916 consecutive patients (mean age 56 years; 67% men) with clinically manifest vascular disease or cardiovascular risk factors, enrolled in the Second Manifestations of ARTerial disease (SMART) study from 1996 to 2009. At inclusion all patients completed the Rose questionnaire. We investigated the prognostic value of four definitions of angina pectoris that were based on the following elements of the Rose questionnaire (1) the full questionnaire; (2) three key questions concerning chest pain; (3) one question about discomfort or pain in the chest; (4) two questions about complaints when slowing down or stopping activities (the definition that is used in the SMART study). All patients were followed for new coronary events and interventions for an average of 4.6 years. Analyses were with multivariable Cox regression models.

Discriminatory ability of the four definitions as assessed with areas under the receiver-operator characteristics curves was similar (range 0.708-0.726) for coronary events in isolation as well as in combination with coronary interventions. The models were assessed for their ability to improve risk stratification compared with each other; differences between definitions are small.

Conclusion. Our data implicate that the use of a subsets of questions of the Rose questionnaire performs equally well compared with the full Rose questionnaire regarding the prediction of coronary events.

Introduction

Angina pectoris is a common complaint that requires further examination to exclude the presence of heart disease. It is characterized by chest pain that is the result of insufficient oxygen supply to the myocardium. The most common etiology is atherosclerotic narrowing of the coronary arteries. In rare cases angina pectoris results from spasms of the coronaries or other causes. In 2003 about 19.200 men (2.4 per 1.000 men) and 17.600 women (2.1 per 1.000 women) for the first time were diagnosed with angina pectoris in the Netherlands.[1] Several studies have indicated that in the general population angina pectoris assessed on clinical grounds predicts coronary death, with relative risks up to 2.6 (95% CI 2.0-3.3) during long-term follow up.[2]

Diagnosis of angina pectoris caused by coronary artery disease on basis of history taking only is challenging. The World Health Organization Rose questionnaire (Table 1) is widely used in epidemiological studies as a validated and standardized method for defining angina pectoris.[3,4] The validity of this questionnaire has been assessed in several studies, comparing it with clinical diagnosis of angina pectoris, ECG abnormalities and as a predictor of mortality due to coronary artery disease.[5-7] It has been found to predict major coronary events in middle aged men and coronary heart disease mortality in both women and men.[8] Most of this prediction is based on exertional chest pain, raising the possibility that some questions of the Rose questionnaire are redundant to diagnose angina pectoris. A study from 2003 challenged the Rose questionnaire and found that a shortened version performed better in identifying postmenopausal women with a medical diagnosis of angina pectoris than the full version.[9] In this specific population the prognostic performance with regard to clinical coronary outcomes was not evaluated.

In prognostic research the Rose questionnaire is also widely used, but has not been validated as in diagnostic research. In the prognostic setting it might also be better to only use a subset of questions instead of the complete questionnaire.

The aim of the current study was to assess the prognostic value of different definitions of angina pectoris based on the Rose questionnaire in patients at high-risk of coronary events.

Methods

Study design and patient population

Patients aged 18 to 79 years, newly referred to the University Medical Center Utrecht, The Netherlands, with traditional risk factors for arterial disease (hypertension, hyperlipidemia, and diabetes mellitus) or with non-disabling symptomatic arterial disease (coronary heart disease, cerebrovascular disease, abdominal aortic aneurysm, or peripheral arterial disease) were included in the Second Manifestations of ARTerial disease (SMART) study. A detailed description of the study has been published previously.[10]

Briefly, patients who gave their written informed consent underwent a standardised vascular screening programme, including a health questionnaire, laboratory assessment, and ultrasonography to investigate the prevalence of additional vascular diseases. The Ethics Committee of the hospital approved the study.

Recruitment in the SMART study is ongoing and annually approximately 800 patients are included. For the current study, data of 7916 consecutive patients included until the March 2009 with at least one half yearly follow up completed were available.

Definitions of prevalent coronary artery, cerebrovascular and peripheral artery obstructive disease were described in detail elsewhere.[11]

Definition of angina pectoris

Angina pectoris was defined on the basis of elements of the Rose questionnaire, which was filled out at inclusion in the SMART study. Four definitions were used (Table 1): (1) on the basis of the complete questionnaire: discomfort at walking uphill or hurrying, or at an ordinary pace on level ground. Furthermore, the pain should be located at the sternum or in the left chest and arm, causing the patient to stop or slow down, and the pain should resolve within 10 minutes when the patient stops or slows down; (2) on basis of three key questions (questions 1, 3 and 4): within this definition patients should have chest pain either during walking uphill or at ordinary pace on level ground; (3) on the basis of only one question; within this definition patients should have had discomfort or pain in the chest; (4) on the basis of two key questions (questions 1 and 5); within this definition the key question is resolution of complaints when slowing down or stopping the activity.

Table 1. Different definitions of angina

Questions for angina	*AP 1 (n=1231)*	*AP 2 (n=2199)*	*AP 3 (n=3651)*	*AP 4 (n=1785)*
1 Do you ever have any pain or discomfort in your chest - Yes/No	Yes	Yes	Yes	Yes
2 Where do you get this pain or discomfort? - mark X on the appropriate place on the chest drawing	On sternum, left side of chest or in left arm	NA	NA	NA
3 When you walk at an ordinary pace on the level does this produce the pain? - Yes/ No/ Unable	Yes	Yes (either this question or question 4)	NA	NA
4 When you walk uphill or hurry does this produce the pain? - Yes/ No/ Unable	Yes	Yes (either this question or question 3)	NA	NA
5 When you get any pain in your chest on walking, what do you do? - Stop/ Slow down/ Continue at same pace/ Not applicable	Stop or slow down	NA	NA	Stop or slow down
6 Does the pain or discomfort in your chest go away if you stand still? - Yes/ No	Yes	NA	NA	NA
7 How long does it take to go away? - 10 minutes or less/ more than 10 minutes	10 minutes or less	NA	NA	NA

NA: Not Applicable, this question does not take part in this definition of angina pectoris

For each patient we determined whether he or she fulfilled the criteria of the four angina definitions. The definitions were determined before analysis started.

Cardiovascular outcomes

To assess the prognostic value of this questionnaire, patients were followed up for coronary events during a period of around 4.6 years. Patients were biannually asked to fill out a questionnaire on hospitalizations and outpatient clinic visits. The primary outcome for the current study was a composite of coronary events, namely coronary death including fatal myocardial infarction, sudden death, fatal heart failure, or non-fatal myocardial infarction. Secondary outcome measure was the composite of the primary outcome and all coronary vascular interventions including coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI). The criteria for each outcome event are displayed in Table 2. For potential outcome events reported by the patient we retrieved hospital discharge letters and the results of relevant laboratory and examinations including 12-lead ECGs. Three members of the SMART Endpoint Committee independently audited all events on basis of available information. This committee consisted of physicians from different departments. In case of disagreement, consensus was reached by consulting other members of the Endpoint Committee. If a patient had multiple events, the first was used for the analyses.

Table 2. Definitions of outcome events

Event	*Definition*
Coronary death	Sudden death: unexpected cardiac death occurring within 1 h after onset of symptoms or within 24 h given convincing circumstantial evidence Fatal myocardial infarction (as defined below) Fatal heart failure
Myocardial infarction	At least two of the following criteria: 1. Chest pain for at least 20 min, not disappearing after administration of nitrates 2. ST elevation > 1 mm in two following leads or a left bundle branch block on the ECG 3. CK elevation of at least two times the normal value of CK and an MB fraction > 5% of the total CK
Vascular intervention	All percutaneous coronary interventions or coronary artery bypass grafting were defined as outcome event. Exceptions are interventions which were already planned before inclusion in the study and performed within six months after inclusion.

Primary endpoint was defined as coronary death and myocardial infarction (cardiac events)
Secondary endpoint was the composite of all three definitions mentioned in this table (cardiac events /interventions)

Data analysis

We employed Cox proportional hazard models for our analyses. In univariable analysis we calculated the hazard ratios and corresponding 95% confidence intervals for each of the four angina definitions. We then constructed four prediction models, in which we sequentially entered variables from the patients' history until no remaining candidate variable had a significance level of 0.15 and into which we forced one of the four angina definitions. These analyses allowed us to assess whether crude and adjusted hazard ratios for the four angina definitions would differ importantly from each other. Next we constructed receiver-operator characteristics (ROC) curves to compare the discriminatory performance of the four multivariable models. In order to evaluate whether the overall performance of the multivariable models improved by inclusion of one of the four angina variables we calculated chi-square statistics on basis of the difference of the -2 log likelihoods of the model with and without the specific angina variable. We tested the prediction models in different subsets of our population; non manifest vascular diseased patients, and patients with qualifying diagnosis for SMART of cerebrovascular disease, coronary artery disease or peripheral artery disease.

The ability of the different prediction models to reclassify study participants in high, moderate and low risk was assessed by dividing them into tertiles of predicted risks. This was done for all four models. The overall improvement in reclassification was quantified as the net reclassification index (NRI).[12] The NRI quantifies the proportion of patients who are correctly moved to higher- or lower risk strata: higher for patients who develop a recurrent event and lower for the patients who do not. Finally we calculated cumulative risks according to the risk tertiles with Kaplan-Meier survival analysis for each model. All analyses were done both for the primary and secondary outcomes.

Results

Baseline results

Of 7916 patients included in the SMART study 1318 (17%) patients had angina pectoris as defined in definition 1, 2376 (30%) according to definition 2, 3960 (50%) according to definition 3 and 1921 (24%) according to definition 4. Baseline characteristics are given in Web Table 1 for all patients

as well as for the four definitions of angina pectoris. The characteristics were comparable between the four predefined definitions of angina pectoris. Compared with all patients of SMART, patients with angina pectoris (definition 1) were older and more often had a history of myocardial infarction, cardiac arrest and cardiac surgery, whereas hyperlipidemia was less common. Obesity was less often present in the whole population compared with patients within one of the definitions for angina pectoris (Web Table 1).

More than half of the patients that fulfilled the criteria of definition 1, 2 and 4 had coronary event as qualifying diagnosis for SMART (63.7%-72.5%), whereas this percentage was somewhat lower in patients that fulfilled the criteria of definition 3 (52.2%). The distribution of the qualifying diagnosis for the SMART study did not differ between the four groups with a different diagnosis of angina pectoris.

Follow up

Mean follow-up time was 4.6 years (36429 person-years). The follow-up was complete for 98.1%. The crude risk of vascular events was comparable between the four groups with a different diagnosis of angina pectoris. The distribution of the components of the composite outcome was similar among the four groups. Definition 1 had a total of 104 ischaemic cardiac events (annual risk 1.59%); for definition 2, 173 events were recorded (annual risk 1.48%); in definition 3, 255 events were recorded (annual risk 1.37%); in definition 4, 153 events were recorded (annual risk 1.60%).

Table 3 summarizes the four Cox regression models. Model 1 was based on variables obtained by history and angina pectoris according to definition 1; model 2 with angina pectoris definition 2; model 3 with angina pectoris definition 3; and model 4 with angina definition 4. For the primary outcome, coronary events, adjusted hazard ratios were in all four groups around 1.2 and the area under the ROC curve (AUC) was roughly 0.7 (Table 3a). Comparing all models with the model without the angina variable gave chi-square values that were statistically significant for angina definition 2 and 4 (Table 4). Adding these angina definitions to the multivariable models was therefore seen as gain in prognostic performance of the model.

Within the secondary outcome, coronary events and interventions, adjusted hazard ratios were around 1.8 in all four definitions of angina and the AUC for all four models was similar around 0.7 (Table 3b). Chi square values were for all four definitions statistically significant.

Table 3a. Ischaemic cardiac events

	AP1		*AP2*		*AP3*		*AP4*	
	HR	*95%CI*	*HR*	*95%CI*	*HR*	*95%CI*	*HR*	*95%CI*
Univariable	1.44	1.16-1.80	1.42	1.17-1.71	1.40	1.16-1.68	1.53	1.27-1.86
Multivariable	1.24	0.99-1.55	1.24	1.02-1.52	1.17	0.95-1.42	1.29	1.05-1.58
Age∞	1.05	1.04-1.06	1.05	1.04-1.06	1.05	1.04-1.06	1.05	1.04-1.06
Sex	1.64	1.30-2.08	1.64	1.30-2.08	1.64	1.30- 2.08	1.64	1.29-2.08
Hypertension treatment	1.43	1.15-1.79	1.43	1.14-1.79	1.42	1.14-1.78	1.42	1.14-1.78
Peripheral artery disease	1.82	1.47-2.24	1.83	1.48-2.25	1.82	1.47-2.24	1.82	1.48-2.25
Aneurysm of aorta	1.85	1.43-2.40	1.86	1.44-2.42	1.86	1.43-2.41	1.88	1.45-2.44
Surgery carotid artery	2.43	1.54-3.14	2.40	1.53-3.77	2.41	1.53-3.78	2.44	1.55-2.83
Myocardial infarction	1.88	1.54-2.29	1.84	1.50-2.24	1.84	1.49-2.26	1.82	1.49-2.23
ROC-AUC	0.73	0.70-0.75	0.73	0.70-0.75	0.73	0.70-0.75	0.73	0.71-0.75

Table 3b. Ischaemic cardiac events and coronary interventions

	AP1		*AP2*		*AP3*		*AP4*	
	HR	*95%CI*	*HR*	*95%CI*	*HR*	*95%CI*	*HR*	*95%CI*
Univariable	2.32	2.04-2.65	2.44	2.17-2.75	2.48	2.18-2.82	2.62	2.32-2.95
Multivariable	1.70	1.48-1.95	1.88	1.65-2.14	1.93	1.68-2.22	1.95	1.72-2.23
Age∞	1.03	1.02-1.04	1.03	1.02-1.04	1.03	1.02-1.04	1.03	1.02-1.04
Sex	1.74	1.49-2.04	1.75	1.49-2.04	1.74	1.49-2.03	1.75	1.50-2.04
Hypertension treatment	1.34	1.16-1.57	1.36	1.17-1.58	1.37	1.18-1.60	1.35	1.16-1.57
Peripheral artery disease	1.36	1.13-1.65	1.39	1.15-1.69	1.41	1.16-1.71	1.42	1.17-1.72
Aneurysm of aorta	1.93	1.38-2.72	1.88	1.34-2.65	1.91	1.36-2.69	1.96	1.40-2.75
Surgery carotid artery	1.74	1.52-2.00	1.65	1.44-1.89	1.57	1.36-1.80	1.64	1.43-1.88
Myocardial infarction	1.47	1.27-1.71	1.39	1.21-1.61	1.40	1.21-1.62	1.40	1.21-1.61
ROC-AUC	0.71	0.70-0.72	0.73	0.70-0.73	0.71	0.69-0.72	0.72	0.71-0.73

∞ age was entered as a continuous variable; the increase in hazard is for each incremental year. ROC-AUC= receiver operator characteristics- area under the curve. AP1-AP4 angina pectoris according to definition given in table 1.

Our analyses were split by sex and adjusted for all variables of the models described in Table 3 (except for sex). The HR for the primary outcome for women was around 1.3 and men around 1.1. For the secondary outcome HR for women was around 2.3 and for men around 1.8 with 95%CI that overlapped each other importantly (Table 5).

