Subarachnoid Haemorrhage

Subarachnoïdale bloeding

epidemiologische studies betreffende etiologie en uitkomst

(met een samenvatting in het Nederlands)

Proefschrift

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voor Mies

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Chapter 1

General introduction

General aspects of subarachnoid haemorrhage

Epidemiology

Subarachnoid haemorrhage (SAH) is bleeding into the subarachnoid space – the area between the arachnoid membrane and the pia mater surrounding the brain. SAH occurs spontaneously or can be caused by traumatic injury of the head. Spontaneous SAH is caused by rupture of an intracranial aneurysm in 85 percent of cases [1]. In this thesis we consider both spontaneous SAH in general and in several studies aneurysmal SAH specifically.

Reliable knowledge about the risks of (aneurysmal) SAH in different populations will help in planning, screening and prevention strategies and in predicting the prognosis of individual patients. The overall incidence of SAH is in general between 5 and 10 per 100,000 person years. However, in Japan and Finland rates are as high as 20 per 100,000 person years [2]. This can partly be explained by genetic and environmental differences between these and other countries [3, 4]. The incidence increases with age; and from midlife onwards incidence is higher in women than in men [2]. The reasons for this higher incidence in women are not clear, but hormonal factors (including hormonal medication) have been suggested as a possible explanation [5,6].

Diagnosis and Treatment

Sudden headache is the most characteristic symptom of SAH; in approximately 75 percent of patients, the onset is within seconds [7]. Often, the headache is accompanied by nausea and vomiting. Headache from SAH is diffuse and described by patients as by far the most severe headache they have ever had. It is, however, not the severity, but the suddenness of onset, which is the characteristic feature – but a feature that patients often forget to mention because it is the severity of the pain for which they seek medical attention [1]. On admission two-thirds of all patients have depressed consciousness, of whom half are in coma [8]. The patient might regain alertness and orientation or might remain with various degrees of lethargy, confusion, or agitation. An acute confusional state can occur and be misinterpreted as psychological in origin [9, 10]. Neck stiffness is a common symptom, caused by the inflammatory response to blood in the subarachnoid space. Neck stiffness takes a couple of hours to appear and might not develop at all in deeply unconscious patients, or in patients with minor SAH [11]. Therefore, absence of neck stiffness can never exclude the diagnosis of SAH in a patient with sudden headache [1].

The first investigation when SAH is suspected is computed tomography (CT) to visualize the SAH. A second step is to examine the presence and localisation of intracranial aneurysms. This can be done by either digital subtraction angiography (DSA) or CT-angiography (CTA). Unruptured intracranial aneurysms (UIA) are present in approximately 2 percent of the general population [12, 13]. The risk of

rupture of an UIA, leading to an aneurysmal SAH is around 2 percent per year [12]. In case of an aneurysm as cause of the SAH, the aneurysm must be treated to prevent rebleeding. The aim of treatment is to exclude the aneurysm from the blood circulation. That aim can be achieved by placing a clip over the neck of an aneurysm during a neurosurgical procedure, or by filling the aneurysm with a coil during an endovascular procedure [14-16]. Other less frequently used treatment modalities comprise wrapping, stenting, or balloon occlusion [17-19]. Causes of spontaneous SAH from other origins include other vascular lesions, inflammatory and non-inflammatory lesions, tumours, and drug or substance use [1, 20]. Patients in whom no cause is found do not undergo any treatment and usually have an excellent prognosis [21].

Aetiology

Systematic reviews have been performed to present current knowledge on risk factors for SAH in a systematic way [22]. Established modifiable risk factors for SAH are hypertension, smoking, and excessive alcohol intake, all of which more-or-less double the risk [23]. However, their roles in underlying mechanisms of SAH occurrence are poorly understood and are essentially limited to their statistical associations [24]. Another group of modifiable risk factors consists of medication. A wide range of chemical substances has been examined with regard to risk of stroke or SAH [6, 23, 25-28]. However, in several studies it remained unclear whether and to what extent the drugs under study were associated specifically with SAH, as this was not separated out [25, 29]. Other studies led to contradicting results [23, 26]. To address some of the uncertainties from previous studies, we examined in this thesis the association between SAH and three drug classes; statins, antithrombotics, and oral contraceptive pills (OCP).

Statins affect the endothelium through increased nitric oxide (NO) bioavailability [30]. Withdrawal of statins causes a rebound effect leading to increases in vascular event rates beyond that of the absence of prevention alone [27]. This observed rebound effect has been attributed to the inhibition of endothelium NO synthase (eNOS) mediated pathways by statins [27]. Withdrawal of statins is supposed to impair the eNOS mediated tension regulation, which in turn may lead to short term increased stress on the vessel wall and risk of SAH.

Use of antithrombotic agents has been associated with increased risk of intracranial haemorrhage (ICH) [28]. It remains unclear whether and to what extent these antithrombotic agents are associated specifically with SAH, as this is regularly not separated out [25, 29].

Since incidence patterns of SAH differ between men and women in different age categories, hormonal influences have been suggested [2]. Others concluded that OCP use promotes endothelial dysfunction [31].

Genetic factors account for approximately 10 percent of spontaneous SAH [32]. Most research in the genetic field has focussed on genetics of intracranial

aneurysms. Candidate genes for intracranial aneurysms identified thus far include genes coding for elements of the extracellular matrix [33], endothelial cells [34], and cell cycle progression [35]. The exact mechanisms leading to rupture of an intracranial aneurysm remain to be clarified. A plausible explanation is a peak in the arterial blood pressure [1]. Such a peak can be caused by heavy physical exercise (including sexual intercourse) or substance use, e.g. cocaine [36-38]. However, in the majority of SAH cases the exact reason for occurrence at a particular point in time remains unexplained [1].

Outcome

Case-fatality after SAH has been estimated between 20 and 50 percent [39, 40]. The percentage of persons dying before they reach a hospital has been estimated between 10 and 15 percent [41, 42]. In those who survive the first episode of SAH, epilepsy develops after discharge in approximately five percent of patients [43, 44]. Cognitive deficits and psychosocial dysfunction in the first year after SAH are common, even in patients who make a good recovery in terms of self care [45-47]. Although improvement can be expected up to one and a half year after SAH, many former patients experience deficits and reduced quality of life 1 to 2 years after SAH [48].

Aims and outline of this thesis

The work in this thesis was conducted as part of the @neurIST project, a European Commission funded project focusing on integration of knowledge on intracranial aneurysms from molecular level up to population level. One of the overall aims of the project was to develop an individualised prediction model that would allow predicting behaviour of an unruptured aneurysm and outcome of eventual rupture of an aneurysm. To reach this final aim, several intermediate questions have to be solved. In this thesis we address several of these.

The aim of this thesis is to provide answers to the following questions:

1. What is the incidence and case-fatality of (aneurysmal) SAH in the general population?
2. Can we estimate the association between prescribed drugs and the risk of (aneurysmal) SAH?
3. Can we identify new genetic risk loci for intracranial aneurysms?
4. Which patient and aneurysm characteristics predict outcome after aneurysmal SAH?

This introductory chapter gave a high level overview of already available knowledge. We consider it to be desirable to obtain further knowledge on several

aspects of (aneurysmal) SAH. Although much has been written on clipping and coiling of intracranial aneurysms, it remains to be established how these treatment modalities are applied in real life clinical settings, and what the outcome is. In the field of aetiology, systematic research in the association between drugs and (aneurysmal) SAH lacks. The role of genetics also needs to be elucidated more. To finally reach the aim of individualized prediction models, effort has to be put in development of prediction models for various outcomes in different stages of the disease.

In **chapter 2** the occurrence of (aneurysmal) SAH in the general population is examined as well as case-fatality and treatment modalities that were applied. The incidence rate tells us how many SAHs occur per 100,000 persons per year. Why these SAHs occur remains to be established, since the exact mechanisms underlying SAH remain incompletely understood. However, there are indications that external factors -amongst which drug use- play a role in the aetiology of SAH.

Therefore, in **chapter 3** we investigated several risk factors for (aneurysmal) SAH. The most important were drugs whose mode of action was thought to interact with the physiology of blood vessels -more specific: arteries- and therefore may play a role in the aetiology of SAH. Statins are protective for cerebrovascular events and withdrawal of statins causes a rebound effect leading to increases in vascular event rates beyond that of the absence of prevention alone [27]. This observed rebound effect has been attributed to the inhibition of endothelium NOS (eNOS) mediated pathways by statins [27]. Withdrawal of statins is supposed to impair the eNOS mediated tension regulation, which in turn may lead to short term increased stress on the vessel wall and risk of SAH. To investigate this potential mechanism we studied the association of statin withdrawal and SAH in **chapter 3.1**. Antithrombotic agents have been associated with increased risk of intracranial haemorrhage [28, 49]. Since their role in precipitating SAH remains to be elucidated, we studied the relation between SAH risk and the use of antithrombotic agents in **chapter 3.2**. A specific subgroup of the population was under study in **chapter 3.3**; since quite some time use of oral contraceptive pills (OCP) has been associated with vascular pathology, amongst which stroke. Also, hormonal factors have been suggested to explain sex differences in SAH incidence [5, 6]. To investigate whether there was an effect on SAH risk in OCP users, we studied the association in a population of women under 50 years of age. **Chapter 3.4** describes the work of a consortium on genetic factors that are associated with aneurysmal SAH. Genetic factors were investigated by pooling genetic data from patients in several populations including data collected by us from IPCI and Erasmus MC cases and controls.

In **chapter 4** we were interested in factors that actually predict outcome of aneurysmal SAH. Prediction of outcome may support clinical decision making and may provide realistic and evidence based expectations to patients and relatives. Predictions may also be used to classify patients according to prognostic

risk, which may be useful to compare outcome between different patient series, to study treatment results over time, or to stratify patients for randomised clinical trials. We developed a prediction model for outcome of aneurysmal SAH based on clinical information that is already available in an early stage of hospital admission. The prediction model was based on a re-analysis of data from the ISAT study, one of the larger clinical trials in the fields of aneurysmal SAH. In **chapter 4.1** we present a prediction model for 60 day case-fatality in patients with an aneurysmal SAH who were in equipoise with regard to best treatment option. As potential predictors we investigated clinical and neuroradiological characteristics of patient and aneurysm. After model selection and internal validation, we developed a prediction model based on two clinical and two neuroradiological characteristics. To extend the usability of our model we expanded the outcome from 60 day case-fatality to outcome on an ordered clinical outcome scale; the modified Rankin scale. Results of this model development exercise are presented in **chapter 4.2**.

The significance and methodological issues of the studies presented in this thesis will be discussed in **chapter 5**. Findings from this thesis will we summarized in **chapter 6**.