Table 4. Contribution of the four angina definitions to the multivariable prediction models

	Primary outcome ∞ *chi square*	*Secondary outcome §* *chi square*
AP1	3.26	52.31‡
AP2	4.50†	89.90‡
AP3	3.24	88.81‡
AP4	5.74†	98.07‡

Chi squared tests with one degree of freedom were calculated as the difference in -2 loglikelihood of models with and without the specific angina pectoris variable. AP1-AP4 angina pectoris according to definition given in table 1.
∞ Ischaemic cardiac events § Ischaemic cardiac events and interventions † p<0.05 ‡ p<0.001

Table 5. Hazard ratios for coronary disease split by sex

	No.patients	*Coronary events*	*HR (95% CI) Primary outcome ∞*	*HR (95%CI) Secondary outcome §*
Men				
AP1	988	88 (8.9%)	1.2 (0.96-1.6)	1.6 (1.4-1.9)
AP2	1728	140 (8.1%)	1.2 (0.9-1.5)	1.8 (1.6-2.1)
AP3	2845	209 (7.3%)	1.1 (0.9-1.4)	1.9 (1.6-2.2)
AP4	1403	124 (8.8%)	1.2 (0.98-1.5)	1.8 (1.6-2.1)
Women				
AP1	330	16 (4.8%)	1.2 (0.7-2.0)	1.9 (1.4-2.7)
AP2	648	33 (5.1%)	1.5 (0.97-2.4)	2.3 (1.7-3.1
AP3	1115	46 (4.1%)	1.3 (0.8-2.0)	2.4 (1.7-3.3)
AP4	518	29 (5.6%)	1.5 (0.96-2.4)	2.6 (1.9-3.5)

All analyses are adjusted for the same variables included in the multivariable models described in Table 3 (except for sex) ∞ Ischaemic cardiac events § Ischaemic cardiac events and interventions

Model performance

We tested the prediction models in different patient groups in our population (Table 6a and b). In patients with qualifying diagnosis of coronary

artery disease the AUC was significantly lower compared with the patients with cerebrovascular disease and those without clinically manifest vascular disease. There is an overlap with patients with peripheral artery obstructive disease. The presence of angina pectoris seems to be the least contributory in the prediction of cardiovascular events or interventions in coronary artery diseased patients.

Table 6a. Areas under the ROC curves; ischaemic cardiac events

	AP1	*AP2*	*AP3*	*AP4*
	AUC-ROC (95% CI)	*AUC-ROC (95% CI)*	*AUC-ROC (95% CI)*	*AUC-ROC (95% CI)*
non manifest vascular disease∞ (n=2129)	0.732 (0.667-0.79)	0.737 (0.673-0.802)	0.737 (0.673-0.801)	0.738 (0.674-0.803)
Cerebrovascular disease§ (n=999)	0.750 (0.696-0.805)	0.750 (0.695-0.804)	0.746 (0.691-0.800)	0.753 (0.699-0.806)
Coronary artery disease§ (n=2427)	0.583 (0.528-0.639)	0.584 (0.529-0.640)	0.586 (0.530-0.641)	0.590 (0.535-0.645)
Peripheral artery disease§ (n=898)	0.681 (0.621-0.741)	0.683 (0.622-0.743)	0.678 (0.618-0.739)	0.681 (0.621-0.741)

Table 6b. Areas under the ROC curves; ischaemic cardiac events and interventions

	AP1	*AP2*	*AP3*	*AP4*
	AUC-ROC (95% CI)	*AUC-ROC (95% CI)*	*AUC-ROC (95% CI)*	*AUC-ROC (95% CI)*
non manifest vascular disease∞ (n=2129)	0.743 (0.697-790)	0.737 (0.690-0.784)	0.737 (0.689-0.786)	0.748 (0.703-0.793)
Cerebrovascular disease§ (n=999)	0.730 (0.689-0.770)	0.729 (0.687-0.770)	0.707 (0.664-0.749)	0.735 (0.694-0.775)
Coronary artery disease§ (n=2427)	0.501 (0.472-0.531)	0.535 (0.507-0.564)	0.514 (0.486-0.542)	0.540 (0.511-0.569)
Peripheral artery disease§ (n=898)	0.685 (0.640-0.731)	0.682 (0.635-0.728)	0.674 (0.628-0.720)	0.687 (0.641-0.733)

AP1-AP4 angina pectoris according to definition given in table 1 ROC-AUC are based on the same variables included in the multivariable models described in Table 3b ∞ According to inclusion diagnosis and medical history § Patient groups defined according to diagnosis at inclusion in SMART[12]

The Kaplan Meier curves according to low, intermediate and high risk are shown in Figure 1a and 1b. On the basis of these risk tertiles we created event-specific reclassification tables.[12] Definition 2 performed slightly better compared with the other definitions, but overall differences were small. NRI was not more than 2% (Table 7a and 7b).

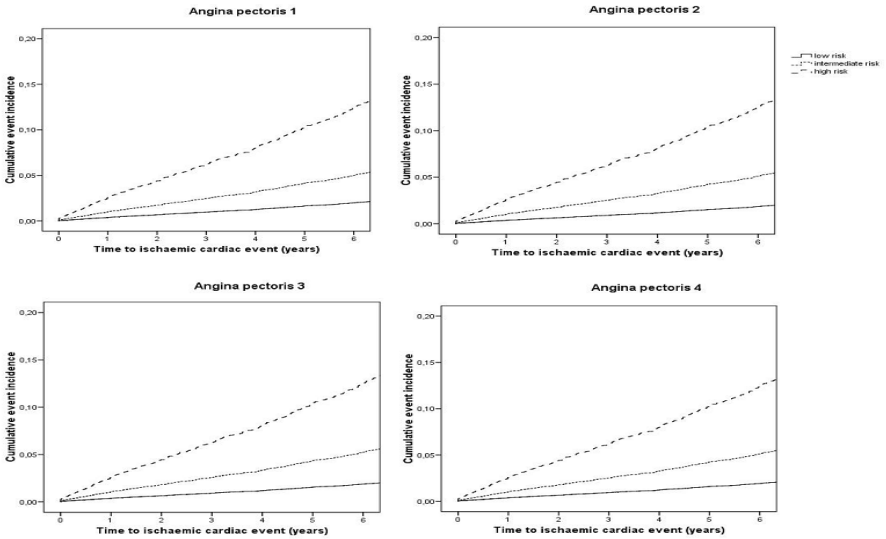

Figure 1a. Kaplan Meier curves for the primary outcome, for low, medium and high risk categories according to four different angina models; AP1-AP4 angina pectoris according to definition given in table 1.

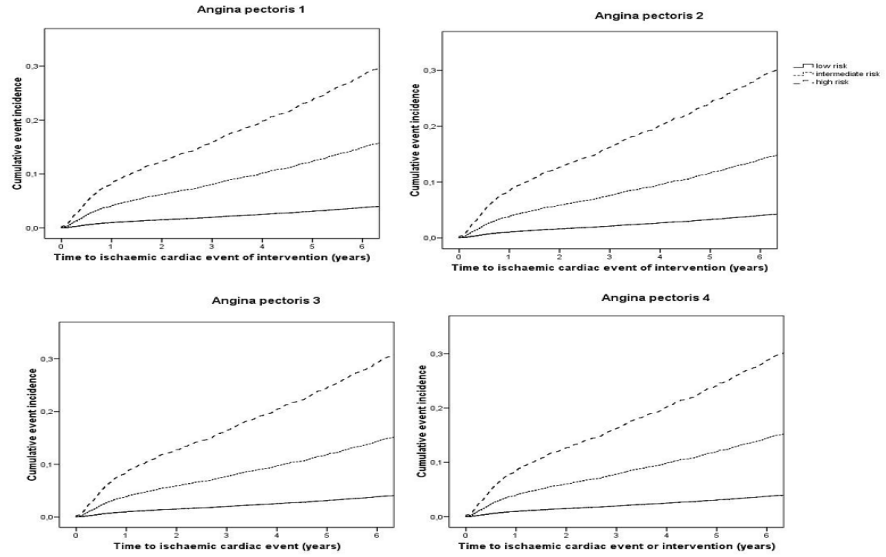

Figure 1b. Kaplan Meier curves for the secondary outcome, for low, medium and high risk categories according to four different angina models; AP1-AP4 angina pectoris according to definition given in table 1.

Table 7a. Net Reclassification Index; cardiac ischeamic events

	AP1	*AP2*	*AP3*
AP2	1.41%		
AP3	0.04%	-1.46%	
AP4	0.50%	-0.91%	0.54%

NRI of 1.41%; definition 2 improved classification for 1.41% of patients with a cardiovascular event, with no net loss for non-events

Table 7b. Net Reclassification Index; cardiac ischaemic events en interventions

	AP1	*AP2*	*AP3*
AP2	1.03%		
AP3	0.32%	-0.71%	
AP4	1.62%	-0.59%	1.30%

AP1-AP4 angina pectoris according to definition given in table 1
NRI of 1.03%; definition 2 improved classification for 1.03% of patients with a cardiovascular event or intervention, with no net loss for non-events

Discussion

We showed that four definitions for angina pectoris had an almost equal discriminatory ability to predict new coronary events and interventions; areas under the ROC curves were essentially the same and reclassification comparing different models was limited. Multivariable adjusted hazard ratios for coronary events were around 1.2 and those for the composite of coronary events and interventions around 1.8. All four definitions improved the multivariable models for the composite outcome more than that for coronary events only. The angina definition based on the combination of ever presence of pain or discomfort in the chest with its disappearance upon decreased exertion (definition 4) tended to contribute most in the prediction of composite outcome. Thus, the full Rose questionnaire was not able to predict coronary outcome better than subsets of questions in patients at high risk for vascular disease.

Since its development in 1962 the Rose questionnaire and its criteria for angina pectoris are widely used for diagnostic and prognostic research.[3] We found a prevalence of angina pectoris according to the Rose questionnaire of 16.6% in our population. Compared with other studies (4.7%-9.6%) this proportion is high, because our study population is at relatively high risk for

future vascular events as compared with population based studies.[9,13-15] In patients with clinically manifest vascular disease in SMART (5299 patients) the percentage of patients with angina pectoris according to definition 1 was up to 22.8%, whereas in patients without clinically manifest vascular disease (2129 patients) the prevalence of angina pectoris was only 4.1%.

Previously a difference of diagnostic performance of the questionnaire between men and women was highlighted before.[7,16] A recent study showed that in postmenopausal women a shortened version of the questionnaire (like our second definition) performed better than the full version in identifying women with a medical diagnosis of angina.[9] When our analyses were split by sex HRs for the primary and secondary outcome were about the same for the two sexes after adjustment for age, treatment for hypertension (only primary outcome) and history of peripheral artery disease, abdominal aneurysm, carotid artery surgery, myocardial infarction and cardiac surgery (only secondary outcome) (Table 5). For this reason other analyses were not split by sex.

There are several studies that related the Rose questionnaire to either presence of atherosclerosis or prognosis. The Rotterdam Coronary Calcification Study found that the questionnaire was strongly associated with the amount of coronary calcification and that this relationship was stronger in men than in women.[14]

Population-based studies have shown that patients with angina pectoris defined according to the Rose questionnaire have an increased risk of coronary heart disease.[17] The odds ratio for major coronary events was 2.1 (95% CI 1.7-2.6).[13] The repeatability of Rose angina was also studied; they found moderate repeatability over 5 years of follow up.[18] Next more repeatability of Rose angina or the exertional part of it was associated with more severe disease.[18] Another study showed that possible angina pectoris (comparable with our definition 2) was related to death from coronary heart disease (OR 2.1; 95% CI 1.2-3.2).[19] In the same study these angina patients had a higher incidence of CABG (OR 4.0; 95% CI 2.0-7.9) and acute myocardial infarction (OR 1.8; 95% CI 1.1-3.1).[19] A recent study showed that in a young general population in Norway a subset of questions of the Rose questionnaire, concerning exertional chest pain also predicted long term coronary heart death (HR 1.50; 95%CI 1.30-3.02).[20]

The main strength of our study is the standardized way in which it was performed. At baseline routine assessment of history with standardized questionnaires was done. The same package of laboratory investigations and further physical examination was done in each patient. During follow up

standardized methods were used to adjudicate outcome events. Together this makes that the patient population used for this study is widely standardized. Another strength is the completeness of data. Since every questionnaire is filled out by the patient self and checked by study nurses for missing answers, which were completed together with the patient when visiting the hospital for baseline examinations, only 36 patients of the 7916 had a missing answer to the first question of the Rose questionnaire. The follow up was complete for 98.1% of all patients.

A limitation is that we did not perform gold standard tests to diagnose angina pectoris in each patient. Since it was not the objective of this study to determine the performance of the questionnaire to diagnose angina pectoris, this is not a real limitation. Another limitation could be the generalisability of the results of this study, since we assessed the Rose questionnaire in a population referred to a University Medical Center, with mostly cardiac events or problems. However we showed that in patients with already existing cardiac problems our prediction rule performed worse than in other subsets of patients. This makes it less likely that the results are greatly influenced by the high number of patients with cardiac disease in this population. A theoretical concern might be the performance of single questions from the full Rose questionnaire on their own. All patients filled out the full questionnaire and we extracted the answers to the separate questions. When only a subset of questions is administered to the patient, other answers might be elicited. We, however, think the questions perform all well on their own, and that this is not a major concern.

The Rose questionnaire is a widely used tool for the assessment of angina pectoris. It has been demonstrated that for diagnostic research a subset of questions can identify patients with angina pectoris at least as good as the full version.[9] Our findings imply that for prediction purposes there is little difference between different definitions of angina with NRI's no larger than 2%. Questions about exertional chest pain performed slightly better. The use of one or two single questions (our definitions 2 and 4) will possibly lead to a better response rate. Further use of the whole Rose questionnaire as a prognostic tool to define angina pectoris in high-risk patients cannot be recommended any more. For patients with manifest coronary artery disease the presence of angina according to one of the definitions does not seem to contribute to the prediction of coronary events.

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Webtable 1. Baseline characteristics of patients with angina according to different definition

	All patients (N=7961)	*AP1 (n = 1318)*	*AP2 (n= 2376)*	*AP3 (n= 3960)*	*AP4 (n= 1921)*
Age (years) (mean, SD)	56.2 (12.6)	59.7 (10.3)	58.7 (11.6)	57.5 (11.4)	59.5 (10.5)
Male sex	5331 (67.1%)	988 (75.0%)	1728 (72.7%)	2845 (71.8%)	1403 (73.0%)
Qualifying diagnosis					
Diabetes mellitus	600 (7.6%)	36 (2.8%)	90 (5.2%)	211 (5.3%)	72 (3.7%)
Hyperlipidaemia	950 (12.0%)	40 (3.0%)	129 (5.4%)	337 (8.5%)	80 (4.2%)
Hypertension	974 (12.3%)	63 (4.8%)	171 (7.2%)	381 (9.6%)	124 (6.5%)
Coronary event	2427(30.7%)	955 (72.5%)	1513 (63.7%)	2067 (52.2%)	1285 (66.9%)
Asymptomatic carotid stenosis	98 (1.2%)	13 (1.0%)	23 (1.0%)	35 (0.9%)	19 (1.0%)
Cerebrovascular event	1150 (14.5%)	61 (4.6%)	142 (6.0%)	325 (8.2%)	109 (5.7%)
Aneurysma of abdominal aorta	299 (3.8%)	29 (2.2%)	58 (2.4%)	108 (2.7%)	44 (2.3%)
Kidney problems	165 (2.1%)	14 (1.1%)	27 (1.1%)	57 (1.7%)	21 (1.1%)
Peripheral artery disease	898 (11.4%)	88 (7.5%)	169 (7.1%)	310 (7.8%)	140 (7.3%)
Other vascular	247 (3.1%)	14 (1.1%)	33 (1.4%)	99 (2.5%)	21 (1.1%)
Rest non-vascular disease	107 (1.3%)	5 (0.4%)	11 (0.5%)	29 (0.8%)	6 (0.4%)
History					
Stroke	850 (10.7%)	67 (5.1%)	136 (5.7%)	274 (6.9)	116 (6.0%)
Carotid surgery	130 (1.6%)	20 (1.5%)	34 (1.4%)	55 (1.4%)	30 1.6%)
Myocardial infarction	1624 (20.5%)	467 (35.4%)	810 (34.1%)	1292 (32.6%)	706 (36.8%)