Chapter 2

Incidence, treatment, and case-fatality of subarachnoid haemorrhage in the Netherlands

Abstract

Background*:* Subarachnoid haemorrhage (SAH) is a devastating disorder and in the majority of cases caused by rupture of an intracranial aneurysm. No actual data are available on the incidence of SAH and aneursymal SAH (aSAH) in the Netherlands and little is known about treatment patterns of aSAH. Our purpose was therefore to assess the incidence, treatment patterns, and case-fatality of (a) SAH within the Dutch general population.

Methods*:* Two population based data sources were used for this retrospective cohort study. One was the nationwide hospital discharge registry (LMR). Cases were patients hospitalized for SAH (ICD-9-code 430) in 2001-2005. The second source was IPCI, a medical record database allowing for case validation. Cases were patients with validated (a)SAH in 1996-2006. Incidence, treatment, and case-fatality were assessed.

Results*:* The incidence rate (IR) of SAH was 7.12 per 100,000 PY (95%CI: 6.94-7.31) and increased with age. The IR of aSAH was 3.78 (95%CI: 2.98–4.72). Women had a twofold increased risk of SAH; this difference appeared after the fourth decade. SAH fatality was 30% (95%CI: 29-31%). Of aSAH patients 64% (95%CI: 53-74%) were treated with a clipping procedure, and 26% (95%CI: 17-37%) with coiling.

Conclusion*:* SAH is a rare disease with substantial case-fatality; rates in the Netherlands are similar to other countries. Case-fatality is also similar as well as age and sex patterns in incidence.

Introduction

Non-traumatic subarachnoid haemorrhage (SAH) is a devastating event, with a case-fatality of around 30 percent [1, 40]. Incidence rates have been assessed in many countries and two patterns can be distinguished: countries with high incidence of around 20 per 100,000 person years (PY), such as Finland and Japan, and countries with low incidence of approximately 5-10 per 100,000 PY [2].

Approximately 85 percent of SAH is a result of rupture of an intracranial aneurysm (IA), although it is not clear whether this percentage is the same over different age and sex categories [1]. Causes of spontaneous SAH from other origins include other vascular lesions, inflammatory and non-inflammatory lesions, tumours, and drug or substance use [1, 20].

The diagnosis of SAH is primarily based on CT imaging and lumbar puncture, eventually followed by angiography; not only to identify an aneurysm as potential cause of the haemorrhage, but also to study the anatomical and morphological configuration of the aneurysm in relation to adjoining arteries, which allows optimal treatment selection [1]. Treatment of aneurysmal SAH (aSAH) consists mainly of either neurosurgical clipping or endovascular coiling. Other less frequently used treatment modalities comprise wrapping, stenting, or balloon occlusion [17-19].

Given the fact that no recent data on age and sex specific incidence of SAH and aSAH in the Netherlands are available, we assessed the incidence of both conditions in the general Dutch population. Moreover, we studied case-fatality, and treatment modalities applied to aSAH patients. This was done in two population based databases; a national discharge database and a smaller medical record database which allowed for assessment of the presence of an aneurysm and treatment modality.

Materials and methods

LMR database

Hospital discharge diagnoses were obtained from the national registry of hospital admissions, the National Medical Registration (LMR), containing information on all admissions in general and academic hospitals throughout the Netherlands (base population: approximately 16.5 million subjects). The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) was used to classify hospital admissions in the Netherlands during the study period. Data used include hospital code, patient sex and age, ICD-9-CM coded discharge diagnosis, up to 9 diagnostic or therapeutic procedures (coded according to the LMR Classification of Diagnostic, Therapeutic, and Surgical Acts), and discharge destination ('home', 'old people's home', 'nursing home', or 'died in hospital').

SAH cases were all admissions with a primary discharge diagnoses (ICD-9-CM) 430 (subarachnoid haemorrhage) during the years 2001 through 2005. Case-fatality was defined as dying in the hospital during hospitalization for SAH.

The denominator for the incidence calculation was the annual mid-year population size as obtained from Statistics Netherlands (CBS, accessed through http://www.cbs.nl, as of June 7th, 2010).

IPCI database

The Integrated Primary Care Information (IPCI) database is a general practice research database with electronic medical record data currently comprising more than one million patients throughout the Netherlands. The patient population is representative of the Dutch population regarding age and sex [50]. Details of the database have been described elsewhere [51]. The system complies with the European Union guidelines on the use of medical data for research and has been proven valid for epidemiological studies [51]. The Scientific and Ethical Advisory Group of the IPCI project approved the study (Project No. 07/02). The database allows for validation of disease by requesting additional information from the general practitioner by questionnaire and copies of original specialist letters.

Potential cases of SAH and aSAH were identified from the IPCI database using an extensive narrative search. All cases were validated by manual review of the electronic medical record and subsequently by review of a questionnaire and specialist letters that were obtained from the GP for each case. The validity of the diagnosis was judged by a medical doctor (R.R.) and a neurologist (D.W.J.D. or F.K.). The judgment of the neurologist was decisive. Case-fatality was defined as dying within a period of 30 days after the date of onset of SAH (index date).

The denominator for the incidence calculation was the number of person years in the IPCI database during the study period (January 1996–September 2006).

Statistical analysis

The incidence rate of SAH and aSAH was calculated by dividing the number of incident cases (numerator), by the total number of accrued person years (IPCI) or persons (LMR) in the study population (denominator). Incidence rates (IR) were calculated in age and sex categories. Confidence intervals (95% CI) for each estimate were based on the Poisson distribution. To estimate case-fatality of aSAH in the IPCI database, Kaplan-Meier survival analysis was used. Incidence rates were used to calculate rate ratios of SAH and aSAH between females and males. All analyses were performed using SPSS software version 15.0 (Chicago, Ill., USA).

Results

LMR: SAH

In the period 2001 to 2005 a total of 5,769 patients (64% female) were admitted to Dutch hospitals with discharge diagnosis 'subarachnoid haemorrhage'. The overall nationwide incidence rate of SAH was 7.12 per 100,000 person-year (PY) (95% CI: 6.94-7.31) (Table 1). The incidence rate rapidly increased with age (Table 1, Figure 1). The overall incidence rate ratio (IRR) of SAH for women compared to men was 1.72 (95% CI: 1.63-1.81). This differential risk occurred gradually and was most pronounced in the fourth and fifth decade (Table 1, Figures 1). Case-fatality for SAH during hospitalization was 30 percent (95% CI: 29-31%), and increased with age, but did not differ between males and females (Table 1).

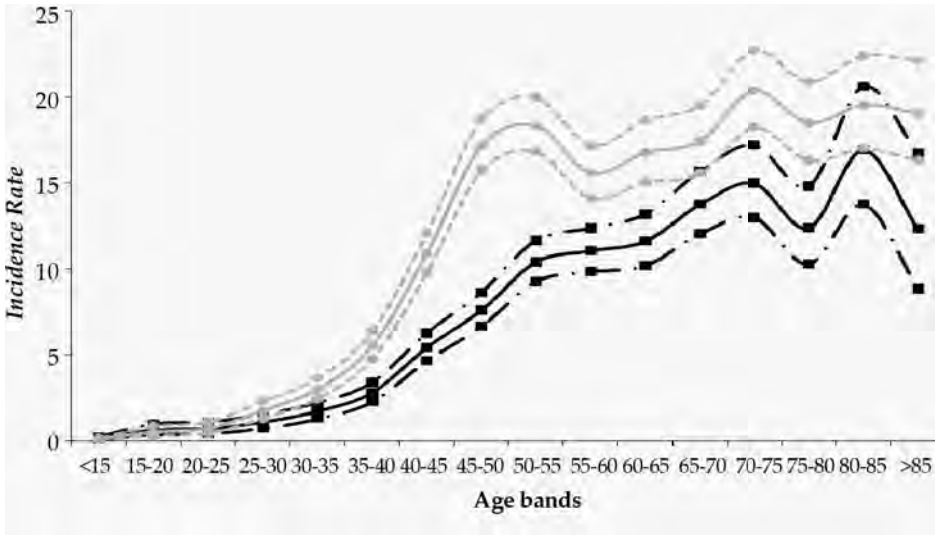

Figure 1. Sex Specific Incidence Rates of SAH in 5 year age bands in LRM data. Solid lines are estimated rates, dashed lines the 95% confidence bands; grey line for women, black line for men.

IPCI database: SAH and aSAH

In the initial source population of 488,118 persons, 107 incident cases of SAH (70% female) were identified after validation (Table 1). Based on these data the observed crude rate was 5.53 per 100,000 PY (95% CI: 4.56-6.66) (Table 1), which translates to a rate of 6.48 per 100,000 PY in the Netherlands after standardization to the Dutch age and sex distribution. The incidence rate increased with age and was similar to the LMR rates up until 64 years; rates were lower after that (Table 1).

Table 1. Incidence rates of (a)SAH by age and sex in the LMR and IPCI databases.

	LMR SAH cases (n)	IR	95% CI	fatality*	IPCI SAH cases (n)	IR	95% CI	fatality§	IPCI aSAH cases (n)	IR	95% CI	fatality§
Age												
< 40	570	1.33	1.23-1.45	19.47	15	1.28	0.75–2.06	20	12	1.02	0.56-1.73	8.3
40 to 64	3305	12.2	11.79-12.62	23.84	69	12.14	9.53–15.3	20.5	49	8.62	6.46-11.3	4.2
65 to 79	1396	16.52	15.67-17.40	37.75	20	12.34	7.78–18.7	36.2	12	7.4	4.04-12.5	9.1
≥ 80	498	18.05	16.51-19.69	58.23	3	9.64	2.67–25.7	100	0	0		
Sex												
Female	3674	8.98	8.69-9.27	31.16	75	7.7	6.10–9.60	29.4	52	5.33	4.03-6.94	7.7
Male	2095	5.23	5.01-5.45	27.26	32	3.34	2.32–4.65	16.5	21	2.19	1.40-3.28	0
Male												
< 40	218	1	0.88-1.14	19.72	3	0.5	0.14-1.34	0	3	0.5	0.14-1.34	0
40 to 64	1218	8.9	8.41-9.41	21.35	21	7.4	4.71-11.09	10	15	5.28	3.09-8.49	0
65 to 79	527	13.81	12.67-15.03	36.05	8	11.78	5.56-22.23	41.7	3	4.42	1.22-11.8	0
≥ 80	132	15.26	12.82-18.03	59.09	0	0			0	0		
Female												
< 40	352	1.68	1.51-1.86	19.32	12	2.09	1.14-3.54	25	9	1.57	0.78-2.86	11.1
40 to 64	2087	15.56	14.91-16.24	25.3	48	16.89	12.60-22.19	25.1	34	12	8.43-16.5	5.9
65 to 79	869	18.74	17.52-20.02	38.78	12	12.75	6.96-21.59	33.3	9	9.56	4.72-17.4	11.1
≥ 80	366	19.32	17.42-21.38	57.92	3	13.65	3.78-36.41	100	0	0		
Total	**5769**	**7.12**	**6.94-7.31**	**29.75**	**107**	**5.53**	**4.56–6.66**	**25.5**	**73**	**3.78**	**2.98-4.72**	**5.6**

* during hospitalization; § within 30 days, using Kaplan-Meier survival analysis.

In 68 percent of all IPCI derived SAH cases (n=73) an aneurysm had been diagnosed (95% CI: 59-76%). The proportion of aneurysms as cause of SAH diminished with age (Figure 2).

The crude observed incidence rate of aSAH was 3.78 per 100,000 PY (95% CI: 2.98-4.72), which would imply a rate of 4.26 per 100,000 for the Dutch population (age and sex standardized). Of the patients with an aneurysm the majority was treated by means of a neurosurgical clipping procedure (64%, 95% CI: 53-74%) and 26 percent (95% CI: 17-37%) by means of endovascular coiling. Five patients (7%) did not receive any treatment because of rapid deterioration and death. In the remaining 3 percent we could not find information on procedures.

Kaplan-Meier survival analysis showed that 26 percent of SAH patients died within 30 days (95% CI: 17-34%) (Table 1). Case-fatality in aSAH patients was 5.6 percent (95% CI: 0.31-10.9) (Table 1). Risks could not be estimated in separate treatment groups due to low numbers.

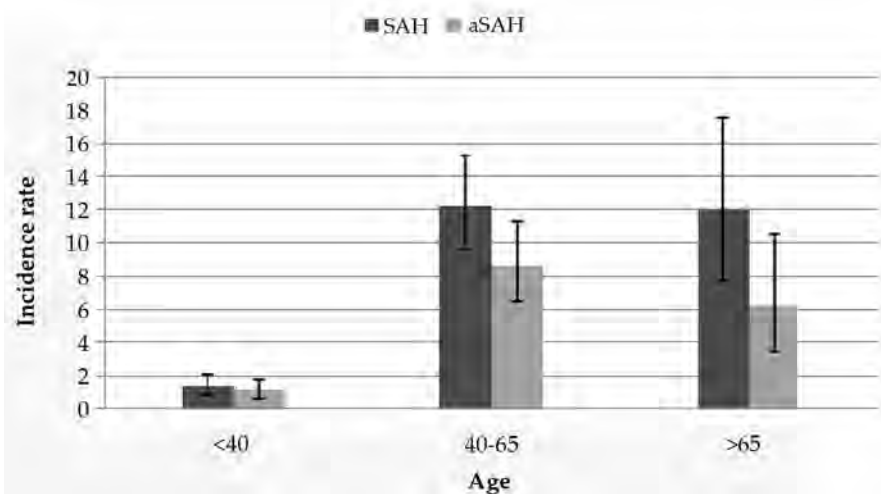

Figure 2. SAH and aSAH incidence rate by age (IPCI database)

Discussion

We used two different population based databases: a hospital discharge database and an electronic medical record database to assess the occurrence, treatment, and case-fatality of SAH and aSAH in the Netherlands. We used both sources to profit from the size in the LMR and the quality of information and validation opportunities in the IPCI database. By using these data we revealed various important observations: first, the crude national incidence rate of SAH was

between 5-7 cases per 100,000 PY, putting the Netherlands in the low incidence countries. Second, about 70 percent of SAHs were of proven aneurysmal origin and this varied slightly by age (lower in high ages). Third, case-fatality of SAH was high: around 26 percent within one month and this increased with age. Fourth, a striking age and gender pattern was observed in the incidence rates. The incidence rates increased rapidly after age 40, but mostly so for women. Fifth, the incidence rates for aSAH increased less rapidly with age than for SAH overall, suggesting a difference in the percentage of aneurysms by age. Sixth, the majority of persons with an aSAH underwent surgical clipping.

Our findings on the rates and case-fatality were similar to previously published population-based studies from other countries. However, often the rates for aSAH are not available. The assumption that 85 percent of SAH is based on aneurysms, may therefore not hold true and certainly not for all age categories. Some of the previous studies have investigated sex specific rates and age-gender interaction, and also reported higher rates in women; however the age dependent change in incidence for women compared to men was reported few times. The reasons for the overall higher incidence in women are not clear, but hormonal factors would be a first logical option [5, 6]. Our finding that the preponderance of women becomes evident around the menopause, during which changes in oestrogen levels take place, further supports this suggestion. Previously, an increase in cardiovascular risk among women after menopause has been recognized [52], for which declining endogenous oestrogen levels have been held responsible [53]. Declining levels of oestrogen might lead to impaired activation of nitric oxide [54], which is hypothesized to be an important factor in the aetiology of SAH through its effects on the vascular endothelium [55, 56].

Being based on observational data the results of our study should be interpreted in the light of potential limitations, such as selection bias and information bias. Selection bias in assessment of rates and case-fatality is negligible in this study since we used population based databases. Selection may have occurred because validation of the discharge diagnoses for the LMR was done only in our hospital. Results of this validation may not be generalizable to all other centres. Thus, the most important limitation is misclassification of the outcome. For a patient to be considered a case in our study, the diagnosis SAH had to be made. Patients who died before reaching medical care were not included in the LMR estimate and it is highly likely that they were also missed in the IPCI database due to lack of a proper diagnosis and specialist information. Previous studies have estimated the percentage of persons dying outside hospitals to be between 11 and 13 percent [41, 42]. This means that the true incidence is potentially 10 percent higher than in our estimations (up to 8 per 100,000 PY). Another potential limitation is the accuracy of the registered diagnosis in the LMR database. Validation of discharge diagnoses in our own hospital showed that 10 percent of the cases were false positive (data not shown). Inclusion of these false positive cases in incidence estimates would

lead to overestimation. In the IPCI database, false positives were unlikely, since cases were validated. In both databases false negative misclassification has not been quantified. We think it is limited in the IPCI database as we applied a very sensitive search on codes and free text to identify potential cases and reviewed all potential cases manually.

Misclassification of mortality was an issue in the LMR database. Since the database only captures data during hospitalizations and is not linked to a death registry, it is not possible to obtain mortality data of patients once they are discharged from the hospital. We therefore chose to report on the mortality during hospital admission only. Nonetheless, the case-fatality is comparable to the case-fatality as estimated from the IPCI database that does capture follow-up and mortality data. This implies that most cases die often immediately and mostly during hospitalization. Case-fatality of aSAH patients is remarkably low and may not represent true fatality of an aneurysmal SAH (Table 1). Severe cases may have died before undergoing imaging; in that case an aneurysm could not be proven. Less severe cases will probably have survived the 30 day period.

The strength of this study is that two separate databases were used to address not only SAH but also aSAH. Both are observational and our study showed that they can be used complementary. Discharge databases are large which allows for fine stratification, but medical record databases allow for depth and more clinical insight. Together they have provided thorough insight in the occurrence, case-fatality, and treatment of (a)SAH.

In conclusion, in this study we showed SAH incidence in the Netherlands is in the range of the low-incidence countries. We demonstrated that the incidence for both SAH and the subgroup of aSAH depends highly on age and sex but the patterns for aSAH might be slightly different than for SAH overall.

Table 2. Published incidence rates of SAH, overall and in sex strata.

		All		Female		male		All	
1st author	**Year**	**IR**	**95% CI**	**IR**	**95% CI**	**IR**	**95% CI**	**case-fatality**	**95% CI**
De Rooij [2]	2007	9.1	8.8-9.5	–	–	–	–	–	–
	2007*	10.5	9.9-11.2	11.5	10.6-12.6	9.2	8.4-10.2	–	–
Benatru [39]	2006	2.12§¶	1.04–3.21	–	–	–	–	26.1†	10.6–55.5
Feigin [57]	2006	10	8.0-12.0	10	7.0-13	10	7.0-13	–	–
Jiang [58]	2006	1.6††	0.8-4.1	1.5††	0.6–6.7	1.6††	0.6–6.5	–	–
Johansen [59]	2006	9	–	10	–	6	–	27.5†	25.5-29.5**
Labovitz [60]	2006	9.7	7.5-12.0	10.4	–	9	–	26.0†	16-40**
Engberg [61]	2007	13.6	–	–	–	–	–	22.7‡	19.8-25.8**
Kozak [62]	2007	27	26-28	33	32-35	20	19-21	26.7	25.2-28.3
Smeeton [63]	2007	6¶¶	5.0-8.0	–	–	–	–	–	–
Ishikawa [64]	2008	–	–	54.4	–	25.6	–	–	–
Islam [65]	2008	7§	4.0-12.0	7	3.0–15	7	3.0–16	25†	0.5–49.5
Koffijberg [42]	2008	12.4	12.2-12.6	14.4	14.2-14.7	10.3	12.2-12.6	31.7†	30.9-32.5**
Vaartjes [66]	2008	7.9**	7.4-8.3**	9.9	9.2–10	5.7	5.2–6.3	–	–
EROS [67]	2009	–	–	3.3¶	0.7-9.2	4.8¶	1.5-11.4	–	–
Sridharan [68]	2009	4.2#	2.2-6.1	–	–	–	–	–	–

*Subset of 18 studies, reporting incidences for men and women separately. † Case-fatality within 28 days. ‡ Case-fatality within 14 days. § Most recent period only. ¶ Standardized to EU population. †† Beijing population only. ** Calculated from data in article. ¶¶ White population only. # Age adjusted.

Chapter 3

Risk

3.1 Withdrawal of statins and risk of subarachnoid haemorrhage

Background and purpose*:* Vascular endothelium, which can be affected by statins, is believed to play a substantial role in subarachnoid haemorrhage (SAH). Our objective was to estimate the association between use and withdrawal of statins and the risk of SAH.
Methods*:* We conducted a population-based case-control study within the PHARMO database. A case was defined as a person hospitalised for SAH (ICD-9-CM code '430') in the period 01.01.98-12.31.06. Ten randomly chosen controls were matched to each case on age, gender, and calendar date.
Results*:* During the study period 1004 incident cases of SAH were identified. Current use of statins did not significantly decrease the risk of SAH (OR=0.77, 95%CI 0.55-1.07). The odds ratio for recent withdrawal compared to none users was 1.62 (95%CI 0.96-2.73). Compared to current use, recent withdrawal was associated with an increased risk of SAH (OR=2.34, 95%CI 1.35-4.05). Interaction analysis showed that the effect of statin withdrawal was highest in patients who had also recently stopped anti-hypertensive drugs (OR=6.77, 95%CI 2.10-21.8).
Conclusions*:* Current use of statins seems to lower the risk of SAH, although the reduction was not significant in new users. Statin withdrawal increased the risk of SAH by a factor 2, even more in patients who had also recently stopped their anti-hypertensive treatment.