	All patients (N=7961)	*AP1 (n = 1318)*	*AP2 (n= 2376)*	*AP3 (n= 3960)*	*AP4 (n= 1921)*
Cardiac arrest	172 (2.2%)	46 (3.5%)	81 (3.4%)	126 (3.2%)	64 (3.3%)
Cardiac surgery	1960 (24.8%)	711 (53.9%)	1127 (47.4%)	1620 (40.9%)	969 (50.4%)
Surgery for aneurysm of abdominal aorta	115 (1.5%)	17 (1.3%)	33 (1.4%)	53 (1.3%)	24 (1.2%)
Vascular surgery legs	375 (4.7%)	57 (4.3%)	122 (5.1%)	180 (4.5%)	93 (4.8%)
Cigarette smoking					
Currently	2475 (31.5%)	319 (24.2%)	624 (25.9%)	1122 (28.5%)	488 (25.5)
Packyears (mean, SD)	18.0 (19.4)	19.4 (19.3)	18.9 (19.5)	18.7 (19.6)	19.3 (19.6)
Blood pressure (mm Hg)					
Systolic (mean, SD)	141.8 (20.9)	140.5 (20.5)	140.4 (20.3)	140.5 (20.4)	140.9 (20.7)
Diastolic (mean, SD)	82.7 (11.7)	80.7 (10.9)	81.1 (11.2)	81.9 (11.4)	80.9 (11.2)
Pulse pressure (mean, SD)	59.1 (15.5)	59.8 (15.5)	59.3 (15.3)	58.6 (15.2)	60.0 (15.7)
Hypertension (current)	2160 (27.3%)	613 (46.7%)	549 (23.1%)	964 (24.4%)	432 (22.5%)
Treatment for hypertension	3479 (44.4%)	289 (22.0%)	1105 (46.7%)	1816 (46.3%)	916 (47.9%)
Cholesterol (mmol/L) (mean, SD)	5.3 (1.4)	4.9 (1.3)	5.0 (1.2)	5.0 (1.3)	4.9 (1.2)
Triglycerides	1.9 (2.1)	1.8 (1.9)	1.9 (1.8)	1.9 (1.9)	1.8 (1.8)
HDL-cholesterol	1.3 (0.4)	1.2 (0.3)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)
LDL-cholesterol	3.1 (1.2)	2.9 (1.0)	2.9 (1.1)	3.0 (1.1)	2.9 (1.0)
Hyperlipidaemia (current)	4263 (55.0%)	555 (42.8%)	1049 (45.0%)	1824 (47.0%)	849 (45.1%)
Treatment for hyperlipidaemia	3417 (43.7%)	819 (62.3%)	1364 (57.7%)	2098 (53.6%)	1159 (60.6%)

	All patients (N=7961)	*AP1 (n = 1318)*	*AP2 (n= 2376)*	*AP3 (n= 3960)*	*AP4 (n= 1921)*
Glucose (mmol/L) (mean, SD)	6.4 (2.2)	6.3 (2.2)	6.3 (2.1)	6.4 (2.1)	6.4 (2.1)
Hyperglycemia	1470 (18.8%)	247 (18.9%)	466 (19.8%)	760 (19.4%)	388 (20.3%)
Diabetes mellitus (current)	1562 (20.7%)	236 (18.4%)	468 (20.4%)	769 (20.4%)	386 (20.8%)
Treatment for diabetes	1350 (17.2%)	201 (15.3%)	397 (16.7%)	653 (16.6%)	327 (17.1%)
BMI (kg/m2) (mean, SD)	26.8 (4.4)	27.3 (4.0)	27.4 (4.2)	27.3 (4.3)	27.5 (4.2)
BMI > 30	1525 (19.3%)	268 (21.7%)	532 (22.4%)	870 (22.0%)	449 (23.4%)
ABI < 0.90	1262 (16.1%)	161 (12.3%)	285 (12.1%)	502 (12.8%)	248 (13.0%)
Carotid stenosis > 50%	901 (11.6%)	131 (10.2%)	230 (9.9%)	376 (9.7%)	201 (10.7%)
Abdominal aorta > 3 cm	376 (4.8%)	58 (4.5%)	96 (4.1%)	163 (4.2%)	79 (4.2%)
Kidney length <9.5 cm	2046 (26.4%)	354 (27.3%)	621 (26.7%)	1013 (26.1%)	499 (26.4%)
Creatinin clearance (Cockroft)					
Normal	3906 (49.8%)	589 (45.0%)	1120 (47.4%)	1919 (48.9%)	871 (45.6%)
Mild impairment	3292 (42.0%)	612 (46.8%)	1061 (44.9%)	1717 (43.7%)	876 (45.9%)
Severe impairment	641 (8.2%)	108 (8.3%)	180 (7.6%)	292 (7.4%)	161 (8.4%)
Hyperhomocysteinaemia	907 (12.5%)	136 (11.0%)	252 (11.2%)	415 (11.1%)	203 (11.3%)

AP1-AP4 angina pectoris according to definition given in table 1
BMI: body mass index, ABI; ankle brachial index

6

Prognostic Modelling in Ischaemic Stroke (PROMISe) Study, additional value of genetic characteristics: Rationale and Design

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Abstract

Background and aim. Prediction of prognosis after cerebral infarction might be improved by genetic information. The aim of PROMISe is to develop two different prognostic models on the basis of traditional vascular risk factors and genetic information in patients who have suffered from cerebral ischaemia of arterial origin (CIAO), one concerning new ischaemic and the other new haemorrhagic events.

Methods. Polymorphisms and haplotypes describing the haemostatic system and those that influence antithrombotic drug activity will be identified in a cohort of 1200 patients with CIAO who will be followed up for a mean of 6.5 years. In total 312 ischaemic and 78 haemorrhagic events are anticipated. With a prevalence of a genetic characteristic of 10% a relative risk of 1.4 (95% confidence interval (CI) 1.1-1.8) for ischaemic events and of 1.8 (95% CI 1.0-3.2) for haemorrhagic events can be estimated with sufficient precision. To determine additional prognostic value of genetic characteristics the area under ROC curves of two separate models will be compared: one based on non-genetic risk factors only, the other also including genetic data.

Introduction

Predicting the prognosis of a patient after a transient ischaemic attack (TIA) or ischaemic stroke on basis of the presence or absence of established cardiovascular risk factors is difficult.[1] There are several prognostic models for ischaemic and haemorrhagic events after cerebral ischaemia of arterial origin (CIAO), but neither of these include genetic information.

Cerebral infarction can be caused by several pathophysiological mechanisms which might be influenced by a single-gene defect. A strong link between classical risk factors for ischaemic stroke, such as hypertension, diabetes mellitus, hypercholesterolaemia, and genetic factors in the prediction of stroke recurrence is suggested.[2] Genetic determinants of bleeding after CIAO have received little attention. Thrombotic and thromboembolic factors are known to be contributing causes for stroke and are the primary interest in this study.

Several studies have been performed to detect an association between polymorphisms and first strokes. Associations between polymorphisms and new vascular events after cerebral ischaemia have received little attention. In order to maximize the chance of detecting such an association, analysis of data according to stroke subtype or separate studies of young individuals is required.[3]

CIAO can be classified into large vessel disease (LVD) and small vessel disease (SVD).[4] Patients with SVD have a lower case fatality and better functional outcome on the short term than patients with LVD, which is probably explained by the difference in amount of brain tissue that is damaged.[5] This assumes also different pathogeneses of these two stroke subtypes.[6] Different genetic risk factors may predispose to these two disease entities and stroke subtype should therefore be taken into account to define the exact phenotype of the patient.

We hypothesise that genetic information will improve the risk stratification with regard to ischaemic and haemorrhagic events after CIAO. We will discuss the background of this hypothesis and describe the protocol of this study that is described to test the hypothesis. Genetic characteristics of special interest are described in tables 1 and 2.

Table 1. Polymorphisms of hemostasis with their relative risk (95% confidence interval)

Gene[*]	*Variant*	*rs number*	*Relative Risk*[#]	*Reference*
F2	G20210A	rs179963	3.5 (0.63-9.75)	[15]
F3	A(-603)G	rs1361600	1.4 (1.1-1.9)[$]	[36]
F5	Arg506Gln	rs6025	1.0 (0.6-3.1)	[14]
			2.6 (1.5-3.7)[#]	[14]
F13A	Val34Leu	rs5985	0.6 (0.4-0.8)	[7]
F13A	Tyr204Phe	rs302447	2.0 (0.6-6.1)	[8]
			2.9 (1.1-7.5)[%]	[11]
F13A	Pro564Leu	rs5982	1.0 (0.5-2.1)	[8]
			1.7 (0.9-3.4)[%]	[11]
F13B	His95Arg	rs6003	0.9 (0.6-1.3)[$]	[9]
			1.6 (1.0-2.6)[&]	[10]
PAI-1	4G/5G	rs1799889	1.6 (1.1-2.2)	[28]
GP1BA	VNTR		1.4 (0.4-4.2)	[32]
GP1BA	Thr145Met	rs6065	1.6 (0.9-2.8)	[32]
GP1BA	T(-5)C	rs2243098	1.6 (1.0-2.5)	[32]
TPA	C7351T	rs2020918	2.6 (1.1-6.4)	[25]
TPA	I/D (alu repeat)	rs4646972	1.8 (1.1-2.8)	[27]
FGB	G(-455)A	rs1800790	2.6 (1.2-5.4)	[22]
FGB	T1689G	rs2227399	0.9 (0.5-1.6)[$]	[23]
FGG	G7874A	rs2066865	1.0 (0.6-1.5)[$]	[20]
			2.4 (1.5-3.9)[&]	[21]
FGG	T9340C	rs1049636	1.1 (0.7-1.8)[$]	[20]
			0.7 (0.4-1.2)[&]	[21]
FGG	G5836A	rs2066865	1.1 (0.7-1.6)[$]	[21]
VWF	Thr789Ala	rs1063856	3.3 (1.5-7.0)	[30]
VWF	G(-1185)A	rs7954855	0.93 (0.77-1.12)[$]	[31]
GP3A	PlA1	rs4634	1.3 (0.9-1.8)[$]	[12]
GP3A	PlA2	rs5918	2.7 (1.4-5.4)[$]	[12]
GP6	Ser219Pro	rs1613662	2.4 (1.1-5.4)[$]	[33]
GP6	Thr249Ala	rs2304167	2.3 (1.2-4.7)[$]	[34]
GP6	His322Asn	rs1671152	1.1 (0.7-1.7)[$]	[34]
GP6	Gln317Leu	rs1654413	0.5 (0.2-1.4)	[35]

Gene[*]	*Variant*	*rs number*	*Relative Risk*[#]	*Reference*
VKORC1	T2255C	rs2359612	2.3 (1.8-2.9)	[37]
			1.7 (1.3-2.2)[%]	[37]
MTHFR	Ala222Val	rs1801133	1.23 (0.96-1.58)	[17]
COX 2	-765G->C	rs20417	0.33 (0.24-0.55)	[29]

[#] relative risk for ischaemic stroke unless otherwise indicated
[$] for myocardial infarction
[&] for deep venous thrombosis
[#] for large vessel disease
[%] for haemorrhagic stroke
[*] description of gene symbols:
F2: coagulation factor II, F3: tissue factor, F5: coagulation factor V, F13A: coagulation factor XIII subunit A, F13B: coagulation factor XIII subunit B, PAI-1: plasminogen activator inhibitor-1, GP1BA: glycoprotein 1-b-alpha, TPA: tissue plasminogen activator, FGA: fibrinogen alpha, FGB: fibrinogen beta, FGG: fibrinogen gamma, VWF: Von Willebrand factor, GP3A: glycoprotein III subunit A, GP6: glycoprotein VI, VKORC1: Vitamin K epoxide reductase complex-1, MTHFR: 5,10-methylenetetrahydrofolate reductase, APOE: apolipoprotein E, COX 2: cyclo-oxygenase inhibitor 2. LD: linkage disequilibrium

Table 2. Polymorphisms of pharmacogenetics with their relative risk/ difference of the mean (95% confidence interval)

Gen[+]	*Variant*	*rs number*	*RR/ mean of difference*[#]	*Reference*
F13A	Val34Leu	rs5985	0.18 (0.09-0.27)[$]	[64]
FGA	Thr312Ala	rs6050	0.3 (0.1 – 0.8) *	[54]
VKORC1	T1173C	rs9934438	1.7 (1.1-2.5)*	[53]
COX1	A-842G/C50T	rs3842787	2.0 (0.6-6.0)	[60]
COX1	C22T	rs1236913	0.5 (0.1-2.7)	[60]
COX1	C644A	rs5788	1.2 (0.4-3.7)	[60]
COX1	C714A	rs5789	Prevalence less than 1%	[60]
GP3A	PlA1	rs4634	4.3 (1.8-10.0)*	[12]
GP1A\2A	807 C/T	rs1126643	12.7 (-0.8-26.2) [$&]	[67]
GP1BA	T(-5)C	rs2243098	0.6 (0.2-1.5)	[60]
GP6	Thr249Ala	rs2304167	4.2 (1.4-12.6)	[60]
CYP2C9	*2	rs1799853	1.4 (1.0-1.9)*	[52]

[#] Relative risk for influence on functionality of acetylsalicacid unless otherwise indicated
* Risk for bleeding associated with antithrombotic treatment
[&] Influence on functionality of clopidogrel
[$] Difference of mean
[+] Description of gene symbols:
F5: coagulation factor V, F13A: coagulation factor XIII subunit A, F13B, FGA: fibrinogen alpha, VKORC1: Vitamin K epoxide reductase complex-1, MTHFR: 5,10-methylenetetrahydrofolate reductase, COX1: cyclo-oxygenase inhibitor 1, GP3A: glycoprotein III subunit A, GP1A/2A: glycoprotein Ia/IIa, GP1BA: glycoprotein 1-b-alpha GP6: glycoprotein VI, CYP2C9: cytochrome P450 2C9

Ischaemic stroke

Many studies are done to determine genetic characteristics of interest for the risk of ischaemic stroke. We will discuss the characteristics of interest for this study, mainly concerning the haemostatic genetics.

Haemostasis and genetics

The Val34Leu polymorphism in subunit A of the factor XIII gene decreased the risk of ischaemic stroke in one study,[7] but showed no protective effect on ischaemic stroke in young women in another study.[8] Furthermore, it appeared to have a gene-gene interaction with the His95Arg polymorphism of the subunit B of the same coagulation factor.[9] On its own this B subunit polymorphism His95Arg showed no association with myocardial infarction.[9] Another study, however, showed a significant association with deep venous thrombosis.[10] On basis of a population-based case control study the Phe204 and Leu564 variants of factor XIII subunit A were suggested to be markers for genetic susceptibility to haemorrhagic stroke in young women.[11] No relation with ischaemic stroke could be found.[8] The Pl(A1/2) polymorphisms of glycoprotein IIIa (GPIIIa) are considered a risk factor for thrombosis in young survivors of myocardial infarction in addition to its interaction with medication .[12] Carriers of the variant of the GPIIIa subunit based on the 33Pro allele (PIA2) had an 1.8-fold increase risk, which was further increased if the carriers also smoked (13.7-fold increase).[13] The Arg506Gln mutation in the factor V gene, also called factor V Leiden polymorphism, is an established risk factor for venous thrombosis, but its role in arterial thrombosis is less clear. An association of factor V Leiden and large-vessel cerebral infarction has been claimed in stroke subtype analysis.[14]

In a small case control study the prothrombin G20210A polymorphism, which is another risk factor for venous thrombosis did show an association with risk of recurrent ischaemic events after clinical manifestation of atherosclerosis (including ischaemic stroke).[15]

An elevated serum homocysteine level is a modest independent predictor for stroke.[16] A C677T mutation in the MTHFR gene leads to moderate increases in the serum homocysteine level. Two recent meta-analyses showed a statistically non-significant trend of the MTHFR polymorphism on the risk of primary ischaemic stroke.[16,17] Interaction of this gene with the contraceptive pill has been described; women with MTHFR 677TT using oral contraceptives had a 5.4-fold (95% CI 2.4-12.0) higher risk of first ischaemic

stroke than women without these risk factors.[18]

The Thr312Ala polymorphism of fibrinogen Aalpha is thought to have thrombotic effects with increased factor XIII cross-linking and formation of thicker fibrin fibers in addition to its effect on major haemorrhagic events occurrence in patients on oral anticoagulant treatment.[19] The fibrinogen gamma haplotype H2 is associated with deep venous thrombosis, however, no association was found between these H2, H3 and H4 haplotypes and myocardial infarction.[20,21] In a cohort study with stroke patients the presence of the 455A allele of the beta-chain of fibrinogen was associated with a 2.5-fold increase in the risk of lacunar stroke, but had no association with large vessel stroke.[22] Other polymorphisms in the β-chain are known, G854A and T1689G, from which the later was found not to be associated with myocardial infarction and peripheral artery disease.[23] G854A was not studied in association with clinical disease, but only showed a modest effect on the plasma levels of fibrinogen.[24]

The C7351T polymorphism of tissue plasminogen activator (TPA) is thought to be associated with the risk on lacunar stroke.[25] In a larger study this effect, however, could not be confirmed.[26] Another polymorphism in TPA is the alu-repeat I/D which was found associated with an increased risk of stroke when at least one D allele is present.[27] Elevated plasminogen activator inhibitor-1 (PAI-1) levels will impair fibrinolytic function and has been implicated in ischaemic stroke. The 4G allele of the PAI-1 polymorphism is associated with an increased risk of ischaemic stroke. Patients with hypertriglyceridaemia and who were 4G homozygous are at the greatest risk of developing stroke.[28]

The 765G→C polymorphism of the cyclo-oxygenase-2 (COX-2) gene has been associated with a considerable decrease in the risk of myocardial infarction and stroke.[29] Other possible initiators of thrombosis are the von Willebrand factor (vWf) in interaction with its specific platelet receptor, the alpha chain of the glycoprotein Ib-IX-V complex (GPIbalpha). The Sma I polymorphism of vWf is suggested to increase the risk of ischaemic stroke.[30] Another four polymorphisms in the vWf gene are described to be in linkage disequilibrium with each other and to form haplotypes. These haplotypes were found not to be associated with myocardial infarction.[31] In GPIbalpha three polymorphisms are described, the Kozak T/C polymorphism, variable number of tandem repeats (VNTR) and HPA2 (Thr145Met). Only the last two showed a trend towards increased risk of ischaemic stroke; the Kozak polymorphism,

however, was significantly overrepresented in patients with ischaemic stroke.[32] The Ser219Pro and Thr249Ala polymorphisms of glycoprotein VI are associated with an increased risk of myocardial infarction. Other polymorphisms in this glycoprotein, His322Asn and Gln317Leu, however, did not show such an association.[33-35] Carriership of the -603 G allele of the tissue factor (TF) is associated with an increased risk for myocardial infarction.[36] The vitamin K epoxide reductase complex subunit 1 (VKORC1) is independent of possible effects on anticoagulation thought to be associated with vascular disease because of vitamin K dependent proteins that play important roles in the coagulation cascade. A Chinese study showed that the VKORC1 T2255C polymorphism was associated with lacunar stroke as well as haemorrhagic stroke.[37]

Intracranial bleeding and genetics

Little is known about genetic predisposition in the risk of (intracranial) haemorrhage. A Pubmed search (MeSH terms 'genetics' and 'cerebral haemorrhage') revealed a total of only 21 articles on this subject, with more than half concerning bleeding because of distinct familial syndromes. This concerns amyloid angiopathies (mostly not associated with genetic disturbance, since familial forms are rare), haemophilia, von Willebrand's disease and genetic disorders causing cerebral vascular malformations.[38] Two well described cerebral angiopathies with known genetic background are Hereditary Cerebral Haemorrhages with Amyloidosis-Dutch type (HCHWA-D) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).[39,40] Patients with HCHWA-D or CADASIL will be excluded from the current study. Whereas apolipoprotein E is found not to be associated with hereditary amyloid angiopathy, the e4 allele is a strong factor for the development of amyloid beta deposition and the e2 allele causes vasculopathic changes due to amyloid-laden vessels.[41] The e4 allele is also associated with lobar intracerebral haemorrhage.[42] Another gene suggested to be especially related to haemorrhagic stroke is the MTHFR. Its C677T and A1298C polymorphisms are found to be genetic risk factors for haemorrhagic stroke, independent from other atherothrombotic risk factors.[43]

Treatment and major bleeding

The major determinants of oral anticoagulant-induced bleeding are the intensity of treatment (expressed as international normalised ratio, INR),

patient characteristics like hypertension, age and the presence of white matter lesions, the concomitant use of drugs that interfere with haemostasis, and the duration of therapy. A recent Cochrane review[44] demonstrated that the risk of major bleeding after TIA or minor stroke is not different during treatment with low (INR 1.4 to 2.8) and medium (INR 2.1-3.6) intensity anticoagulation as compared with antiplatelet therapy (also no benefit for anticoagulation could be proved). High intensity anticoagulation (INR 3.0-4.5), however, did increase the risk on major bleeding complications importantly compared with antiplatelet therapy.

In the CAPRIE trial, clopidogrel was superior to aspirin in the overall population of patients with recent ischaemic stroke, recent myocardial infarction, or symptomatic peripheral arterial disease reducing the relative risk for the primary outcome (ischaemic stroke, myocardial infarction, or vascular death) by 8.7% versus aspirin.[45] Subgroup analysis for patients with ischaemic stroke showed a non-significant risk reduction of clopidogrel compared with aspirin. No major differences in safety were reported. In the MATCH trial life-threatening bleedings were more frequent in the group receiving aspirin and clopidogrel compared with the clopidogrel alone group (absolute risk increase 1.3 [95%CI 0.6-1.9]).[46] Recently the CHARISMA study showed no benefit for the contribution of clopidogrel and aspirin above aspirin therapy.[47] Major bleeding occurred more often in patients treated with clopidogrel and aspirin, for moderate bleedings this effect was statistically significant with a relative risk of 1.62 (95% CI 1.27-2.08). Also, platelet glycoprotein IIb/IIIa receptor inhibitors have been associated with haemorrhagic complications.[48]

A number of recent studies has suggested that efficacy and safety of antithrombotic drugs depend on genetic make-up. In 200 patients on long-term warfarin polymorphisms encoding for the cytochrome P4502C9 (CYP2C9) protein were determined.[49] The patients with at least one variant allele (31%) had higher risks of above-range INRs and a more than double incidence of major bleeding than those with the wild-type genotype. These clinical data fit with in-vivo observations on impaired warfarin clearance with CYP2C9 variants.[50] An American research group incorporated information on eight polymorphisms in the CYP2C9 system in a multivariate-dosing model of warfarin.[51] They found that clinical (lower body surface area), demographic (age) and pharmacogenetics (presence of CYP2C9*2 or *3 alleles) factors can estimate the maintenance warfarin dose and overanticoagulation with bleeding events confirmed by other studies.[49] Since vitamin K is an important factor

in oral anticoagulation it is imaginable that polymorphisms in the VKORC1 gene also influence therapeutic action of oral anticoagulants and concomitant bleeding risk.[52]

Recently we found that patients who had a major haemorrhage while treated with oral anticoagulation less frequently had the heterozygous (AG) or homozygous (GG) form of the fibrinogen Aalpha Thr312Ala polymorphism than similar patients treated who did not develop a major haemorrhage (odds ratio 0.32; 95% CI 0.13 – 0.75).[53] It can be questioned whether this has to do with the anticoagulation or that this single nucleotide polymorphism (SNP) on its own has a link with the occurrence of haemorrhages. Acetylsalicylic acid (aspirin) treatment of patients diagnosed with atherothrombotic disease reduces the number of ischaemic events in high-risk patients by about 25%.[54]

The response to aspirin treatment varies among individuals and may be caused by so-called aspirin resistance that can be defined in two ways. From a clinical point of view aspirin resistance is the inability of aspirin to protect individuals from cardiovascular thrombotic events such as stroke.[55] However, many cardiovascular events that occur in patients treated with aspirin may not be preventable by aspirin. Furthermore, the diagnosis of clinical resistance can only be made in retrospect because an ischaemic event must occur before a diagnosis of clinical resistance can be considered. From a pharmacological point of view aspirin resistance is defined as failure of aspirin to inhibit thromboxane A_2 (TxA_2) production.[56] In activated platelets, COX-1 utilizes arachidonic acid (AA) to produce prostaglandins, which are further converted to TxA_2, a potent platelet activator. The poor response to aspirin resulting in less inhibition of platelet activator, has been associated with new cardiovascular events.[57] There are a few potential mechanisms that could be considered responsible for aspirin resistance. It is possible that genetic variants have several effects on antiplatelet therapy. Two SNPs in complete linkage disequilibrium in the COX-1 gene were described.[58] Healthy volunteers who were heterozygous for the -A842G/C50T haplotype showed greater inhibition of prostaglandin H(2) formation by aspirin than common allele homozygotes.[58] Another study found a relation between aspirin non-response and the –A842G polymorphism of COX-1 and C13254T polymorphism of GPVI, depending on the method of assessment of aspirin response.[59] Other studies showed conflicting results concerning involvement of the COX polymorphisms in the effect of treatment with aspirin.[60-62] Inhibition of factor XIII activation by aspirin was found enhanced in carriers of the Leu34 polymorphism of the factor XIIIA sub-unit, indicating

that patients with this polymorphism might have a greater benefit of low dose aspirin in the reduction of new vascular events than those without this polymorphism.[63]

GPIa/IIa is the major platelet collagen receptor and plays an important role in platelet function as deficiency for this receptor or the presence of inhibitory antibodies directed against it result in impaired haemostasis with bleeding tendency.[64,65] Two linked polymorphisms (C807T and G873A) within the Ia gene (ITGA2) have been described in association with an increased expression of the GPIa/IIa receptor. In particular the T807 and A873 alleles are associated with this increased expression and a high density of these receptors has been associated with thrombotic risk. A recent study found that the T allel of the 807 C/T polymorphism of the GP Ia gene is associated with a higher degree of collagen-induced platelet aggregation in patients undergoing coronary stenting treated with a standard 300 mg clopidogrel loading dose.[66] This suggests that carriers of the T allele may have a more extensive platelet adherence despite the use of antiplatelet treatment, setting these individuals at a higher thrombotic risk. In a sub-study of a trial on the oral GPIIb/IIIa antagonist orbofiban an important interaction between treatment (placebo and orbofiban) and the Pl(A) polymorphism of glycoprotein IIIa for bleeding was found.[67]

Study population

Polymorphisms and haplotypes describing the haemostatic system and influencing antithrombotic drug activity will be identified in a cohort of 1200 patients who had CIAO. Patients originating from the European / Australasian Stroke Prevention in Reversible Ischaemia Trial (ESPRIT)[68] and the Second Manifestations of ARTerial disease (SMART)[69] study will be followed for new ischaemic and haemorrhagic events. The current study is an extension of the POLARIS study, in which the genetic characteristics of patients with or without new vascular events are studied in contrast to this study where a prognostic model is made with this information.[70]

In ESPRIT patients with a TIA or minor ischaemic stroke (Rankin grade ≤ 3) of presumed arterial origin were randomised between oral anticoagulation (INR 2.0-3.0), the combination of dipyridamole (400mg daily) plus aspirin (in any dose between 30-325mg daily) and aspirin only. A total of 3376 patients from more than 10 countries was recruited and this study was completed recently.[68,71,72] For the current study only patients randomised in

the University Medical Center Utrecht (UMC Utrecht), The Netherlands, are included.

The SMART study is a single-centre prospective cohort study among patients, newly referred to the UMC Utrecht with: (1) clinically manifest atherosclerotic vessel disease, or (2) marked risk factors for atherosclerosis. Objectives of SMART are to determine the prevalence of concomitant arterial disease at other sites, and risk factors in patients presenting with a manifestation of arterial disease or vascular risk factor and to study the incidence of future cardiovascular events and its predictors in these high-risk patients.[69] Blood samples are taken at cohort entry. Currently about 700 patients have been enrolled; approximately 850 had cerebrovascular disease at entry.

To complete the dataset for PROMISe additional patients are selected from the Utrecht Stroke Database (USDB) from the UMC Utrecht. In the USDB extensive baseline data have been collected for consecutive patients visiting the UMC Utrecht for TIA or stroke since 1991. Blood samples have been taken from 1999 onwards and are stored in the Neurology Blood Bank. Follow-up data will be collected by contacting these patients or their general practitioners.

Study design

The design of the PROMISe study is twofold. Substudy A will address genetic characteristics (SNPs or haplotypes) that already have been suggested in the literature to be candidates of future risk of ischaemic or haemorrhagic events, either directly, or via interaction with antithrombotic drugs or other environmental factors. Many of the candidate genetic characteristics have not been properly studied in the context of prognosis after cerebral ischaemia. The developments in this field will be closely followed and new promising candidates will be added to the list to be studied. Stroke at baseline is subclassified into small and large vessel disease. The classification of the subtypes is described elsewhere.[6] Subtype adjudication will be blinded to genotype.

In sub-study B two risk models (one for ischaemic and one for haemorrhagic events) will be developed on basis of the extensive baseline data obtained in the 1200-patient-cohort described in sub-study A. Based on the current literature, especially those characteristics known from other studies to be strong predictors of either of the two outcomes will be entered

into two separate risk models. Next to already know non-genetic risk factors like smoking, hypertension, diabetes, history of vascular disease, hypercholesterolaemia, male gender, and a high age, a couple of genetic risk factors already studied several times and found associated with vascular diseases will be taken into account in these models.

Outcome

Primary outcome measures are of two kinds. Firstly ischaemic events defined as vascular death including sudden death, death from stroke (fatal haemorrhage excluded),[73] non fatal ischaemic stroke or non fatal myocardial infarction are of interest. Secondly haemorrhagic events, including fatal haemorrhage, non-fatal intracerebral haemorrhage and any haemorrhage requiring hospitalization will be used.

Outcome events will be adjudicated independently by three physicians blinded to genotype. If the initial classifications do not correspond, consensus will be reached by means of discussion.

Statistical considerations

Polymorphisms and haplotypes describing the haemostatic system and influencing antithrombotic drug activity will be determined in the 1200-patient-cohort. The patient included last will be followed for at least one year; mean follow-up of the cohort will be about 6.5 years (7800 patient years). With an average annual event rate for first ischaemic events of 4% and of 1% for major haemorrhages a total of 312 ischaemic events is expected and 78 major haemorrhages.

On the assumption of a type I error of 5%, a type II error of 20%, a prevalence of a genetic characteristic of 10% in the study cohort, a relative risk of 1.4 for ischaemic events can be estimated with sufficient precision - the 95% confidence interval would range from 1.1 to 1.8. With similar assumptions, a relative risk of 1.8 for haemorrhagic events would yield a 95% confidence interval from 1.0 to 3.2. Separate analyses will be done according to the type of qualifying ischaemic cerebral events (LVD and SVD). Within the cohort about half of the patients is expected to have small vessel disease and the other half large vessel disease. This implies that relationships between genetic characteristics and ischaemic or haemorrhagic outcomes need to be somewhat stronger to be determined with sufficient precision. With the same assumptions used above a relative risk of 1.5 (95% CI 1.0-2.1) for ischaemic events would

be demonstrable among patients with small (or large) vessel disease and of 2.3 (95% CI 1.1-4.8) for haemorrhagic events. The relative risks of ischaemic and haemorrhagic events associated with a genetic characteristic will be assessed by hazard ratios obtained from Cox proportional hazard regression models. Precision of the hazard ratios will be described by means of 95% confidence intervals.

The model for ischaemic events will allow the inclusion of a sufficiently large number of predictors given the expected number of events and the rule of thumb requiring at least ten outcome events for each predictor.[74] Likewise, the model for haemorrhagic events may incorporate a maximum of about 7 predictors given 78 anticipated haemorrhages. For the construction of the prediction models variables with a p-value <0.15 will be included. To adjust for overfitting bootstrapping will be performed, yielding adjusted (shrunk) regression coefficients.[75] With the shrunk coefficients two prediction rules will be made. To determine the additional prognostic value of genetic characteristics for ischaemic and haemorrhagic events the area under the ROC curve of two separate models will be compared: one based on non-genetic risk factors only, the other also including genetic data.

Validation of risk models

On basis of the data of the three trials in patients with CIAO that were coordinated in the department of neurology of the UMCU simulation studies will be done for external validation of the prognostic models. These trials are the Dutch TIA Trial, SPIRIT and ESPRIT.[71,76,77] A total of more than 5000 patients in these trials were treated with aspirin, which until recently was considered to be the standard therapy for secondary prevention after CIAO. In these trials extensive baseline data were recorded, but no data on the genetic characteristics of all patients are available. However, assuming that genetic characteristics found to contribute to the prediction models are distributed according to Mendelian randomisation over the patients, for all patients of the three trials a complete dataset can be generated. With this information prognostic performance of the two prediction rules will be evaluated.

Current status

For the current study only patients randomised in the UMC Utrecht will be included. A total of 870 patients has been included already and their follow-up is known until mid-2006. A total of 1200 patients are needed and

should be followed for at least one year for this study.

Genetic information about other known genetic risk factors for stroke, like inflammation and stress inducing mechanisms will be added in future research.

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Appendix

Addendum on Prognostic Modelling in Ischaemic Stroke Study, Additional Value of Genetic Characteristics.