Introduction

Subarachnoid haemorrhage (SAH) is a severe neurological disorder [1]. Modifiable risk factors for SAH are hypertension, smoking, and excessive alcohol intake [1]. Drugs may comprise another class of risk factors, but their role in SAH aetiology has not been systematically evaluated. Considering the potential role of vascular endothelium in SAH [55], drugs affecting the endothelium might be expected to alter SAH risk. Statins affect the endothelium through increased nitric oxide bioavailability and have been shown to ameliorate SAH-induced vasospasm [30, 69]. Although statins decrease cerebrovascular event rates during use, *withdrawal* of statins causes a rebound effect leading to increases in vascular event rates beyond that of the absence of prevention alone [27]. This observed rebound effect has been attributed to the inhibition of endothelium NOS (eNOS) mediated pathways by statins [27]. Withdrawal of statins is supposed to impair the eNOS mediated tension regulation, which in turn may lead to short term increased stress on the vessel wall and risk of SAH.

We hypothesized that statins influence the risk of SAH during use (*decrease*) and shortly after withdrawal (*increase*). To examine these hypotheses we conducted a population-based case-control study in the general Dutch population.

Patients and methods

Setting

Data were obtained from PHARMO Record Linkage System (RLS), a population based system with dispensing data and hospitalizations regarding a well-enumerated population and follow-up. For a detailed description of the database we refer to previous work [70].

The source population included all persons in the PHARMO RLS database who had at least three years of valid database history prior to the index date (see below). Persons were followed from inception or start of the study period (January 1st, 1998) until transferral out of the database, death, diagnosis of SAH, or the end of the study period (December 31st, 2006).

Cases and controls

Study outcome was hospitalisation for a first SAH, as recorded in the Dutch National Medical Registration (LMR) with ICD-9-CM code '430'. To assess the positive predictive value (PPV) of the LMR information discharge letters of 42 patients were evaluated.

For each case 10 controls were obtained from the source population and matched to the case on year of birth, gender, and index date (ID).

Drug exposure and covariates

The drugs of primary interest were statins. Use was classified as 'no', 'current' (use at ID), 'recent withdrawal' (stopping up to 30 days before ID), and 'past' (stopping > 30 days before ID). To inspect protopathic bias we looked at 'recent starters' (current use less than 30 days), to limit prevalent user bias (healthy users) current use was restricted to short term use (< 3 years).

We considered diabetes mellitus (DM), a history of vascular events, and hypertension as covariates. Use of insulin without a history of use of oral anti-diabetic agents was considered a proxy for having DM type I. Use of oral anti-diabetic agents either or not followed by use of insulin was considered a proxy for having DM type II. A history of vascular events was defined by use of two or more prescriptions of nitrates, or of either aspirin or clopidogrel or hospitalisation for ischemic cardio- or cerebrovascular events (CVD). Past use of antihypertensives was considered a proxy for uncontrolled hypertension.

All drugs with potential effect on the eNOS pathway were considered as co-variates (table 1). We also studied agents known for increased bleeding risk, as well as NSAIDs, coxibs, tricyclic antidepressants (TCA), benzodiazepines, antipsychotics, anti-epileptic drugs, female sex hormones, and antibiotics.

Statistical analysis

Conditional logistic regression analysis was used for matched case-control data to estimate odds ratios (OR) and 95% confidence intervals (CI) for SAH associated with exposure to drugs. Analyses were performed with reference to 'no use', and subsequently to 'current use' to better adjust for confounding by indication. In the adjusted model we included all factors that changed the effect estimate with more than 5%.

Sensitivity analyses were conducted on time intervals for recent withdrawal, information bias due to hospitalization (patients hospitalized in days before) and new users to avoid a healthy user bias. Effect modification was investigated for gender, a history of ischemic CVD, and use of antihypertensives.

Association measures for products with exposure in less than 3 cases or 3 controls are not reported.

Table 1. Characteristics of the study population.

	Cases		*Controls*		*OR*	*95% CI*
	No	*%*	*No*	*%*		
Total	**1004**	**100**	**10033**	**100**		
Mean age (SD) (years)	58 (14)		58 (14)		Matched	
<50 years	293		2928			
50-65 years	383		3828			
>65 years	328		3277			
Gender, female	670	67	6695	67	Matched	
Diabetes mellitus I	12	1.2	84	0.8	1.42	0.77-2.62
Diabetes mellitus II	57	5.7	649	6.5	0.87	0.66-1.16
History of CV events	152	15.1	1254	12.5	**1.30**	**1.07-1.59**
Anti-hypertensive drug use[1]						
Current use	254	25.3	2560	25.5	1.05	0.89-1.25
Recent withdrawal	20	2	231	2.3	0.91	0.57-1.46
Past use	152	15.1	1197	11.9	**1.34**	**1.10-1.62**
Current use of medication with increased bleeding risk[2]						
Vitamin K antagonists	39	3.9	283	2.8	**1.43**	**1.01-2.03**
Platelet aggregation inhibitors	111	11	1032	10	1.18	0.94-1.48
Heparin	13	1.3	96	1	1.35	0.75-2.41
SSRIs	32	3.2	335	3.3	0.96	0.67-1.40
NSAID	80	8	357	3.6	**2.34**	**1.77-3.09**
Use for > 30 days	37	3.7	297	3	1.3	0.90-1.87
COX-2 selective inhibitors	13	1.3	56	0.6	**2.35**	**1.27-4.36**
Use for > 30 days	7	0.7	43	0.4	1.65	0.74-3.70
Current use of other drugs						
TCAs	10	1	117	1.2	0.85	0.44-1.64
Benzodiazepines (BZD)	102	10.2	764	7.6	**1.43**	**1.13-1.80**
BZD > 30 days	74	7.4	740	7.4	1.04	0.80-1.36
Antipsychotics	10	1	119	1.2	0.83	0.44-1.59
Anti-epileptics	15	1.5	121	1.2	1.26	0.73-2.17
Estrogens	15	1.5	142	1.4	1.05	0.61-1.80
Progesterone	3	0.3	68	0.7	0.42	0.13-1.35
Combinations of estrogens and progestins	77	7.7	676	6.7	1.29	0.96-1.74
Antibiotics	19	1.9	145	1.4	1.36	0.83-2.23

	Cases		*Controls*		*OR*	*95% CI*
	No	*%*	*No*	*%*		
Total	**1004**	**100**	**10033**	**100**		
Use of medication affecting endothelium						
Anti-migraine						
Current use	6	0.6	23	0.2	**2.69**	**1.09-6.60**
Use > 30 days	2	0.2	20	0.2	-	-
Recent withdrawal	7	0.7	46	0.5	1.58	0.71-3.53
Past use	59	5.9	406	4.0	**1.50**	**1.13-1.98**
No use	932	92.8	9558	95.3	Ref.	
Systemic Corticosteroids						
Current use	14	1.4	112	1.1	1.24	0.70-2.17
Recent withdrawal	3	0.3	79	0.8	0.38	0.12-1.19
Past use	182	18.1	1872	18.7	0.96	0.81-1.14
No use	805	80.2	7970	79.4	Ref.	
Nitrates[2]						
Current use	28	2.8	264	2.6	1.10	0.74-1.66
Recent withdrawal	3	0.3	41	0.4	0.75	0.23-2.44
Past use	85	8.5	707	7.0	1.24	0.97-1.59
No use	888	88.4	9021	89.9	Ref.	
β-blockers						
Current use	117	11.7	1280	12.8	0.93	0.75-1.14
Recent withdrawal	12	1.2	139	1.4	0.88	0.48-1.59
Past use	135	13.4	1114	11.1	**1.23**	**1.01-1.50**
No use	740	73.7	7500	74.8	Ref.	
Calcium channel blockers						
Current use	56	5.6	569	5.7	1.02	0.76-1.36
Recent withdrawal	6	0.6	60	0.6	1.03	0.44-2.40
Past use	74	7.4	563	5.6	**1.35**	**1.05-1.75**
No use	868	86.5	8841	88.1	Ref.	
RAS drugs						
Current use	126	12.5	1168	11.6	1.14	0.93-1.40
Recent withdrawal	10	1	123	1.2	0.86	0.45-1.65
Past use	66	6.6	422	4.2	**1.65**	**1.25-2.17**
No use	802	79.9	8320	82.9	Ref.	

[1] compared to no use
[2] current use compared to no use

Results

Our study population comprised 1004 cases of SAH and 10,033 matched controls. The PPV of the discharge diagnosis was 88% (95%CI 75%-95%). The PPV did not differ significantly (p=0.26) between statin exposed and unexposed cases.

Past use of antihypertensives was associated with an increased risk of SAH (table 1). NSAIDs, coxibs, and benzodiazepines increased the risk; however, exclusion of recent starters weakened the effect, which points to protopathic bias. Current use of statins was associated with a reduction in SAH risk, but this effect was not statistically significant when restricting to new users (table 2). Exclusion of patients hospitalized in the 30 days prior further attenuated the effect (OR=0.79, 95%CI .56-1.12).

Compared to no use recent withdrawal of statins was associated with a non-significant increased risk of SAH (table 2). Recent withdrawal of statins was associated with a 2-fold increase in risk when compared to current users of statins: $OR_{adjusted}$ =2.34 (95%CI 1.35-4.05) (table 2), this remained when current users were restricted to new users (OR=2.23, 95% CI 1.24-4.02) (not shown). The effect of withdrawal was most outspoken after withdrawal of atorvastatin. Sensitivity analyses on different risk windows of withdrawal showed that the association measure is highest when withdrawal is closest to the index date, i.e. persons who stopped more recently.

Stratification by gender did not reveal significant differences, although women seem to be more susceptible to statin withdrawal (table 3). A history of CVD did not modify the association, although the effects of current use and withdrawal were stronger in patients with CVD (table 3). Use of antihypertensives modified the effect of statins; in persons who stopped statins and antihypertensives the risk of SAH was more than six-fold increased compared to current users of both statins and antihypertensives (table 3).

Discussion

In this study we showed that statins are associated with the risk of SAH. Whereas current use of statins may protect against SAH, withdrawal is associated with a clear increase in risk, especially if patients have also stopped antihypertensive drugs. The observed results support our hypothesis that statins have an effect on vascular endothelium. An important finding is the interaction between stopping of statins and having stopped use of antihypertensives, especially since statins and antihypertensives are often prescribed concomitantly and non-adherence frequently affects both types of drugs [71]. The apparent protective effect during current use of statins requires confirmation in additional studies with richer clinical details as the results were susceptible to analyses aiming to evaluate healthy user bias.