In 2010 we decided to change the research plans of the PROMISe study importantly, although the rationale of the study, to determine the additional value of genetic information for prediction of outcome after stroke of arterial origin, did not change.[1] With this letter we would like to inform you on the background of this change of plans.

Many articles concerning single nucleotide polymorphisms (SNPs) and their association with ischaemic stroke have been published. The findings of these studies, however, were often not reproducible in larger and different studies rendering the original finding not reliable. Moreover, cerebral ischaemia of arterial origin is a complex disease in which many genetic variants play an important role. Therefore, we decided to determine a broader range of genetic variants and decided to genotype our patients with the ImmunoChip that had become available at reasonable cost in the meantime.[2] On this chip multiple SNPs are plated (~200.000) of which ~2500 originate from the largest genome wide association case control study in ischaemic stroke by the Welcome Trust Case Control Consortium-2 (WTCCC2).[3] These SNPs had shown promising results, but were still under further research for replication at the time of development of the ImmunoChip.

The initial plan was to determine the additional prognostic value of the genetic information with receiver operating characteristic curves; by comparison of a model based on classical risk factors only and one also containing the genetic information. This plan did not change; the only change concerned the amount of genetic information studied.

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7

Additional prognostic value of genetic information in the prediction of vascular events after cerebral ischemia of arterial origin: The PROMISe Study

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In preparation

Structured Abstract

Background. Patients who have suffered from cerebral ischemia have a high risk of recurrent vascular events. Models aimed to estimate this risk are based on classical risk factors and have limited prognostic value. Given that cerebral ischemia has a heritable component, addition of genetic information might improve performance of these risk models. Our aim was to develop and compare two prognostic models: one containing traditional vascular risk factors, the other also including genetic information.

Methods and Results. We studied 1020 patients with cerebral ischemia and genotyped them with the Illumina Immunochip. Median follow-up time was 6.5 years; the annual incidence of new ischemic events (primary outcome, n=198) was 3.0% and of major bleedings (n=55) 0.8%. The prognostic model based on classical vascular risk factors had an area under the receiver operating characteristics curve (AUC-ROC) of 0.65 (95% confidence interval 0.62-0.69). When we added a genetic risk score based on a meta-analysis of genome-wide association data of ischemic stroke, the AUC-ROC remained the same. Similar results were found for the secondary outcomes ischemic stroke and major bleeding.

Conclusions. We found no additional value of genetic information in a prognostic model for the risk of ischemic or hemorrhagic events in patients with cerebral ischemia of arterial origin. Therefore, the genetic component in the etiology of stroke should be explained by a complex, polygenic architecture, where many genes of weak effect likely act in concert to influence the heritable risk of an individual to develop (recurrent) cerebrovascular accidents.

Introduction

Patients who suffered from cerebral ischemia have an increased (long-term) risk of new cerebrovascular and cardiovascular events. The American Heart Association recommends the Framingham risk score as a prediction model for major vascular events.[1, 2] Recently this model was compared with six other models in terms of calibration and discrimination.[3] Almost all models slightly overestimated the risk for major events in low and high risk patients. Addition of genetic information might improve these models, but so far this has not yet been evaluated.

Several studies have been performed with a candidate gene approach to find an association between single nucleotide polymorphisms (SNPs) and first ischemic strokes, with often conflicting results, possibly due to small sample sizes in earlier studies and heterogeneity of the stroke subtypes. Two loci (PITX2 and ZFHX3) were found to be associated with atrial fibrillation and cardioembolic stroke risk.[4, 5] Subsequently, two loci (9p21 and HDAC9) were identified as robust associations with large vessel stroke.[6, 7] These findings were recently confirmed in a meta-analysis of genome-wide association studies (GWAS) carried out by the METASTROKE consortium.[8] These common genetic polymorphisms account only for a small increase in disease risk, suggesting that large sample sizes will be needed to find additional susceptibility alleles.[9]

Associations between genetic polymorphisms and recurrence of vascular events following an initial episode of cerebral ischemia have received little attention. The aim of our study was to assess the additional value of genetic information in prognostic models in a hospital based cohort of patients who have suffered from cerebral ischemia of arterial origin (CIAO). We benefited from continuing efforts in the international stroke genetics community by incorporating results from the METASTROKE study.[8] With the observed effect estimates for selected SNPs (enriched for association with ischemic stroke), we calculated a genotype-based score for each individual in our cohort. Because the data from the GWAS were collected independently from our cohort, we were able to directly and rigorously assess the prognostic value of our prediction models.

Methods

Study design and patient population

The rationale of this study is described elsewhere in detail.[10] We collected data of patients with non-disabling cerebral ischemia of arterial origin, who were referred to the University Medical Center Utrecht, The Netherlands and were included in the SMART (Second Manifestations of Arterial disease) study, or the Utrecht Stroke Database (USDB). A detailed description of the SMART study was published previously.[11] Briefly, patients who gave their written informed consent underwent a standardised vascular screening programme, including a health questionnaire, laboratory assessment, and ultrasonography to investigate the prevalence of additional vascular diseases. Patients were followed up with bi-annual questionnaires. In the USDB extensive baseline data have been collected for consecutive patients visiting the University Medical Center Utrecht for TIA or stroke since 1991. Blood samples were taken from 1999 onwards and stored in the Neurology Blood Bank. Follow-up data were collected by contacting these patients or their general practitioners. The Ethics Committee of the hospital approved both studies. Patients with non-atherosclerotic causes of cerebral ischemia or with potential source of embolism in the heart were excluded from this study. We therefore included only patients with cerebral ischemia of arterial origin.

For the current study, the data of 1125 patients were available. These patients were included between April 1994 and May 2009.

Outcome

The primary outcome event was defined as a composite of the first occurrence of myocardial infarction, ischemic stroke or vascular death not due to hemorrhage. Secondary outcome events were recurrent ischemic stroke and major hemorrhage (Table 1). For potential outcome events reported by the patient we retrieved hospital discharge letters and the results of relevant laboratory and radiology examinations. Three members of the SMART Outcome Committee independently audited events on basis of available information. This committee consisted of physicians from different departments. In case of disagreement, consensus was reached by consulting other members of the Outcome Committee. Potential outcomes in patients included from the USDB were audited similarly.

Genotyping

DNA samples available from both studies and stored in a -70 degrees Celsius freezer were transported to the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands ,where the samples were genotyped with the Illumina Immunochip.[12] The goal of the Immunochip was to provide a cost-effective genotyping platform for deep follow-up replication studies. It includes about 200,000 SNPs, selected based on the association results (low p-values) from a wide range of GWAS of immune-related diseases as well as the diseases covered by the second round of the Wellcome Trust Case Control Consortium (WTCCC-2), including ischemic stroke (http://www.wtccc.org.uk/ccc2/wtccc2_studies.shtml). About 2500 SNPs from an early analysis of the ischemic stroke GWAS by WTCCC-2 were contributed to the Immunochip design. We used these specific SNPs for our further analyses.

Table 1. Definitions of outcome events

Event	*Definition*
Ischemic stroke	Relevant clinical features that caused an increase in impairment of at least one grade on the modified Rankin scale1 associated with a relevant infarction on a repeat brain scan
Myocardial infarction	At least two of the following criteria: 1. Chest pain for at least 20 min, not disappearing after administration of nitrates 2. ST elevation > 1 mm in two following leads or a left bundle branch block on the ECG 3. CK elevation of at least two times the normal value of CK and an MB fraction > 5% of the total CK
Vascular death: not due to hemorrhage	Sudden death: unexpected coronary death occurring within 1 h after onset of symptoms or within 24 h given convincing circumstantial evidence. Terminal heart failure Fatal myocardial infarction or ischemic stroke
Major hemorrhagic event	Intracranial bleeding: intraventricular, intracerebral, epidural, subdural or subarachnoidal bleeding seen on brain imaging. Retinal bleeding: typical complaints, changes with fundoscopy and impaired sight Rupture of an aneurysm of the abdominal aorta Severe extracranial hemorrhage: causing death or requiring intervention or admission to the hospital

Primary endpoint was defined as all fatal and non-fatal ischemic events.
Secondary endpoints were ischemic stroke and major bleeding complication separately.

Quality control steps consisted of filtering of SNPs and individuals with >5% missing data, followed by filtering of SNPs with a minor allele frequency (MAF) <1% or deviation from Hardy-Weinberg equilibrium (HWE; p < 10–6). We then used individual-pairwise identity-by-state estimates to remove (unknown) related and potentially contaminated samples. We pruned SNPs by their pairwise linkage disequilibrium to arrive at a set of independent SNPs. Data processing and quality control filtering were performed in PLINK.[13] Principal components analysis was used to check genetic clustering of all individuals against reference individuals from the HapMap.[14]

Individual genetic risk scores

For each individual patient we calculated a genetic risk score with PLINK.[13] We calculated two different risk scores. The first risk score was based on the observed effects (odds ratios) in our own data (association analysis between SNPs and occurrence of a new ischemic event; genetic risk score I, 1600 SNPs). For the second risk score (genetic risk score II, 1501 SNPs) we used the observed effects independently obtained from the GWAS of ischemic stroke led by the METASTROKE consortium.[8] The number of SNPs varied between the two calculated risk scores because of the different quality control steps in the independent populations (Figure 1). The risk score was calculated for each individual separately and was defined as the sum of the ln(OR) multiplied by the number of risk alleles carried for each SNP considered in a given individual.

Statistical analysis

We used logistic regression in PLINK to assess SNPs for associations with recurrent ischemic or hemorrhagic events. The prediction models were built with Cox regression analyses in SPSS. In univariable analysis we calculated the hazard ratios and corresponding 95% confidence intervals (CI) of different stroke risk factors. We then constructed the prediction model, in which we sequentially entered variables from the patients' history until no remaining candidate variable had a significance level of 0.10 and into which we forced the two genetic risk scores separately. Next we constructed receiver-operator characteristics (ROC) curves to compare the discriminatory performance with its area under the curve (AUC) of the model with and without genetic information. All analyses were done for the primary and secondary outcomes.

Table 2. Baseline characteristics

	Included patients n=1020*		*Excluded patients n=105*
	Patients with primary outcome n=198	*Patients without primary outcome n=822*	
Age (years) (mean, SD)	66 (10)	62 (11)	63 (11)
Male sex	150 (76%)	518 (63%)	68 (65%)
Qualifying diagnosis			
TIA	75 (38%)	283 (34%)	30 (28%)
Stroke	93 (47%)	451 (55%)	66 (62%)
Transiant monocular blindness	24 (12%)	79 (10%)	6 (6%)
Retinal infarction	6 (3%)	9 (1%)	3 (3%)
Subtype diagnosis			
LVD	141 (71%)	552 (67%)	57 (54%)
SVD	57 (29%)	270 (33%)	48 (46%)
History			
Stroke	53 (27%)	161 (20%)	17 (16%)
Carotid surgery	14 (7%)	21 (3%)	3 (3%)
Myocardial infarction	37(19%)	80 (10%)	9 (9%)
Vascular surgery	51 (26%)	119 (15%)	16 (15%)
Hypertension	99 (50%)	391 (48%)	50 (47%)
Diabetes	37 (19%)	106 (13%)	19 (18%)
Hyperlipidemia	60 (30%)	280 (34%)	36 (34%)
Cigarette smoking			
Curentlty	35 (18%)	198 (24%)	28 (26%)
Never or ever	159 (81%)	559 (73%)	71 (67%)
Blood pressure (mm Hg)			
Systolic (mean, SD)	157 (28)	149 (25)	153 (28)
Diastolic (mean, SD)	85 (14)	84 (13)	84 (14)
Glucose (mmol/L) (mean, SD)	6.6 (2.4)	6.4 (1.9)	6.5 (2.0)

* Patients were excluded because of quality concerns

Results

Baseline

Data were available of 1125 patients presenting with transient or non-

disabling manifestations of cerebral or retinal ischemia and with information on genetic variants. Of these, 105 patients had to be excluded from further analysis because of genotyping quality concerns or unexpected relatedness between patients. These patients were similar to the remaining patients with respect to baseline characteristics (Table 2).

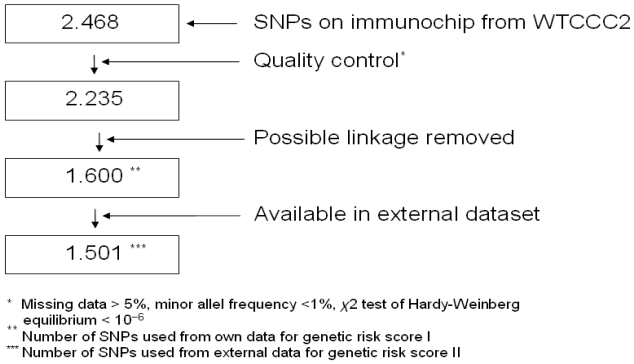

Figure 1. SNP selection

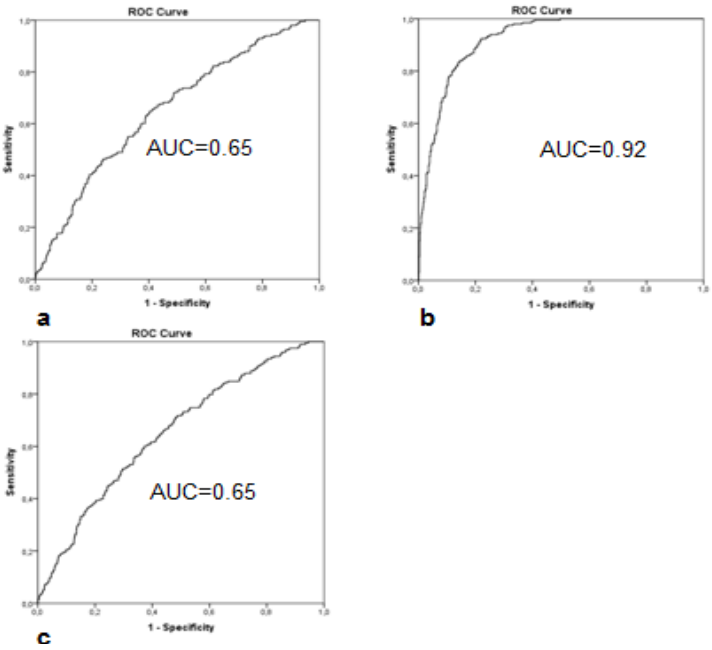

Figure 2. ROC curves primary outcome a) including classical risk factors only; b) classical risk factors plus genetic risk score I; c) classical risk factors plus genetic risk score II.

At baseline patients had a mean age of 63 years and 66% was male (Table 2). The index event was a minor stroke in 53% of the patients, 35% had had a TIA and 12% suffered from an ischemic ocular event. Almost 50% of the patients had hypertension and 20% suffered from an earlier stroke. Patients with a recurrent ischemic vascular event had overall more risk factors for vascular disease than patients who had no recurrence.

Follow up

The median follow-up time was 6.5 years (6630 person-years). The follow-up was complete in 99.5% of the patients. The annual risk of ischemic events was 3.0% (198 events). Half of the events were a fatal or non-fatal ischemic stroke (98
patients), 65 events concerned fatal or non-fatal myocardial infarction and the remaining 35 were other vascular death. The annual risk for major bleeding was 0.8% (55 events). Most of the hemorrhages were in the gastro-intestinal tract.

Prognostic value of classical vascular risk factors

The different AUC-ROC values for the primary and two secondary outcomes are displayed in Table 3 and for the primary outcome the ROC-curves are displayed in Figure 2. For the primary outcome the prognostic model consisted of the variables age, sex, history of stroke, myocardial infarction, intermittent claudication, diabetes mellitus and vascular surgery. The AUC-ROC was 0.65 (95%CI 0.62-0.69). For the secondary outcome, ischemic stroke, the AUC-ROC was 0.61 (95%CI 0.60-0.67), this included the vascular risk factors age, history of stroke, myocardial infarction, and hypertension. For major bleeding the AUC-ROC was 0.69 (95%CI 0.62-76) with the vascular risk factors age, history of stroke, myocardial infarction, hypertension, hypercholesterolemia and vascular surgery.

Prognostic value of classical risk factors and genetic risk score I or II

The vascular risk factors used in these models remained the same as the ones used in the first model. After addition of genetic risk score I the AUC-ROC improved to 0.92 (95%CI 0.91-0.94) for the primary outcome. For ischemic stroke the AUC-ROC improved to 0.78 (95%CI 0.73-0.83) and for major bleedings it became 1.00.

When genetic risk score II was added the AUC-ROC for the primary outcome was essentially identical to that of the model with only classical

vascular risk factors. For cerebral ischemia and hemorrhagic events the AUC-ROC showed similar patterns (Table 3).

Table 3. Cox proportional hazard models and AUC-ROC

	M1		M2		M3	
Indicator	HR	95% CI	HR	95% CI	HR	95% CI
Demographic characteristics						
Male	**1.47**	**1.06-2.04**	1.35	0.86-2.11	1.17	0.65-2.01
Age	**1.05**	**1.03-1.06**	**1.02**	**1.00-1.04**	**1.06**	**1.02-1.08**
History						
Stroke	**1.49**	**1.09-2.04**	**1.50**	**0.97-2.34**	**1.77**	**1.00-3.13**
Angina pectoris	1.14	0.71-1.84	0.88	0.41-1.90	1.37	0.59-3.20
Myocardial infarction	**1.78**	**1.25-2.55**	**1.72**	**1.03-2.87**	**1.91**	**0.99-3.70**
Hypertension	1.08	0.82-1.43	**1.56**	**1.05-2.33**	**2.09**	**1.20-3.56**
Intermittent Claudication	**1.76**	**1.14-2.72**	1.55	0.80-2.97	1.67	0.71-3.90
Diabetes Mellitus	**1.37**	**0.96-1.97**	1.34	0.81-2.24	0.84	0.38-1.86
Hypercholesterolemia	0.77	0.57-1.04	0.91	0.60-1.39	**1.64**	**0.97-2.79**
Smoking	0.98	0.68-1.41	0.98	0.60-1.63	1.06	0.54-2.06
Carotid surgery	1.91	1.11-3.29	0.64	0.77-3.75	1.93	0.70-5.35
Vascular surgery	**1.79**	**1.30-2.46**	1.50	0.93-2.40	**2.39**	**1.35-4.25**
AUC-ROC						
Only classical risk factors	0.65	0.62-0.69	0.61	0.55-0.67	0.69	0.62-0.76
Plus genetic risk score I*	0.92	0.91-0.94	0.78	0.73-0.83	1.00	1.00-1.00
Plus genetic risk core II**	0.65	0.62-0.69	0.61	0.56-0.67	0.70	0.61-0.74

Table displays univariable analyses of risk factors for vascular disease for different endpoints. The bold numbers are accounted in the multivariable model with and without genetic risk scores.
AUC-ROC= Area Under Curve of the Receiver Operating Characteristics Curve
M1= primary outcome
M2= secondary outcome, ischemic stroke
M3= secondary outcome, hemorrhagic event
*Based on regression coefficients calculated in own dataset
**Based on regression coefficients from external data7

Discussion

In our cohort of patients with cerebral ischemia of arterial origin we found no additional value of genetic information in the prediction of new ischemic events or hemorrhages. The overall prognostic performance of the known classical risk factors for vascular diseases is poor (AUC-ROC

0.65;95%CI 0.62-0.69), consistent with similar prediction models described in the literature.[3] The inclusion of a genetic risk score based on SNPs that could be associated with ischemic stroke did not result in a prognostic model that improved risk stratification.

Despite recent progress in the identification of reproducible associations between SNPs and stroke risk, these associated variants explain only a small fraction of the heritability.[4-7] It is still poorly understood to what extent these variants influence recurrent risk.

The major strength of our study is the cohort of well phenotyped ischemic stroke patients from one medical center with uniform follow-up data. The origin from a single hospital could also be seen as a limitation with respect to the generalisability of the result. However, we feel that our cohort is representative since the results for classical risk factors for atherosclerosis affecting prognosis, like previous stroke, diabetes mellitus or hypertension, are consistent with other studies.

Our study also has limitations. We had to drop almost 10% of our population due to quality concerns of the genetic data. These patients, however, had similar clinical characteristics as the remaining patients. Furthermore we included only non-disabled patients on the (predefined) basis that this patient group is the one most relevant, with the highest chance of survival and long-term prospects for recovery, and therefore represent the main focus of our study.

Despite their large number, the selection of the SNPs with their observed effects that were included in our prognostic models might also be considered a limitation of the study. With the addition of a risk score based on our own dataset to the prognostic model we found a major improvement in the prognostic performance. This, however, can be entirely attributed to statistical overfitting of the data, since we used the observed effects in our own population to generate the risk score. In contrast, with external data from the METASTROKE consortium, there was no significant improvement at all.

We were not aware of another dataset on patients with cerebral ischemia of arterial origin followed prospectively for the occurrence of ischemic events or bleedings in whom also genetic data were available. We therefore decided to use the observed effect estimates from the METASTROKE study, even though this study focused on prevalent (ischemic) stroke cases in the population instead of the occurrence of recurrent vascular events. It is certainly possible that genes influencing the risk of a first cerebral ischemic event differ from those genes associated with long-term prognosis after an ischemic event. The selection of

SNPs and the external data set could therefore be seen as a limitation of our study. Our results illustrate the complexity of finding susceptibility alleles for a clinical phenotype that is so complex and heterogeneous such as ischemic stroke.

Platelet function, response to pharmacotherapy and genetics may be associated with outcome and recurrent risk. The debate about the role of aspirin resistance and the way patient tailored treatment strategies could be implemented in daily practice is still ungoing.[15-17] The same is true for other antithrombotic treatment strategies as, for example clopidogrel, for which genetic association with its metabolism is reasonably well understood.[18] For future studies, when more variants have been robustly identified that influence ischemic or hemorrhagic outcome, they might be used to improve the prognostic models. Finally, another limitation of our study is the small sample size, and the lack of subtype information (e.g. small and large vessel disease stroke).

Future stroke genetic studies should take into account that different stroke subtypes exist and that phenotyping is important to determine genetic risk factors in these etiologically different groups of patients.[19] It remains unresolved to what extent genetic variants of modest effect (even if many more are identified as a result of larger discovery efforts) could contribute meaningfully to prognostic models for such complex phenotypes as stroke.

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A common HDAC9 polymorphism is associated with large vessel ischemic stroke: a replication study

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Abstract

Background. Recently a genetic variant in HDAC9 (rs11984041) was found to be associated with ischemic stroke due to large vessel disease (LVD). We aimed to replicate this finding.
Methods. Patients from the Secondary Manifestation of ARTerial disease (SMART) study were genotyped for this single variant. Cases were patients with LVD, controls were all patients without clinically manifest vascular disease. Association analyses were performed with PLINK.
Results. A total of 357 cases en 1902 controls were included in this study. SNP rs11984041 was significantly associated with LVD stroke (OR 1.36; 95%CI;1.06-1.74; p-value 0.01). The association was not significant for all-stroke or small vessel ischaemic stroke.
Conclusions. This study confirms that rs11984041 at the HDAC9 locus is associated with LVD.

Introduction

Cardiovascular and cerebrovascular diseases including myocardial infarction and stroke have the highest mortality worldwide.[1] With increased aging, and expected rise in risk factors for vascular disease such as hypertension, hyperlipidemia and overweight the burden of cardiovascular disease undoubtedly will increase in the coming years. In addition to these classical vascular risk factors a genetic background has been suggested for this devastating disease.[2]

The search for genetic variants that reproducibly predispose to ischemic stroke has been slow and difficult, in part due to clinical heterogeneity of stroke subtypes.[3] The genetic basis of stroke is complex, likely involving the concerted action of many genes, where each gene plays only a modest role. Recently a genome wide association study (GWAS) in ischemic stroke and its subtypes was performed, and a robust association was reported between a common single nucleotide polymorphism (SNP; rs11984041) in the gene encoding histone deacetylase (HDAC9) and large vessel ischemic stroke.[4] In the study we aimed to replicate this finding.

Methods

Study population

Patients aged 18 to 79 years, newly referred to the University Medical Center Utrecht, The Netherlands, with classical risk factors for arterial disease (hypertension, hyperlipidemia, diabetes mellitus) or with symptomatic arterial disease (coronary heart disease, cerebrovascular disease, abdominal aortic aneurysm, or peripheral arterial obstructive disease) were included in the Second Manifestations of ARTerial disease (SMART) study. A detailed description of the study was published previously.[5] The ethical committee of our hospital approved this study and all patients gave written informed consent. These patients were included between September 1996 and March 2010 and were followed biannually. Cases were patients who had an ischemic stroke at baseline or during follow-up. The remaining patients served as controls, excluding all patients with any form of manifest vascular disease including cerebrovascular disease, myocardial infarction, peripheral artery obstructive disease or abdominal aneurysms.

Definitions

We used stroke subtypes defined according to the TOAST criteria.[6] For patients in whom no imaging data were available we used only clinical criteria (signs of cortical dysfunction such as aphasia, neglect or monoparesis for LVD and one of the classical lacunar syndromes for SVD) to classify the stroke subtype. In case clinical information was unclear the etiology of the stroke was classified as undetermined.

Cerebral infarction due to small vessel disease (SVD) was defined as suffering from infarcts of < 15 mm in diameter localized in the deep regions of the brain or in the brain stem with no other apparent cause of ischemic stroke. All other visible infarcts > 15 mm were classified as large vessel disease (LVD) unless another potential cause of stroke was found. The differentiation between SVD and LVD was based on clinical features if imaging was uninformative or unavailable. Motor or sensory deficit of only the face, arm or leg was classified as LVD. Motor or sensory deficits of two or three of these areas without disorders of cognitive functioning were classified as SVD as were patients with an ataxic hemiparesis or a dysarthria-clumsy hand syndrome. Cerebellar syndromes were classified as LVD and brainstem syndromes as SVD. Patients with symptomatic ipsilateral carotid stenosis were classified as LVD independent of their clinical features. Patients with an infarction of cardiac origin of their stroke, patients with more than one possible cause or patients with multiple causes of stroke were included only in the total stroke group.

Data and statistical analysis

Wet-lab genotyping was carried out by KBiosciences, Hertfordshire, UK. (www.kbioscience.co.uk), using their proprietary KASPar technique. Investigators reviewing genotyping data were blinded by patient status. We tested the SNP for missingness, Hardy-Weinberg equilibrium and association using PLINK.[7] Consistent with the reported association, we assumed an additive model and performed logistic regression analysis in PLINK yielding odds ratios and corresponding 95% CIs. We tested the SNP for association with LVD, SVD and all-strokes, using the same set of control individuals in all analyses. In a sensitivity analysis we restricted the analyses to patients who fulfilled the TOAST criteria for stroke subtype classification. Furthermore we corrected for baseline differences in the LVD association analyses (age, sex, hypertension, hyperlipidemia, diabetes mellitus and smoking habits).

Results

Baseline results

We included 608 patients with an ischemic stroke; 357 patients (59%) suffered from LVD and 193 patients (31%) from SVD. A total of 1902 control patients were included. Baseline characteristics are described in Table 1. Compared with the control patients the stroke patients were older and were more often men. LVD and SVD stroke patients were similar according to risk factors for vascular disease, except for the presence of a carotid stenosis, which was more present in LVD patients.

Table 1. Baseline characteristics

	All ischemic stroke (n=608)	*LVD stroke (n=357)*	*SVD stroke (n=193)*	*Control patients (n=1902)*
Mean age in years (mean, SD)	60.6 (10.9)	61.7 (13.0)	58.8 (11.5)	47.2 (13.0)
Male sex	418 (69%)	244 (68%)	136 (71%)	958 (50%)
Imaging available	356 (59%)	208 (58%)	125 (65%)	-
History				
Stroke	335 (55%)	198 (56%)	105 (54%)	0
Carotid surgery	26 (4%)	22 (7%)	2 (1%)	0
Myocardial infarction	93 (15%)	56 (16%)	24 (12%)	0
Cardiac surgery	64 (10%)	36 (10%)	18 (9%)	0
Vascular surgery legs	48 (8%)	27 (8%)	17 (9%)	0
Current smoking	220 (36%)	128 (36%)	79 (41%)	471 (25%)
Hypertension	173 (28%)	105 (29%)	48 (25%)	655 (35%)
Hyperlipidemia (current)	334 (55%)	218 (60%)	83 (43%)	1381 (72%)
Diabetes Mellitus (current)	119 (20%)	70 (20%)	45 (24%)	501 (26%)
BMI (kg/m2) (mean, SD)	26.3 (3.7)	26.2 (3.5)	26.5 (4.1)	27.2 (5.3)

LVD = Large vessel disease, SVD = small vessel disease, SD, BMI = body mass index.

Table 2. Association analyses for rs11984041

	MAF	*Genotype rate (TT/TC/CC)*	*HW p-value*	*Current study OR (95% CI)*	*p-value*	*GWAS OR (95%CI)*
LVD (n = 357)	0.1246	7/75/275	0.4659	1.36 (1.06-1.74)	0.01	1.42 (1.28-1.57)
SVD (n = 193)	0.09067	1/33/159	1	0.95 (0.66-1.37)	0.80	1.13 (1.00-1.28)
All stroke (n = 608)	0.1127	9/119/480	0.5423	1.22 (0.99-1.50)	0.07	-
Controls (n=1902)	0.09464	15/330/1557	0.6883	-	-	-

MAF=minor allel frequency, HW= Hardy Weinberg, LVD=Large vessel disease, SVD=Small vessel disease.

Genetic results

The total genotyping rate of rs11984041 in all individuals of the SMART cohort was 97.6%, and the genotypes were in Hardy-Weinberg equilibrium. The variant was associated with LVD subtype (OR 1.36;95%CI 1.06-1.74; p=0.01) but not with all-stroke nor the SVD subtype (Table 2*).* The sensitivity analyses showed the same results for LVD patients (n=208) OR 1.46 (95%CI 1.07-1.98), SVD patients (n=125) OR 0.88 (95% CI 0.55-1.39) and for all stroke cases (n=356) OR 1.23 (95%CI 0.95-1.59) (Webtable 1). Adjustment for baseline differences did also not alter the results (age and sex adjusted OR 1.34, 95%CI;1.02-1.77).

Discussion

In this study we confirmed an association between the genetic variant rs11954041 in the *HDAC9* gene with LVD. We did not observe a significant association for the SVD subtype or for all-stroke. This is consistent with the initial report of the association for this SNP.[4] The effect size in our study is comparable to that found in the original study (Table 2).

The hospital-based origin of our patients may limit the generalisability of our findings. The differences in baseline characteristics between stroke cases and control subjects can be explained by the selection of individuals without clinically manifest disease. The control subjects showed an elevated prevalence for hyperlipidemia and hypertension and were younger. The association of

rs11954041 with LVD is unlikely to be mediated through hypertension or hyperlipidemia (as an intermediate risk factor), especially considering that this SNP association has not been reported for either trait in large genetic studies.[8,9]

The mechanism by which this common variant increases LVD stroke risk is not clear yet. The functional role of *HDAC9* (as the nearest gene to the SNP) or any other nearby gene is not understood. The association with only large artery stroke and not with other stroke subtypes emphasizes the notion that different stroke phenotypes likely have different pathogenic mechanisms.[10]

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Appendix

Webtable 1. Sensitivity analysis

	All subtyping		*subtyping based on TOAST*	
	Number of cases	*OR (95%CI)*	*Number of cases*	*OR (95%CI)*
LVD	357	1.36 (1.06-1.74)	208	1.46 (1.07-1.98)
SVD	193	0.95 (0.66-1.37)	125	0.88 (0.55-1.39)
All stroke	608	1.22 (0.99-1.50)	356	1.23 (0.95-1.59)

LVD = large vessel disease, SVD = small vessel disease

9

General Discussion

General Discussion

In this chapter I summarise the main results of this thesis and put them into perspective; I discuss strengths and limitations and outline future perspectives for research and clinical practice.

Introduction

Atherosclerosis is a problem of all vessels in the human body. The main aim of the study described in this thesis was to gain more insight in the interplay between atherosclerosis, vascular risk factors and the type of vascular lesion that has been caused by atherosclerosis.