Results of this study should be seen in the light of limitations inherent to observational studies with non-random treatment-assignment. Information bias may occur on the level of outcome or exposure. Despite errors in exposure assessment due to non-compliance, information bias is likely to be non-differential since data were gathered prospectively without knowledge of the hypothesis studied and withdrawal effects were not observed for other non-CNS and non-endothelium affecting drugs [72, 73]. Misclassification of exposure due to hospitalization prior to the index date was ignorable.

Table 2. Statin use and the association with SAH.

	Cases N	*%*	*Controls N*	*%*	*$OR_{unadjusted}$*	*95% CI*	*$OR_{adjusted}$*	*95% CI*
Statin use with reference to no use								
Current use[†]	82	8.2	976	9.7	0.84	0.66-1.07	**0.71**	**0.54-0.93**
Simvastatin	38	3.8	412	4.1	0.92	0.65-1.29	0.79	0.55-1.13
Pravastatin	12	1.2	182	1.8	0.66	0.36-1.19	**0.53**	**0.29-0.97**
Fluvastatin	2	0.2	34	0.3	–			
Atorvastatin	25	2.5	287	2.9	0.87	0.57-1.32	0.73	0.47-1.13
Cerivastatin	0	0.0	4	0.0	–			
Rosuvastatin	5	0.5	57	0.6	0.87	0.35-2.20	0.76	0.30-1.92
New users only (< 3 years)[$]	46	4.6	503	5.0	0.91	0.66-1.24	0.77	0.55-1.07
Recent withdrawal[†]	19	1.9	103	1.0	**1.83**	**1.12-3.01**	1.62	0.96-2.73
Past use[†]	32	3.2	263	2.6	1.21	0.83-1.76	0.96	0.65-1.42
No use[†]	871	87	8691	87	Ref.		Ref.*	
Statin withdrawal with reference to current use of statins								
Current use	82	8.2	976	9.7	Ref.		Ref.[§]	
Recent withdrawal	19	1.9	103	1.0	**2.19**	**1.28-3.75**	**2.34**	**1.35-4.05**
Simvastatin	5	0.5	41	0.4	1.44	0.56-3.73	1.51	0.58-3.92
Pravastatin	4	0.4	19	0.2	2.52	0.83-7.69	2.54	0.83-7.77
Fluvastatin	1	0.1	1	0.0	–			
Atorvastatin	7	0.7	32	0.3	**2.60**	**1.12-6.06**	**2.88**	**1.22-6.80**
Cerivastatin	0	0	0	0.0	–			
Rosuvastatin	2	0.2	10	0.1	–		–	
Past use	32	3.2	263	2.6	1.45	0.94-2.23	1.42	0.92-2.20
No use	871	87	8691	87.0	1.20	0.94-1.53	1.23	0.95-1.58

[†] 'current use' = use at index date; 'recent withdrawal' = stopping up to 30 days before index date; 'past use' = cessation of drug use more than 30 days before index date; 'no use' = drug is never used. [$] Statins are currently used shorter, resp. longer than three years. *Adjusted for having a history of cardio- or cerebrovascular disease and use of platelet aggregation inhibitors, nitrates, and RAS medication. [§] Adjusted for hypertension.

Table 3. Interaction between use of statins and antihypertensives, prior CVD or gender on the association with SAH

statin use	*Effect modifier*	*Cases n (%)*	*Controls n (%)*	*Odds ratio (95% CI)*
	use of antihypertensive medication			
Current use	Current use	60 (6.0)	683 (6.8)	Ref.*
Recent withdrawal	Current use	6 (0.6)	40 (0.4)	1.75 (0.71-4.33)
Current use	Recent withdrawal	1(0.1)	16 (0.2)	–
Recent withdrawal	Recent withdrawal	5 (0.5)	27 (0.3)	2.37 (0.77-7.29)
Current use	Past use	5 (0.5)	89 (0.9)	0.70 (0.27-1.82)
Recent withdrawal	Past use	5 (0.5)	8 (0.1)	**6.77 (2.10-21.8)**
	Prior CVD			
No use	No CVD	788 (92.5)	8088 (92.1)	–
Current use	No CVD	39 (4.6)	472 (5.4)	0.76 (0.53-1.09)#
Recent withdrawal	No CVD	10 (1.2)	60 (0.7)	1.76 (0.64-4.82)**
No use	CVD	83 (54.6)	603 (48.1)	–
Current use	CVD	43 (28.3)	504 (40.2)	0.64 (0.37-1.12)#
Recent withdrawal	CVD	9 (5.9)	43 (3.4)	(0.98-4.49)**
	Gender			
No use	Male	286 (85.6)	2752 (82.4)	–
Current use	Male	33(9.9)	445 (13.3)	0.67 (0.43-1.05)#
Recent withdrawal	Male	6 (1.8)	45 (1.3)	1.82 (0.70-4.73)**
No use	Female	585 (87.3)	5939 (88.7)	–
Current use	Female	49 (7.3)	531 (7.9)	0.77 (0.55-1.08) #
Recent withdrawal	Female	13 (1.9)	58 (0.9)	**2.69 (1.36-5.31)** **

* reference is current use of statins and current use of antihypertensives for all categories, adjusted for use of platelet aggregation inhibitors, RAS drugs and nitrates. **compared to current use and adjusted for use of hypertensive drugs. #compared to no use and adjusted for use of platelet aggregation inhibitors, RAS drugs and nitrates

Misclassification of the outcome was a concern in our study as there is no information on the overall PPV of the discharge diagnosis. We demonstrated that the PPV was quite good and unrelated to exposure. Since we could not validate all cases, outcome misclassification due to inclusion of false positives is a fact but limited and non-differential, resulting in an attenuation of the association. SAHs that were fatal before reaching the hospital were not included in our study because of the nature of the hospitalisation database. Therefore the results cannot be generalized to these types of fatal SAH. Confounding by indication might play a role in studying relations between statins and SAH, especially when

evaluating potential beneficial effects. For the withdrawal analysis we addressed confounding by indication by comparing withdrawers to current users instead of no users. For current use of statins we conducted several analyses to inspect healthy user effects: exclusion of prevalent users and stratification for primary/secondary prevention. Healthy user bias may explain at least part of the association between current use of statins and SAH. Hypertension is a known risk factor for SAH. In this study we did not have information on actual blood pressure; we took stopping antihypertensives as proxy. Recent studies show that non-adherence with antihypertensives is indeed associated with increased systolic blood pressure [74], which supports the assumption that patients who stopped have uncontrolled blood pressure.

The observation that the withdrawal effect is specific for statins, and does not occur in other drugs, encourages us to think of it as a true association; were the withdrawal effect due to reversed protopathic bias or immeasurable time bias, the ORs would have increased for other drugs.

In conclusion, we found that statins are associated with SAH, the risk increases substantially in patients who stop their statins and this effect was particularly pronounced in patients who had also stopped anti-hypertensive drug use.

3.2 Platelet aggregation inhibitors, vitamin K antagonists, and risk of subarachnoid haemorrhage

Background*:* Use of platelet aggregation inhibitors and vitamin K antagonists has been associated with an increased risk of intracranial haemorrhage (ICH). Whether use of these antithrombotic drugs is associated with an increased risk of subarachnoid haemorrhage (SAH) remains unclear, especially since confounding by indication might play a role.
Objective*:* Our aim was to investigate whether use of platelet aggregation inhibitors or vitamin K antagonists increase the risk of SAH.
Methods*:* We applied population-based case-control, case-crossover, and case-time-control designs to estimate the risk of SAH while addressing issues both of confounding by indication and time varying exposure within the PHARMO Record Linkage System database. This system includes drug dispensing records from community pharmacies and hospital discharge records of more than three million community dwelling inhabitants in the Netherlands. Patients were considered a case if they were hospitalised for a first SAH (ICD-9-CM code 430) in the period between January 1st 1998 and December 31st 2006. Controls were selected from the source population, matched on age, sex, and date of hospitalisation. Conditional logistic regression was used to estimate multivariable adjusted odds ratios and 95% CIs for the risk of SAH during use of platelet aggregation inhibitors or vitamin K antagonists. In the case-crossover and case-time-control designs we selected 11 control periods preceding the index date in successive steps of one month in the past.
Results*:* 1004 cases of SAH were identified. In the case-control analysis the adjusted odds ratio (OR) for the risk of SAH in current use of platelet aggregation inhibitors was 1.32 (95% CI: 1.02-1.70) and in current use of vitamin K antagonists 1.29 (95% CI: 0.89-1.87) compared to no use. In the case-crossover analysis the ORs for the risk of SAH in current use of platelet aggregation inhibitors and vitamin K antagonists were 1.04 (95% CI: 0.56-1.94) and 2.46 (95% CI: 1.04-5.82), respectively. In the case-time-control analysis the OR for platelet aggregation inhibitors was 0.50 (95% CI: 0.26-0.98) and for vitamin K antagonists 1.98 (95% CI: 0.82-4.76).
Conclusion*:* Use of platelet aggregation inhibitors was not associated with an increased SAH risk; the modest increase observed in the case-control analysis could be due to confounding. Use of vitamin K antagonists was associated with an increased risk of SAH. The increase was most pronounced in the case-crossover analysis and therefore cannot be explained by unmeasured confounding. Vitamin K antagonists should be prescribed cautiously in patients with known unruptured aneurysms.

Introduction

Non-traumatic subarachnoid haemorrhage (SAH) is a severe neurological disorder with relatively low incidence. SAH is caused by a ruptured aneurysm in 85 percent of patients [1]. Due to its occurrence at younger age and high case fatality, SAH causes more loss of productive life years than ischemic and haemorrhagic stroke [1]. The classical cardiovascular disease risk factors such as age, gender, family history, hypertension, and smoking are risk factors for SAH [1, 23]. Use of platelet aggregation inhibitors and oral anticoagulants (vitamin K antagonists) has both been associated with increased risk of intracranial haemorrhage (ICH) [28]. The indication for platelet aggregation inhibitors is mostly secondary prevention of cardiovascular and cerebrovascular disease. These share many risk factors with SAH and may therefore cause confounding by indication. Vitamin K antagonists are indicated mainly for prevention and therapy of thrombo-embolic events. A review on oral anticoagulants versus antiplatelet therapy for preventing stroke in patients with non-valvular atrial fibrillation and no history of stroke or transient ischemic attacks (TIA) showed a doubling of risk on ICH in oral anticoagulant users compared to users of platelet aggregation inhibitors [25]. This finding suggests that a difference in association might be expected, due to different haemostatic mechanisms. It remains unclear whether and to what extent these antithrombotic agents are associated specifically with SAH, as this is regularly not separated out [25, 29]. A recent population based case-control study indicated an increased risk of SAH in users of platelet aggregation inhibitors, but the authors acknowledge the vulnerability of their study to residual confounding [75].