Why atherosclerosis causes problems in the brain in some people and problems in the heart in others is not well known. Due to the extensive non-invasive diagnostic possibilities nowadays better knowledge can be obtained about involvement of other vascular beds in patients presenting with symptoms associated with diseased vessels in one vascular territory or one organ. Secondary preventive therapy for these "silent" atherosclerotic lesions may help in the prevention of major ischaemic events, but the best management for these patients is largely unknown. Different sets of vascular risk factors predispose for different vascular beds to atherosclerosis. Moreover, prognosis concerning new vascular events differs between vascular beds (Chapter 2). Especially the risk for major bleeding is less in patients with coronary artery disease than in patients with cerebral or peripheral artery disease (Chapter 3). Ischaemic stroke of arterial origin is a complex disease that can be caused by small vessel or large vessel disease. These different subtypes of ischaemic stroke have a different prognosis in the long-term (Chapter 4). The best way to collect information about the prognosis of patients with ischaemic disease in the heart is uncertain. Different scales are available. For defining angina pectoris the single question whether the patient ever had had chest pain is performing as well as the whole Rose questionnaire in predicting new cardiac ischaemic events (Chapter 5).

Genetic information may be useful to predict the prognosis of patients with atherosclerosis. Unfortunately, information about the presence of 1500 promising single nucleotide polymorphisms, has no additional value next to classical vascular risk factors in predicting ischaemic events or major bleedings in patients with ischaemic stroke of arterial origin (Chapter 7). The genetic variant HDAC9 is associated with large vessel ischaemic stroke and not with other stroke subtypes (Chapter 8). This observation emphasizes the notion

that different stroke subtypes might have different pathogenesess as was also postulated in Chapter 4.

Differences in atherosclerotic diseases; risk factors and prognosis

The first part of the thesis focused on differences in risk factors and prognosis of atherothrombotic disease in different vascular beds and proper subtyping of cerebral ischaemic disease. With the current diagnostic techniques more and more is known about atherosclerosis and its different clinical manifestations. Studies on secondary preventive treatment after an atherothrombotic event already showed differences in major bleeding risk in patients with different subtypes of cerebrovascular disease (CVD).[1,2] It is well known that patients with atherosclerosis in one vascular bed, are at higher risk of developing atherosclerosis somewhere else in the vascular system.[3,4] Here, I further explore the differences between atherosclerotic diseases with regard to aetiology, prognosis and treatment.

Is there really a difference in atherosclerotic disease?

In the chapters 2 and 3 we focussed on atherosclerotic diseases in different vascular beds. We found that patients with peripheral obstructive artery disease (PAOD) more often smoked and more often suffered from hyperlipidaemia and hypertension. Patients with coronary artery disease (CAD) were more obese. Despite treatment hyperlipidaemia and hypertension were still frequently present in patients with CVD. During follow-up patients with PAOD and patients with CVD had a two times higher risk of new vascular events than those with CAD. The risk of major bleedings was also higher in patients with PAOD and in patients with CVD patients than in patients with CAD. Thus patients with CAD appeared to be different from patients with CVD or POAD. One could speculate that the vessels of the myocardium could be considered as a central vascular system whereas the carotid artery together with the femoral artery can be seen as a more peripheral vascular system and that differences between these two vascular systems might form part of the explanation for the differences that were found.

Treatment of vascular risk factors is very important for the prognosis in different atherosclerotic diseases. Especially for patients with PAOD, who have the highest risk for future cardiovascular events and have even a higher risk for ischaemic coronary events than patients with CAD, treatment of risk factors and possibly supervision by a nurse practitioner is beneficial to alter this high-

risk.[5,6] Also in patients with CVD vascular risk factors including hypertension and hypercholesterolaemia were not treated sufficiently. Concerning secondary preventive treatment, different strategies might be beneficial in this heterogenic group of atherosclerotic patients, especially when taking into account the higher risk of major bleedings in patients with PAOD and CVD compared with patients with CAD. We tried to better understand this difference, but baseline characteristics did not help us. The use of statins or antithrombotic treatment did also not explain the higher risk of major bleeding, neither did haemostatic genetic variants.[7] Possibly, patients with PAOD and CVD have more fragile vessels, which are more prone to rupture together with atherosclerotic disease of this vessel, causing haemorrhagic events.

Ischaemic stroke of arterial origin can be subdivided into cerebral small vessel (SVD) and cerebral large vessel disease (LVD). Whether these subtypes are really different diseases with a different pathogenesis and outcome is still under debate.[8-11] LVD stroke is according to our findings probably more similar to PAOD than is SVD stroke, which is independent of carotid abnormalities.[12] At baseline we found no differences in risk factors for cardiovascular disease between patients with non-disabling cerebral ischemia associated with SVD or LVD. Patients with SVD had an increased risk of recurrent events, especially ischaemic strokes. These differences in long-term prognosis, especially with respect to recurrent stroke, indicate that optimal prevention is also highly indicated for patients with formerly thought 'benign' SVD stroke. New ischaemic strokes mostly remained "true to type", i.e. a patient with an initial SVD stroke more often suffered from a new SVD stroke than from a new LVD stroke. These findings suggest a different pathogenesis in patients with LVD or SVD. Somewhat in contrast with this statement treatment with antithrombotics has been demonstrated equally effective in SVD and LVD stroke prevention.[13] During the follow-up in our study we were not informed about secondary preventive treatment taken by the patients. Compliance with such treatment might be different for patients with relatively mild SVD stroke with minor symptoms, who are possibly less likely to continue their medication compared with the more motivated disabled LVD patients. Encouraging patients to keep on taking their secondary preventive medication remains important, but whether it explains the difference in long-term outcome between SVD and LVD stroke patients remains an unanswered question.

Methodological considerations

All above described studies are mainly based on patients originating from the Secondary Manifestation of ARTerial disease (SMART) study.[14] These patients received optimal medical and, if necessary, surgical treatment, according to guidelines. A limitation of the SMART study is that it has limited generalisability because it is based on a cohort of a single university hospital. However, for most research questions this should not be a major limitation. In our etiologic research (our chapters 2, 3 and 8) this is not a major issue, with comparisons of different vascular patients at a certain time-point. Etiologic research focuses on unraveling the mechanism of the disease for which generalisability is less an issue. However, maybe the patient population presenting with symptoms in a university medical center is different compared with presenting in a general hospital with the same problem. For example possibly patients with more severe or complicated disease will attend the university medical center. To test for this we performed sub-analyses in specific subsets with different severity of the disease in our population and found no different results, this supports the generalisability of our data. For prognostic research (our chapters 4, 5 and 7) the one-hospital based concept could limit the generalisability, especially when the study findings are not validated in another independent dataset to confirm the results. In all our prognostic studies sensitivity analyses in subsets of the study population were performed (or external data were used as in chapter 7) yielding similar results across study strata and suggesting good generalisability. Another limitation of SMART is the lack of information on medication change during follow-up. With all patients originating from one hospital, we assumed that all patients were treated according to the local protocols with the same preventive measurements and advices, so no differences in between patients with the same disease should exist in this respect.

Implications for patient care and future perspectives

Patients with atherosclerotic disease should receive optimal secondary preventive treatment, tailored to the kind of atherosclerotic disease, and should be motivated to continue the use of this medication. Furthermore they should be motivated to keep their risk factors for vascular disease under control. This is especially true for patients with PAOD, who have the worst risk factor profile and the most future events. Modern techniques like the internet could play a role in risk factor management.[15] The reason for the occurrence of more major

bleedings in patients with CVD and PAOD, despite the probably less frequent use of anticoagulant therapy has not become clear from our research. Further research to determine characteristics of patients at risk for major bleeding is warranted to tailor secondary preventive treatment in more detail to the individual patient.

Prognosis and Genetics of Ischaemic Stroke

In the second part of the thesis I focus on genetic information and ischaemic stroke. The additional prognostic effect of genetic information was determined in a cohort of patients with ischaemic stroke of arterial origin (CIAO). At the start of this study we aimed to focus on single SNPs, that had been shown to be associated with ischaemic stroke or atherothrombotic disease as described in chapter 6. However, this initial plan was changed importantly during the subsequent years. Because of conflicting results of the performance of the single SNPs in the literature and the developments in the field of genetic research, we decided to change to a broader view on genetics. We chose to genotype our patients with the ImmunoChip,[16] which contains ~200.000 SNPs, from which ~2500 originate from the largest genome wide association case control study in ischaemic stroke by the Welcome Trust Case Control Consortium (WTCCC2).[17] We hoped to improve our prognostic models with a selection of SNPs that could be associated with ischaemic stroke.

Prognosis of ischaemic stroke: additional information from genetics?

Because of data quality concerns we had to remove about 10% of the data of patients included initially. The characteristics of these excluded patients were similar concerning stroke subtype and vascular risk factor profile as those of the included patients.

The overall prognostic performance of the classical vascular risk factors was poor (AUC-ROC 0.65; 95%CI 0.62-0.69). This performance is not different from models described in the literature.[18] The reason for this poor prognostic performance is not clear. Possibly, the inclusion of dichotomous variables instead of continuous variables worsens its performance, or the proper variables were not included. For example for angina pectoris a diagnostic questionnaire is available, but nothing was known about its performance for the long-term prognosis.[19] For this reason we decided to assess the prognostic performance of the Rose questionnaire. We found that the single question whether a patient ever had had chest pain performed similar in predicting

coronary events in comparison with the whole questionnaire. For this reason a simplified variable could be added to the prognostic models. This variable, however, did not contribute significantly to our prognostic models.

Since external validation of prognostic models is necessary to assess the robustness of a model developed in a single cohort, we looked for ways to replicate our findings. The limited size of our cohort did not allow us to split the data into a discovery and replication set. With the absence of an external dataset with information on prognosis after cerebral ischaemia and genetic information available, we searched for an alternative. To this end we used the available results of a comparison between ischaemic stroke patients and the general population.[17]

During the development of the ImmunoChip ~2500 SNPs were plated on this chip because of promising results of the largest genome wide association study (GWAS) on ischaemic stroke by the WTCCC2.[17] We added the information on these SNPs to the prognostic model in two different ways. First we added genetic information based on our own association data and then found an impressive rise in the area under the receiver operating characteristics curve (from 0.65 with classical vascular risk factors only to 0.92 with adding genetic information). Second, we added the results of the association analyses of ischaemic stroke patients in the general population for the same ~2500 SNPs and found no improvement (AUC-ROC remained 0.65). This last method was performed as an external validation, which was not perfect since the comparisons are somewhat different in our population and the external data population.

Overall we had to conclude that in our cohort of patients with well-defined ischaemic stroke of arterial origins genetic information had no additional value with respect to the prediction of new ischaemic events.

Methodological considerations on prognostic genetic research

The lack of improvement of the model with adding genetic information in our second approach can have several reasons. We might have added genetic information not relevant for the prognosis of patients already presenting with cerebral ischaemia. The selection of SNPs included in our model was based on the results of a GWAS studying the relation of SNPs with ischaemic stroke in the general population, i.e. for first events.

Since literature about genetics and first ischaemic stroke is conflicting and often not reproducible in independent cohorts, knowledge on genetics

and ischaemic stroke is still limited. Possibly future studies can show more robust findings concerning genetic variants and association with ischaemic stroke. Results from large genetic studies might be disappointing because of the heterogeneity of stroke. Multiple subtypes and causes of ischaemic stroke are described, possibly all with different genotypes predisposing for the disease. Subtyping of the broad group of stroke patients is becoming more and more important for future research.[20,21]

Within these different subtypes of stroke possibly gene-environment interactions and classical vascular risk factors might differ as well. This will decrease the number of patients with the same disease, i.e. same subtype of stroke and comparable or known life-style factors and therefore will hamper large scale studies.

One of the interesting points described in our initial study design but not further explored is that pharmacogenetics might explain a part of the risk of ischaemic events or bleedings. The debate about the role of aspirin resistance and the way patient tailored treatment strategies could be implemented in daily practice is still ungoing.[22-24] The same holds true for other antithrombotic treatment strategies such as clopidogrel, for which much is known on the genetic dependent metabolism.[25] In our study these pharmacogenetic variants were not determined. When an association of these variants with ischaemic or bleedings has become clear, they might be used to improve the prognostic models.

Implications for patient care and future perspectives

Unfortunately with the negative result of our study, use of genetic information in the prediction of new vascular events for patients with cerebral ischaemia of arterial origin cannot be recommended. With ungoing research in the field of genetics, more genetic variants can be discovered. Addition of information on these specific variants to models for specific stroke subtypes might improve future models.

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Summary

Summary

Stroke is the second most common cause of death and a leading cause of adult disability worldwide. Of all strokes 80% is an ischaemic stroke. Atherosclerosis or local thrombosis in the carotid or intracranial vessels are the main causes of cerebral ischaemia of arterial origin.

Patients with a recent stroke have an increased long-term risk of cardiovascular events. It is important to identify patients at early risk of recurrent events to guide rapid institution of secondary prevention therapy. In the long term knowledge of prognosis after stroke enables appropriate risk-benefit evaluation. For patients at increased risk of ischaemic events the amount of antithrombotic or oral anticoagulant therapy might better be more intense.

Currently available prognostic models are mainly/primarily designed to estimate the risk of rcurrent events in the early days after stroke and are not useful for long-term predictions. The discriminatory value of these prognostic models is modest with receiver operating characteristics areas under the curves of around 0.7. Addition of stronger predictors or implementation of continuous variables may improve these models.

A strong link between classical risk factors for ischaemic stroke, such as hypertension, diabetes mellitus, hypercholesterolaemia, and genetic factors in the prediction of stroke recurrence has been suggested.

This thesis consists of two major parts. In the first part we clarify the prognosis of patients with different types of vascular disease, we searched for the best way to define angina pectoris in prognostic research and further explored two types of cerebral ischaemia of arterial origin: large and small vessel disease. Part two describes prognostic models with additional genetic determinants.

Part I

Chapter 2 describes a cohort study with long-term follow-up of patients with non-disabling, but clinically manifested vascular disease. Those who presented with cerebrovascular disease (CVD) or peripheral arterial obstructive disease (PAOD) resembled each other more closely than those with coronary artery disease (CAD), both at cardiovascular risk profile at baseline and the events during follow-up. More cardiovascular risk factors (i.e. hypertension,

hyperlipidaemia and cigarette smoking) were found in patients with CVD and PAOD than in those with CAD. Accordingly, but remarkably, during follow-up the patients with PAOD had both a higher death rate and a higher event rate of ischaemic coronary events than patients with CAD. Another striking finding was the relatively high bleeding risk in patients with CVD or PAOD compared with patients with CAD. In *Chapter 3* we describe this difference in bleeding risk in more detail and we tried to find a reason for this. Risk factors for haemorrhagic events (i.e. female sex, kidney dysfunction) were more frequent in the CVD/PAOD patient group compared with CAD patients. However, the higher prevalence of risk factors did not explain the difference, neither did a selection of prothrombotic genetic variants (HR 1.74; 95%CI 1.14-2.61). The reason for the difference in haemorrhagic risk between patients with PAOD/CVD and CAD is not clear and we can only speculate about the origin of our findings. Possibly patients with PAOD and CVD have more fragile small vessels which rupture easily due to atherosclerotic changes. Nevertheless the choice for antiplatelet or oral anticoagulants as secondary preventive therapy should be individualised for each patient.

In *Chapter 4* we tested the Rose Angina Questionnaire and its performance in prognostic research. We tested the whole questionnaire as well as 3 different predefined subsets of this questionnaire. We studied 7916 patients (mean age 56 years; 67% men) with clinically manifest vascular disease or cardiovascular risk factors, enrolled in the Second Manifestations of ARTerial disease (SMART) study. At inclusion all patients completed the Rose questionnaire. All patients were followed for new coronary events and interventions for an average of 4.6 years. Discriminatory ability of the four definitions as assessed with areas under the receiver-operator characteristics curves was similar (range 0.708–0.726) for coronary events in isolation as well as in combination with coronary interventions. We concluded that the use of a subset of questions of the Rose questionnaire performs equally well as the full Rose questionnaire to predict coronary events.

Chapter 5 describes two different phenotypes of ischaemic stroke, cerebral small vessel and large vessel disease (respectively SVD/LVD). We included 971 patients with transient ischaemic attack or non-disabling ischaemic stroke of arterial origin. Classification of SVD/LVD was primarily based on brain imaging. Patients with SVD smoked more often and more often had non-disabling stroke than TIA and per definition no ocular events at inclusion. Carotid disease was hardly ever found in SVD patients. During

a mean follow-up of 6.3 years new vascular events occurred in 56 of 312 SVD patients (3.3%/year) and in 128 of 659 LVD patients (2.9%/year). Ischaemic stroke recurred less in LVD patients (HR 0.60; 95%CI 0.39-0.94, age and sex adjusted). During the first year of follow-up no difference in prognosis was found between the two groups. The long-term follow-up showed a worse prognosis for patients with SVD. This is in contrast to earlier reports in literature. Possibly this finding could be attributed to the inclusion of non-disabled patients. We feel that these patients care most for future and need good secondary preventive treatment. Possibly a different pathogenetic mechanism underlies these two similar looking diseases.

Part II

In *Chapter 6* the background of PROMISe (Prognostic Modelling in Ischaemic Stroke study) is explained. Due to developments in the genetic field of research our initial plan has slightly changed. At first we aimed to perform a candidate gene approach for searching interesting and possibly associated single nucleotide polymorphisms (SNPs) for ischaemic events. We ended up with a chip array study, with a broader view on genetics. The way of analyzing the data with different risk models for both new ischaemic and haemorrhagic events was left unchanged. This change of plans is described in the addendum to the initial design paper.

Chapter 7 describes the different prognostic models with and without genetic information. We included a total of 1020 patients with cerebral ischaemia of arterial origin. These patients were genotyped with the Immunochip. Genetic information based on a selection of 1500 most promising SNPs on the chip showed no additional value to classical vascular risk factors in predicting ischaemic events or major bleedings in patients with ischaemic stroke of arterial origin.

In *Chapter 8* we present the results of a replication study of the HDAC9 genetic variant in large vessel ischaemic stroke. This variant was first described in a genome wide association study by the WTCCC2. We replicated this finding in our own population with an association with large artery ischaemic stroke and not with other stroke subtypes.

Chapter 9 is the general discussion of this thesis. The results are mentioned short and they are put into perspective. Strengths and limitations

are discusses as well as future perspective for research and clinical practice. For each individual patient a decision about secondary preventive measures should be made concerning potential risks and benefits. Especially patients with cerebral small vessel disease ought to be motivated to continue secondary preventive treatment, because of their higher recurrent ischaemic complication rate in long-term. At present, the additional value of genetic information in prognostic models is not clinically relevant. Probably, future studies provide more insight into genetics and ischaemic stroke and this information may be applied to improve prognostic models.

Nederlandse Samenvatting

Samenvatting

Een beroerte is een van de meest voorkomende oorzaken van overlijden en blijvende invaliditeit wereldwijd. Van alle beroerten is 80% een herseninfarct of een TIA (ischemie) en 20% betreft een bloeding in de hersenen. Secundaire preventie na een TIA of een herseninfarct is van groot belang om de kans op toekomstige vasculaire complicaties te verkleinen. Sommige van deze preventieve behandelingen gaan op zichzelf gepaard met een zeker risico, bijvoorbeeld operatieve risico's bij het verwijdering van aderverkalking in de halsvaten of het bloedingsrisico bij intensieve antistollende therapie. In de klinische praktijk moet er regelmatig een afweging worden gemaakt of deze risicovolle ingrepen verantwoord zijn. Hierbij is een nauwkeurige inschatting van de prognose van de individuele patiënt van groot belang. Het discriminerende vermogen van prognostische modellen op basis van klassieke vasculaire risicofactoren, zoals bijvoorbeeld diabetes mellitus, hoge bloeddruk, leeftijd en geslacht is echter beperkt. Toevoegen van nieuwe variabelen in deze modellen zou het discriminerende vermogen kunnen verbeteren.

Individuen met een familielid, die vaatproblemen heeft, hebben zelf een groter risico op het doormaken van vaatziekten zoals ischemie dan mensen zonder deze positieve familieanamnese. Dit suggereert dat er naast de klassieke risicofactoren ook een familiare, genetische component een rol speelt in de ontwikkeling van ischemische complicaties zoals een herseninfarct.

Dit proefschrift bestaat uit twee delen. In het eerste deel verkrijgen we meer duidelijkheid over de prognose na verschillende atherosclerotische ziekten en twee subtypen van het herseninfarct en bekijken we de definiëring van angina pectoris voor prognostisch onderzoek kritisch. Deel twee richt zich op de prognostische modellen. Met behulp van genetische factoren proberen we de tot nu toe matig voorspellende modellen te verbeteren.

Deel I

Hoofdstuk 2 beschrijft een cohort onderzoek van mensen met drie verschillende type atherosclerotische vaataandoeningen; cardiale ischemie, perifeer vaatlijden en cerebrale ischemie. Patiënten met perifeer vaatlijden rookten vaker, hadden vaker suikerziekte en gestoorde glucose-waarden dan de andere patiënten. Hypertensie kwam vaker voor bij patiënten met cerebrale ischemie en patiënten met perifeer vaatlijden. Patiënten met cardiaal lijden hadden meer hypercholesterolemie. Tijdens de mediane follow-up van 4

jaar vonden wij dat patiënten met cerebraal of perifeer vaatlijden een bijna tweemaal grotere kans hadden op het doormaken van nieuwe ischemische complicaties in vergelijking met de patiënten met cardiaal vaatlijden. De meeste complicaties vonden plaats in hetzelfde vaatbed als waar de eerdere vaatproblemen waren, dus patiënten met een doorgemaakte cerebrale ischemie hadden de grootste kans nogmaals cerebrale problemen te krijgen. Grote bloedingscomplicaties kwamen significant minder voor bij patiënten met cardiaal vaatlijden in vergelijking met de andere twee groepen. In *hoofdstuk 3* gaan we dieper in op dit verschil van bloedingsrisico en proberen we hiervoor een verklaring te vinden. Het was weliswaar zo dat risicofactoren voor het krijgen van een bloeding (vrouwelijk geslacht en nierfunctiestoornissen) meer voorkwamen bij patiënten met perifeer of cerebraal vaatlijden; correctie voor deze factoren leverde echter geen verklaring op voor het hogere bloedingsrisico met een hazard ratio 1.74 (95% betrouwbaarheidsinterval (BI) 1.14-2.61). We bestudeerden ook enkele prothrombotische genetische variaties, met als hypothese dat afwezigheid van deze varianten zou kunnen lijden tot een hogere bloedingneiging. In deze varianten bleek ook geen verklaring voor het verschil in bloedingsrisico te zitten. Het blijft gissen waarom dit verschil in bloedingsrisico bestaat. Het is mogelijk dat patiënten met perifeer en cerebraal vaatlijden meer vaatproblemen hebben in de kleinere vaten, die makkelijker aanleiding kunnen geven tot een bloeding. Concluderend kunnen we stellen dat voor iedere atherosclerotische patiënt een individueel risicoschatting moet worden gemaakt alvorens te beslissen hoe en met welke intensiteit die patiënt zijn secundaire preventie moet krijgen.

In *hoofdstuk 4* onderzochten we de prognostisch voorspellende waarde van de Rose angina vragenlijst. Wij vergeleken deze totale vragenlijst, die ontwikkeld is voor het stellen van de diagnose angina pectoris, met verschillende subsets van deze vragenlijst. In een cohort van 7916 patiënten, geïncludeerd in de Secondary Manifestation of ARTerial disease (SMART) studie (gemiddelde leeftijd 56 jaar; 67% mannelijk geslacht), onderzochten we vier definities van angina pectoris die we van te voren hadden vastgesteld. Deze patiënten werden gemiddeld 4.6 jaar gevolgd, waarbij nieuwe cardiale ischemische complicaties werden bijgehouden. We berekenden voor iedere patiënt een individuele risicoscore op basis van de verschillende definities en bekeken of deze score geassocieerd was met het optreden van ischemische cardiale complicaties. Bij het voorspellen van ischemische cardiale complicaties bleek het niet uit te maken of de gehele Rose vragenlijst gebruikt werd of

maar een gedeelte hiervan. Zelfs het beantwoorden van maar één vraag "of er ooit pijn op de borst was geweest" voorspelde even goed als het beantwoorden van de totale vragenlijst. Het gebruik van deze enkele vraag is makkelijker en mogelijk ook betrouwbaarder dan het gebruik van de volledige vragenlijst.

Hoofdstuk 5, daarin worden twee verschillende types van het herseninfarct beschreven; een infarct van de kleine vaten (lacunair infarct) en een infarct van de grote vaten door atherosclerose. Wij keken hierbij alleen naar patiënten met een niet invaliderend herseninfarct (n=971) en richtten ons op de prognostische verschillen van deze twee groepen. Patiënten met een lacunair infarct rookten vaker, hadden vaker een klein herseninfarct dan een TIA en hadden per definitie geen oculaire events in vergelijking met de patiënten met een grote vaten infarct. Symptomatische atherosclerose van de arteria carotis kwam minder vaak voor bij patiënten met een lacunair infarct. In totaal traden er gedurende de gemiddelde follow-up van 6.3 jaar vasculair ischemische complicaties op bij 56 van de 312 lacunaire infarct patiënten (jaarlijks risico: 3.3%/jaar) en bij 128 van de 659 van de patiënten met een grote vaten herseninfarct (jaarlijks risico: 2.9%/jaar). In het eerste jaar van de follow-up was er nauwelijks verschil tussen de beide groepen qua nieuwe complicaties. Op de lange termijn bleek in ons cohort dat de patiënten met een eerder lacunair infarct een slechtere prognose hadden. Dit is in tegenstelling tot wat er tot nu toe beschreven is. Deze bevinding zou kunnen worden verklaard door onze patiëntenselectie van patiënten met een niet-invaliderende herseninfarct. Het wordt echter steeds duidelijker dat patiënten met lacunaire infarcten op de langere termijn meer, vooral cognitieve, problemen hebben dan voorheen gedacht. Dit pleit voor een ander onderliggend mechanisme bij het ontstaan van deze twee subtypen herseninfarcten.

Deel II

In *hoofdstuk 6* beschrijven we de achtergrond van het PROMISe onderzoek (Prognostic Modelling in Ischaemic Stroke). Gezien de snelle ontwikkelingen op het gebied van genetisch onderzoek zijn onze initieel opgestelde ideeën gaandeweg aangepast en zijn we van een kandidaatgen studie naar een chiparray studie overgegaan. De gedachtegang betreffende het hoe te ontwikkelen van de verschillende risicomodellen bleef hierbij echter onveranderd. Deze verandering is nader toegelicht in het addendum.

In *hoofdstuk 7* worden de verschillende prognostische modellen beschreven. We includeerden 1020 patiënten. Genetische informatie, gebaseerd

op 1500 SNPs die het meest veel belovend leken op basis van eerder onderzoek, bleken de voorspellende waarde van de verschillende modellen niet te doen toenemen. De additionele waarden van de genetische informatie voor het voorspellen van nieuwe vasculaire complicaties is nihil en niet klinisch relevant.

Hoofdstuk 8 beschrijft de replicatie van een HDAC9 variant bij patiënten met een grote vaten herseninfarct. Deze bevinding werd eerder beschreven in de grootste genoomwijde associatie studie uitgevoerd door het Welcome Trust Case Control Consortium (WTCC2). Ook in onze populatie van patiënten met een beroerte bleek deze genetische variant geassocieerd met het grote vaten herseninfarct en niet met andere subtypes van het herseninfarct.

In *hoofdstuk 9*, de algemene beschouwingen, worden de resultaten van dit proefschrift en hun implicaties voor de patiëntenzorg en verder wetenschappelijk onderzoek besproken. Voor atherosclerotische ziekte geldt dat goed moet worden nagedacht wat de risico's en voordelen zijn van het starten van secundaire preventieve behandeling. Met name de mensen met een lacunair infarct lijken goed gemotiveerd te moeten worden om de medicatie te continueren, gezien het grotere risico op toekomstige vasculaire complicaties. Het additionele effect van genetische informatie in prognostische modellen lijkt niet klinisch relevant te zijn. Mogelijk dat er in de toekomst meer genetische informatie met betrekking tot het herseninfarct ontdekt zal worden, en dat het toevoegen van deze informatie de prognostische modellen kan verbeteren.

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List of abbreviations

List of abbreviations

ABI	ankel brachial index
AP	angina pectoris
AUC	area under the curve
BMI	body mass index
CABG	coronary artery bypass grafting
CAD	coronary artery disease
CADASIL	cerebreal authosomal-dominant arteriopathy with subcortical infarcts and leucoencephalopathy
CI	confidence interval
CIAO	cerebral ischaemia of arterial origin
CK	creatine kinase
COX	cyclooxygenase
CT	Computed Tomography
CVD	cerebral vascular disease
DNA	desoxyribo nucleic acid
ECG	electrocardiography
GWAS	genome wide association study
HCHWA-D	hereditary cerebral haemorrhages with amyloidosis- Dutch type
HDAC9	histone deacetylase
HR	hazard ratio
HWE	hardy weinberg equilibrium
IMT	intima media thickness
INR	international normalized ratio
LVD	large vessel disease
MAF	minor allel frequency
MD	Medical Docter
MI	Myocardial Infarction
mRS	modified Rankin Scale
NA	not applicable
NRI	net reclassification index
PAD/PAOD	peripheral artery obstructive disease
PCI	percutaneous coronary intervention
PCR	polymerase chain reaction

PROMISe	Prognostic Modelling in Ischaemic Stroke
REACH	Reduction of Atherothrombosis for Continued Health
ROC	Receiver Operating Characteristics
SCS	Symptomatic Carotid Stenosis
SD	Standard Deviation
SMART	Secondary Manifestation of ARTerial disease
SNP	single nucleotide polymorphism
SVD	small vessel disease
TIA	transient ischaemic attack
TOAST	Trial of Org 10172 in Acute Stroke Treatment
UMC	University Medical Center
USDB	Utrecht Stroke Database
WTCCC	Welcome Trust Case Control Consortium

List of publications

List of publications

Achterberg S, Kappelle LJ, de Bakker PIW, Traylor M, Algra A for the SMART study Group and the METASTROKE Group. Additional prognostic value of genetic information in the prediction of vascular events after cerebral ischemia of arterial origin: The PROMISe Study. *In preparation*

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Curriculum Vitae

Sefanja Achterberg werd geboren op 17 april 1981 te Veghel. Na haar eindexamen van het gymnasium aan het Zwijsen college te Veghel begon zij in 1999 met veel enthousiasme aan de studie geneeskunde aan de Katholieke Universiteit van Nijmegen. Al vroeg werd haar interesse voor de neurologie gewekt en in het bijzonder ook voor de wetenschappelijke kant van de geneeskunde. Zij verrichtte onder leiding van dr. Verhagen, internist-oncoloog UMC st. Radboud, een klein onderzoek naar palliatieve sedatie in en om Nijmegen en zette haar eerste stapjes in de neurologie bij dr. A. Kappelle en dr. L. Dorresteijn met het bijwonen van spreekuren en het meedenken op wetenschappelijk gebied. Dit resulteerde uiteindelijk in een wetenschappelijke stage in Uppsala, Zweden, in het laboratorium van dr. A. Östman, alwaar zij basaal geneeskundig onderzoek verrichtte naar therapeutische mogelijkheden tegen de hersentumor glioblastoma multiforme. Als afsluiting van haar co-schappen werd een tropen co-schap gevolgd in Berekum, Ghana, waarna zij in 2006 haar arts-examen behaalde. Na korte werkervaringen op de afdelingen neurologie in het Jeroen Bosch Ziekenhuis te Den Bosch en het Rijnstate Ziekenhuis te Arnhem begon zij in mei 2006 als AGNIO in het Universitair Medisch Centrum te Utrecht op de afdeling neurologie, afdeling C3Oost. De interesse in de wetenschappelijke kant van de geneeskunde bleek ook hier te kunnen worden gevoed en in oktober 2006 startte zij met het onderzoek onder leiding van prof. dr. A. Algra en prof. dr. L.J. Kappelle wat heeft geleid tot dit proefschrift. In 2007 begon ook officieel de klinische opleiding tot neuroloog in het UMC Utrecht (opleiders prof. dr. J van Gijn en prof. dr. J.H.J. Wokke). Gedurende dit gehele traject is zij gelukkig samen met haar vriend Luc Pigmans, samenwonend sinds 2006 te Zeist en in augustus 2012 werd hun dochter Karlijn geboren.

Appendix

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