To quantify the associations while addressing the issue of residual confounding, we applied case-control, case-crossover, and case-time-control designs in a population-based database.

Patients and methods

Setting

Data were obtained from the PHARMO Record Linkage System (RLS) database. This includes drug-dispensing records from community pharmacies and hospital discharge records of more than three million community dwelling inhabitants of 50 demographically defined areas in the Netherlands. For all residents, the computerized drug-dispensing histories contain data concerning the dispensed drug, type of prescriber, dispensing date, dispensed amount, prescribed dosing regimens, and the legend duration of use (prescription length). All drugs are coded according to the Anatomical Therapeutic Chemical (ATC) Classification [76]. The hospital records include detailed information concerning discharge diagnosis, procedures, dates of hospital admission and discharge, and discharge destination

or dying in the hospital before discharge. Diagnoses are classified according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). For a detailed description of the database, which is representative for the Dutch population, we refer to previous work [70].

Source population

The source population included all persons in the PHARMO RLS database who had at least three years of valid database history. Persons were followed from inception or start of the study period (January 1st, 1998) until transferral out of the database, death, diagnosis of SAH, or the end of the study period (December 31st, 2006), whichever came first.

Cases and controls

Study outcome was hospitalisation for SAH, registered as primary diagnosis code 430 according to ICD-9-CM, as recorded in the PHARMO RLS database. Patients were considered a case if they were hospitalised for SAH in the period between January 1st 1998 and December 31st 2006. Previously, we have reported on a small validation study, in which we demonstrated that the positive predictive value (PPV) of this discharge diagnosis was 88 percent [56].

Case-control design

For each case, a set of controls was obtained from the source population by means of incidence density sampling [77]. Controls had to be alive at the index date (calendar date of hospitalisation for SAH of the case), and have no history of SAH. A random sample of up to 10 controls was drawn from the set of all eligible controls, matched to the case on year of birth, sex, and index date.

Case-crossover design

To control for suspected residual confounding by both measured and unmeasured factors that are stable over time (in our study, a one year period), we additionally performed a case-crossover study. In this design, the case acts as its own reference by its own past experience, instead of using matched external controls [78]. Thus, confounders (measured and unmeasured) that are stable over time in one person cancel out. As control periods, we selected the 11 periods preceding the index date in successive steps of one month in the past.

Case-time-control design

In Figure 1, we show the changes in prescriptions rates for platelet aggregation inhibitors and vitamin K antagonists over the study period in the PHARMO RLS database. The strong time trend in user prevalence observed in this figure triggered the use of a case-time-control design, as this design accounts for time-trends in exposure prevalence that may act as confounder in a case-crossover design [79].

This design includes both cases and controls (as defined for the case-control design). Each of them is considered twice, once for the case period and once for the control periods (as defined for the case-crossover design). Within each case subject, exposure frequencies from the case and control periods are compared through a matched odds ratio (OR), as in the case-crossover design. Among control subjects, the frequencies of exposure during the case period and the control periods are an estimate of the exposure distribution in the underlying cohort during different periods of time, and therefore the matched odds ratio between periods measures the time trend in exposure. The case-time-control design is based on two main assumptions: 1) the case-crossover odds ratio is the product of the odds ratio due to the causal effect of the exposure on the outcome and the odds ratio due to the time trend in exposure prevalence, and 2) the latter is the same among cases and controls. Thus, the case-time-control odds ratio is the case-crossover odds ratio divided by the time trend odds ratio.

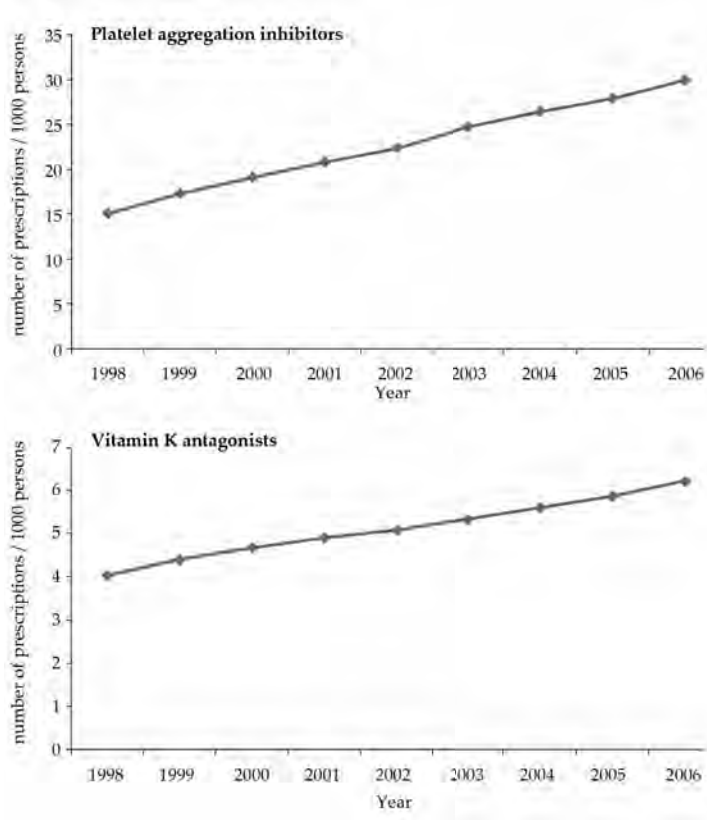

Figure 1. Exposure prevalence: number of prescriptions per 1000 persons for platelet aggregation inhibitors and vitamin K antagonists per calendar year. Data from the PHARMO RLS database, 1998-2006.

Study drugs and definition of exposure

The primary drugs of interest were platelet aggregation inhibitors and vitamin K antagonists (see ATC codes in appendix). Heparin was left out of our analyses due to insufficient numbers. Exposure data were obtained from drug dispensing files. For platelet aggregation inhibitors, duration of use was obtained by dividing the total amount of dispensed units by the prescribed amount per day. In the case-control study, use was classified as "no", "current" (use at index date), "recent past" (stopped less than 30 days before index date), and "distant past" (stopped more than 30 days before index date). In sensitivity analyses the exposure window of current use was extended to use within seven or fourteen days preceding the index date. In the case-crossover and case-time-control studies, "distant past" was merged with "no" use, because of the one month period we chose as control period. Since the registered dosing regimen for vitamin K antagonists in the pharmacy may differ from the tailored dosing regimen of the thrombosis service, we manually examined all prescription histories for the case-crossover study, since this design is sensitive to misclassification of the duration of a prescription.

Covariates

We considered diabetes mellitus, hypertension, and a history of vascular events as covariates. Use of insulin without a history of use of oral anti-diabetic agents was considered a proxy for having diabetes mellitus type I (DM I). Use of oral anti-diabetic agents either or not followed by use of insulin was considered a proxy for having diabetes mellitus type II (DM II). In this study we did not have information on blood pressure but considered antihypertensive drug use as proxy. Use of anti-hypertensive agents was considered a proxy for being in a state of controlled hypertension. Past use of antihypertensive drugs was considered a proxy for being in a state of uncontrolled hypertension. A history of vascular events was defined by use of two or more prescriptions of nitrates or hospitalization for ischemic cardiovascular or cerebrovascular events (CVD) in a three year period preceding the index date.

We also studied SSRIs, NSAIDs, and COX-2 selective inhibitors, because of their potential effects on bleeding risk [80, 81]; and the pain and neuropsychiatric agents: tricyclic antidepressants (TCAs), benzodiazepines, antipsychotics, anti-epileptics, and antimigraine drugs; as well as female sex hormones, antibiotics, and statins as these have been associated with vascular events [26, 56, 82].

Statistical analysis

We compared cases and controls with regard to demographic characteristics and drug use. Next, we applied conditional logistic regression analyses to matched case-control data, case-crossover data, and case-time-control data to estimate matched odds ratios and 95 percent confidence intervals (95% CI) for the association between exposure to drugs and SAH risk. Analyses were performed with reference

to no use. In the adjusted model we included all factors that changed the risk estimate with more than 5 percent. For all three study designs we performed sensitivity analyses to examine the effect of extension of the exposure window on the observed associations. The periods of current use were extended to cessation of use up to seven and fourteen days before the index date. We examined interaction of the study drugs with antihypertensive drugs, statin withdrawal, long term (> 30 days) use of platelet affecting drugs (NSAID, SSRI), or antibiotics which increase the dose/effect of vitamin K antagonists [83]. To examine whether a history of vascular events was an effect modifier, we performed a stratified analysis. In the case-crossover study we conducted an additional sensitivity analysis on exposure misclassification by excluding case-controls sets with a small unexplained 'gap' in the prescriptions for vitamin K antagonists, since it might be that this gap does not represent a true interval of abstinence.

Analyses were performed using SPSS software version 15.0 (Chicago, Ill., USA). A two-sided p-value of less than 0.05 was considered significant. To avoid presentation of unstable estimates, association measures for products with exposure in less than 3 cases or 3 controls are not reported.

Results

Case-control analysis

Our study population comprised of 1004 cases of SAH and 10,033 matched controls. Due to matching on year of birth and gender, mean age and distribution of sex in cases and controls were the same (table 1). Past use of antihypertensive drugs increased the risk of SAH, OR=1.34 (95% CI: 1.10-1.62). Current use of NSAIDs and COX-2 selective inhibitors (coxibs) seemed to be associated with SAH risk. However, exclusion of recent starters of NSAIDs or coxibs reduced the associations, pointing to protopathic bias. Current use of SSRIs was found to be unrelated to SAH, OR=0.96 (95% CI: 0.67-1.40).

Use of platelet aggregation inhibitors was associated with an increased risk of SAH in the case-control analysis: the adjusted OR was 1.32 (95% CI: 1.02-1.70) (table 2). Use of vitamin K antagonists seemed to increase the risk of SAH, although the association was not statistically significant. The adjusted OR was 1.29 (95% CI: 0.89-1.87) (table 2). Extension of the exposure window of current use to seven or fourteen days after the end of the prescription yielded higher point estimates for both platelet aggregation inhibitors and vitamin K antagonists. For the latter, the association reached the significance threshold in the fourteen days period.

In the case-control analysis, we found no interaction between antihypertensive drugs, statin withdrawal, long term use of NSAIDs or SSRIs, or antibiotics and platelet aggregation inhibitors or vitamin K antagonists (data not shown).

Table 1. Characteristics of the cases and controls.

	Cases		*Controls*		***OR***	*95% CI*
	N	*%*	*N*	*%*		
Total	**1004**	**100**	**10033**	**100**		
Mean age (SD) (years)	58 (14)		58 (14)		Matched	
<50 years	293	29	2928	29		
50-65 years	383	38	3828	38		
>65 years	328	33	3277	33		
Gender, female	670	67	6695	67	Matched	
Diabetes mellitus I	12	1.2	84	0.8	1.42	0.77-2.62
Diabetes mellitus II	57	5.7	649	6.5	0.87	0.66-1.16
Antihypertensives[1]						
Current use	254	25.3	2560	25.5	1.05	0.89-1.25
Recent past	20	2	231	2.3	0.91	0.57-1.46
Past use	152	15.1	1197	11.9	**1.34**	**1.10-1.62**
No CV event history	870	86.7	8868	88.4	1	
History of CV events	152	15.1	1254	12.5	1.30	1.07-1.59
Current use of other drugs[1]						
SSRI	32	3.2	335	3.3	0.96	0.67-1.40
NSAID	80	8	357	3.6	**2.34**	**1.77-3.09**
Use > 30 days	37	3.7	297	3	1.30	0.90-1.87
COX-2 selective inhibitors	13	1.3	56	0.6	**2.35**	**1.27-4.36**
Use > 30 days	7	0.7	43	0.4	1.65	0.74-3.70
TCA	10	1	117	1.2	0.85	0.44-1.64
Benzodiazepines	102	10.2	764	7.6	**1.43**	**1.13-1.80**
Use > 30 days	74	7.4	740	7.4	1.04	0.80-1.36
Anti-psychotics	10	1	119	1.2	0.83	0.44-1.59
Anti-epileptics	15	1.5	121	1.2	1.26	0.73-2.17
Antimigraine	6	0.6	23	0.2	**2.69**	**1.09-6.60**
Use > 30 days	2	0.2	20	0.2	-	
Estrogens	15	1.5	142	1.4	1.05	0.61-1.80
Progesterone	3	0.3	68	0.7	0.42	0.13-1.35
Combinations of estrogens and progestin	77	7.7	676	6.7	1.29	0.96-1.74
Antibiotics	19	1.9	145	1.4	1.36	0.83-2.23
Statins	82	8.2	976	9.7	0.84	0.66-1.07

[1] current use compared to no use

Case-crossover analysis

In the study population of 1004 cases, the 1004 risk periods directly preceding the SAH were compared to 11,044 control periods occurring in successive steps of one month in the past. Use of platelet aggregation inhibitors was not associated with SAH in this design: OR= 1.04 (95% CI: 0.56-1.94). These findings were not susceptible to changes in the risk period (table 2). Use of vitamin K antagonists was significantly associated with SAH; the OR was 2.90 (95% CI: 1.27-6.65). Extension of the risk period showed similar ORs (table 2). Exclusion of cases with an unexplained 'gap' in the prescription regimen (due to misclassification of duration due to thrombosis service regimens) did not change the associations materially (data not shown).

Table 2. Case-control, case-crossover, and case-time-control analyses.

Current use	**Case-control**				**Case-crossover**		**Case-time-control**	
	Cases	***Controls***	***OR***	***95% CI***	***OR*²**	***95% CI***	***OR*²**	***95% CI***
Platelet aggregation inhibitors								
No use (any time prior)	810	8443	1		1		1	
Use at index date	111	1032	1.32[1]	1.02-1.70	1.04	0.56-1.94	0.50	0.26-0.98
Use at index or stopping for max. of 7 days	116	1059	1.34[1]	1.04-1.71	1.07	0.58-1.97	0.53	0.27-1.02
Use at index or stopping for max. of 14 days	121	1079	1.37[1]	1.07-1.75	1.09	0.60-2.00	0.54	0.28-1.05
Vitamin K antagonists								
No use (any time prior)	909	9221	1		1		1	
Use at index date	39	283	1.29[3]	0.89-1.87	2.90	1.27-6.65	1.98	0.82-4.76
Use at index or stopping for max. of 7 days	40	292	1.38[3]	0.97-1.96	2.78	1.22-6.31	1.97	0.82-4.71
Use at index or stopping for max. of 14 days	45	313	1.44[3]	1.03-2.02	3.03	1.35-6.81	2.07	0.87-4.89

[1]adjusted for hypertension and statin use and for use of vitamin K antagonists; [2] estimates of platelet aggregation inhibitors are adjusted for use of vitamin K antagonists and *vice versa*; [3] adjusted for use of RAS medication and use of platelet aggregation inhibitors.

Case-time-control analysis

For all controls from our case-control analysis, we selected index periods and control periods, analogous to what was done to the cases in the case-crossover design. Analysis of these 10,033 index periods and 110,363 control periods yielded the time trend odds ratio. By dividing the case-crossover odds ratio by the time

trend odds ratio we obtained the case-time-control estimate of the SAH risk in our population. This is mathematically identical to exponentiate the difference of the betas. Confidence intervals were calculated by exponentiate this difference plus or minus 1.96 times the square root of the sum of the squared standard errors of the case-crossover and time trend betas. For platelet aggregation inhibitors this OR was 0.50 (95% CI: 0.26-0.98) and for vitamin K antagonists 1.98 (95% CI: 0.82-4.76) (table 2). Extension of the exposure windows did not change the odds ratios substantially.

Discussion

In our study we applied three study designs to examine the association between use of antithrombotic drugs and the risk of SAH. The case-crossover and case-time-control designs were applied to address the issues of residual confounding and time bias, respectively. We found consistently that use of vitamin K antagonists was associated with an increased risk of SAH; although the estimates and confidence intervals varied. Use of platelet aggregation inhibitors was not associated with SAH in this study. The increased risk observed in the case-control design disappeared when confounding by indication was taken into account in the case-crossover design. The effect decreased even further when taking time varying exposure prevalence into account in the case-time-control design. The findings for platelet aggregation inhibitors seem very susceptible to confounding and time bias.

Hence, our findings suggest that there is no association between use of platelet aggregation inhibitors and SAH in the general population. Iso *et al*. reported on a doubled risk of SAH in female aspirin users [84]. However, this finding was based on only twelve cases and considered women using fifteen or more tablets of aspirin (325 mg.) per week. Schmidt *et al*. reported on a case-control study indicating an increased risk of SAH in users of platelet aggregation inhibitors [75]. With regard to size and demographics, their study population was similar to ours. The odds ratios they found in their case-control study were also quite similar to what we found in our case-control analysis. Both studies point to a small increase in risk of SAH in platelet aggregation inhibitor users. Our study is an addition in two important aspects; first, we investigated the effect of anticoagulants additional to platelet aggregation inhibitors, and secondly, we addressed issues related to confounding more thoroughly. Schmidt *et al*. alluded to residual confounding as alternative explanation but they did not address it further. We showed that residual confounding actually explained the observed increase in risk for platelet aggregation inhibitors.

Our findings on vitamin K antagonists are in line with what one would expect based on similar findings on ICH [25, 28, 29]. Due to their pharmacological action,

antithrombotic drugs could convert a 'warning leak' into a life-threatening, major rupture, but it is not self-evident that these drugs *cause* lesions or ruptures. In a reported series on aneurysmal SAH, anticoagulants indeed seemed to increase the extent of the bleeding. As a consequence of more severe initial bleeding, the risk of death or dependency in anticoagulated patients versus those not receiving anticoagulants was almost doubled [85]. Whether the observed association represents a causal relation between use of vitamin K antagonists and SAH cannot be established definitively based on our data.

Hypertension is a known risk factor for SAH [23]. In this study we did not have information on blood pressure but considered antihypertensive drug use as a proxy. Current use of antihypertensive drugs was not associated with SAH, as the blood pressure of these patients is likely to be fairly controlled. But patients who formerly used antihypertensive drugs are likely to suffer from uncontrolled blood pressure; recent studies show that non-adherence to antihypertensive therapy is associated with high systolic blood pressure [74]. Indeed, former use of antihypertensive drugs was associated with increased risk of SAH.

With regard to other potential associations reported in table 1, it is unlikely that they represent true associations. Although NSAIDs, coxibs, and anti-migraine medication have platelet or vascular effects, it is most likely that the reported associations are due to protopathic bias, i.e. this medication is used to treat a prodrome of the disease under investigation. In case of SAH these prodromes are most likely headache and neck pain due to a 'warning leak'. This explanation is supported by the sub analyses in patients using these agents for more than 30 days; in these patients the association could not be found which argues against a causal relation.

The main strengths of our study are the size and the quality of the prospectively registered pharmacy data, which allow for estimation of the effects of timing of drugs. Despite these strengths the results of this study should be seen in the light of its limitations, inherent to any observational study with non-randomly assigned intervention. Due to the use of a population-based database of healthcare records selection bias is unlikely. Information bias may occur on the level of outcome as well as on the level of exposure. Despite errors in exposure assessment due to non-compliance, information bias is likely to be non-differential since data were gathered prospectively without having knowledge of the hypothesis studied [72, 73, 86]. Misclassification of the outcome was a source of concern in our study as there is no information on the overall PPV of the discharge diagnosis. In a small validation study we demonstrated that the PPV of this discharge diagnosis was 88 percent [56]. Disease misclassification due to inclusion of false positive cases is therefore limited and most likely non-differential, resulting at most in a small attenuation of the association. The use of a registry-based approach renders our study vulnerable to potential confounders that are not covered by our database. In

the case-control design we could not adjust for lifestyle factors such as smoking, alcohol abuse, obesity, and actual blood pressure.

But we addressed this residual confounding by conducting a case-crossover design. We considered application of the case-crossover design appropriate due to the nature of the association under investigation. The case-crossover design is particularly suitable when the exposure is intermittent, the effect on risk is immediate and transient, and the outcome is abrupt [78]. The outcome SAH fulfils the requirement of abruptness. The effect of antithrombotic drugs on bleeding tendency is in the order of magnitude of hours after administration, and the effect decreases in two to six days. The exposure to antithrombotic drugs is intermittent in the sense that the administration can be stopped and started at any moment, especially for vitamin K antagonists.

The case-time-control design was applied because of the time varying exposure prevalence of both platelet aggregation inhibitors and vitamin K antagonists (Figure 1).

Of note is that the beneficial effects of well-indicated antithrombotic drugs in appropriate patients should not be disregarded. However, any benefits could be offset by an increased risk of haemorrhages [87]. This may be the case in sub groups of high risk patients – patients harbouring an unruptered intracranial aneurysm, for instance. In those patients caution might be warranted with regard to prescribing vitamin K antagonists.

Our results are of clinical relevance for the growing patient group with a recent TIA or minor stroke in whom a work-up for carotid or intracranial stenosis reveals an intracranial aneurysm [88]. Our results suggest that platelet aggregation inhibitors can be prescribed safely to these patients.

In conclusion, use of platelet aggregation inhibitors was not associated with an increased SAH risk; the modest increase observed in the case-control analysis could be due to confounding. Use of vitamin K antagonists was associated with an increased risk of SAH in some designs. The increase was most pronounced in the case-crossover analysis and therefore cannot be explained by unmeasured confounding. Vitamin K antagonists should be prescribed cautiously in patients with known unruptured aneurysms.

Appendix

Platelet aggregation inhibitors:
All B01AC drugs were eligible, in PHARMO only the following drugs in this class were used during the study period: B01AC04 (clopidogrel), B01AC05 (ticlopidine), B01AC06 (acetylsalicylic acid), B01AC07 (dipyridamole), B01AC08 (carbasalate calcium), B01AC30 (combinations).

Vitamin K antagonists:
All B01AA drugs were eligible, in PHARMO only the following drugs in this class were used during the study period: B01AA04 (phenprocoumon), B01AA07 (acenocoumarol).

3.3 Oral contraceptives and risk of subarachnoid haemorrhage

Background: The question whether use of oral contraceptive pills (OCP) affects the risk on (aneurysmal) subarachnoid haemorrhage (SAH) is relevant since female sex as a risk factor for SAH changes after menopause.
Objective*:* To estimate the association between OCP use and the risk of (aneurysmal) SAH.
Methods: We conducted population-based case-control studies in two different databases in the Netherlands. One was the PHARMO Record Linkage System database which includes drug dispensing records from community pharmacies and hospital discharge records of more than three million community dwelling inhabitants in the Netherlands. The second was the Integrated Primary Care Information (IPCI) database, a database with longitudinal medical records of currently more than one million persons. We identified cases from these two non-overlapping populations. In PHARMO a case was defined as a female under age of 50 years hospitalised for SAH (ICD-9-CM code '430') in the period 01.01.98-12.31.06. Ten randomly chosen controls were matched to each case on age, sex, and index date. In IPCI, cases were females below 50 years with a validated aneurysmal SAH. All eligible controls, obtained from the source population by means of incidence density sampling, were matched to the case on age, sex, and index date.
Results: In PHARMO the odds ratio (OR) for the SAH risk in OCP users was 1.79 (95% CI: 1.22-2.64). Subdivision according to progestin content of the OCP showed an OR for levonorgestrel use of 1.88 (95% CI: 1.24-2.85) and for desogestrel 1.99 (95% CI: 1.11-3.54). In IPCI the results were very similar; the OR for OCP use was 2.11 (95% CI: 0.82-5.42). The OR for levonorgestrel use to 3.19 (95% CI: 1.20-8.48).
Conclusions: Use of OCP seems to be associated with an increased risk of (aneurysmal) SAH.

Introduction

Subarachnoid haemorrhage (SAH) is a severe neurological disorder. Due to its occurrence at younger age and its higher case fatality, SAH causes more loss of productive life years than ischemic and haemorrhagic stroke, although its incidence is much lower [1]. Ruptured aneurysms are the cause of the SAH in approximately 85 percent of patients [1].
Non-modifiable risk factors such as age, sex, and family history play an important role in the risk of SAH [1, 23]. Modifiable risk factors have been identified as well, providing opportunities for intervention. Established modifiable risk factors for SAH are hypertension, smoking, and excessive alcohol intake [1]. Another class of potentially modifiable risk factors comprises the use of drugs, but their role in SAH aetiology has not been systematically evaluated. The fact that the age-specific incidence rate of SAH is higher in postmenopausal women than in men, but lower in premenopausal women than men suggests an influence of sex hormones [2]. In addition, endothelium-dependent arterial vasodilatation increases significantly during the ovulatory phase of the menstrual cycle, when oestrogen levels are highest in naturally cycling young women [89-91], and some progestins may antagonize the beneficial vascular effects of oestrogen in postmenopausal women [92-95]. Each progestin can have different effects on the vasculature depending on the parent molecule from which it was created, its chemical structure, pharmacokinetics, activity and specificity to given receptors [96-98].

Several studies on the association between use of OCP and the risk of subarachnoid haemorrhage in premenopausal women have been conducted. Results of these previous investigations were inconclusive; a meta-analysis and a systematic review led to contradictory conclusions [23, 26]. The meta-analysis of observational studies by Johnston *et al*. suggests that OCP use produces a small increase in the risk of SAH [26]. The systematic review by Feigin *et al*. [23] considered only one extra study with regard to OCP use (Mhurchu *et al*. [6]), which showed no effect of OCP use on SAH risk. The conclusion of the systematic review was that use of OCP does not significantly affect the risk of SAH [23]. These overviews did not examine the effect of different progestin contents. Additionally, the study of Mhurchu *et al*. dichotomised the OCP group in 'ever users' and 'never users' which may result in severe misclassification and inability to demonstrate an effect [6].

To investigate the association between SAH and OCP use, and specifically the progestin contents of OCP, we conducted two population-based case-control studies. We were interested in SAH in general and more specifically in aneurysmal SAH. The first study was performed in a discharge database linked to drug dispensing records (PHARMO). The second in an electronic medical record database (IPCI). The latter database was smaller, but allowed for more detailed

validation of aneurysms as the underlying cause of rupture. During the study period the databases did not overlap in their underlying source population.

Patients and methods

Setting

Data were obtained from the PHARMO Record Linkage System (RLS) and the Integrated Primary Care Information database (IPCI).

PHARMO RLS includes, amongst other databases, the drug-dispensing records from community pharmacies and hospital discharge records of more than three million community dwelling inhabitants of 50 demographically defined areas in the Netherlands.

For all residents, the computerized drug-dispensing histories contain data concerning the dispensed drug, type of prescriber, dispensing date, dispensed amount, prescribed dosing regimens, and the legend duration of use (prescription length). All drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification [99]. The hospital records include detailed information concerning diagnoses, procedures, and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). For a detailed description of the database, which is representative for the Dutch population, we refer to previous work [70].

The Integrated Primary Care Information (IPCI) database is a general practice research database with electronic medical record data currently comprising more than one million patients throughout the Netherlands. This patient population is representative of the general Dutch population regarding age and sex [50]. In the health care system in the Netherlands, everyone is registered with one general practitioner who is the gatekeeper for medical care and information. The electronic records contain anonymous and coded information on patient demographics, symptoms and diagnoses (using the International Classification for Primary Care (ICPC-codes [100]) and free text terminology), referrals, clinical and laboratory findings, and hospitalizations. Summaries of hospital discharge letters or additional information from medical specialists are entered in a free text format and hard copy letters can be requested. Information on drug prescription comprises brand name, amount, strength, indication, prescribed daily dose and the Anatomical Therapeutic Chemical (ATC) classification. To maximize completeness of the data, GPs participating in the IPCI project are required not to use paper-based records in addition. The system complies with European Union guidelines on the use of medical data for research and is valid for pharmaco-epidemiological research [51]. The Scientific and Ethical Advisory Group of the IPCI project approved the study (Project No. 07/02).

Source population

In PHARMO the source population included all females who had at least three years of valid database history and were 50 years of age or younger. Because of our research interest in OCP we were primarily interested in premenopausal women. Since the mean age of menopause is 51 year, a maximum age of 50 was chosen [101]. Persons were followed from inception or start of the study period (January 1st, 1998) until transferral out of the database, death, diagnosis of SAH, or the end of the study period (December 31st, 2006), whichever came first.

In IPCI the source population comprised females below 50 years of age, with at least 1 year of valid history in the IPCI database during the study period (January 1996-September 2006). Follow-up started at the beginning of the study period or on the date on which 1 year of valid history was available, whichever date was latest. Follow-up was terminated when the person transferred out of the practice, turned 50 years of age, the date of last data supply by the GP, death, diagnosis of SAH or at the end of the study period, whichever came first.

Cases and controls

In the PHARMO database the study outcome was hospitalisation for a first SAH. Cases were females hospitalised for the first SAH during the study period, as recorded in the Dutch National Medical Registration (LMR) with ICD-9-CM code 430. For each case a set of controls was obtained from the source population by means of incidence density sampling [77]. Controls had to be alive at the index date (calendar date of hospitalisation for SAH of the case) and have no history of SAH. A random sample of up to 10 controls was drawn from the set of all eligible controls, matched to the case on year of birth, sex, and index date.

In IPCI, cases were female patients under the age of 50 with an aneurysmal SAH confirmed by lumbar puncture, neuroradiological investigation, surgery, or autopsy. Potential cases were identified from the computerized records by searching for ICPC codes and free text searches. The computerized medical records of all potential cases were manually evaluated by a medical doctor (RR) to exclude false positive records and to assess the date of occurrence of the subarachnoid haemorrhage (index date). For all remaining cases specialist letters were requested from the GP. These letters were evaluated by two medical doctors (RR, DD). Each case was either classified as definite or excluded. Patients with subarachnoid haemorrhage originating from a source other than an intracranial aneurysm, were excluded; patients with proven SAH in whom an aneurysm could not be identified were also excluded. The doctors were blinded to drug exposure throughout the validation process. For each case a set of controls was obtained from the source population by means of the incidence density sampling method [77]. Women with a suspected, though unproven SAH or with a SAH of unknown origin were censored at the date of diagnosis and were ineligible as controls thereafter. All eligible controls were matched to the case on year of birth, sex, and index date.

Study drugs and definition of exposure

The group of drugs of primary interest were oral contraceptive pills (OCP). Drug prescriptions were obtained from dispensing files (PHARMO) or prescription files (IPCI). The dosage of a prescription or dispensing was calculated based on the strength of one unit and prescribed dosing regimen. Duration of use was obtained by dividing total number of dispensed units by prescribed number per day while taking into account the stop weeks. OCP use was classified as 'no use', 'current use' (use at index date or within 90 days previous [102]), and 'past use' (cessation of drug use more than 90 days before index date).

To investigate differences in effects we also classified the exposure categories of OCP according to their progestin content.

Covariates

We considered diabetes mellitus (DM) and concomitant use of other medications as covariates. Antihypertensive agents were studied as a proxy for hypertension. We also studied agents known for increased bleeding risk (antithrombotics and SSRI), as well as NSAID, coxibs, tricyclic antidepressants (TCA), benzodiazepines, antibiotics, anti-migraine drugs, systemic corticosteroids, and statins (table 1).

Statistical analysis

Analyses were performed using SPSS software version 15.0. (Chicago, Ill., USA). A two-sided P-value of less than 0.05 was considered significant. The two case-control studies were analysed separately.

First, we compared cases and controls with regard to demographic characteristics and drug use. Next, we used conditional logistic regression analysis for matched case-control data to estimate matched odds ratios (OR) and 95 percent confidence intervals (95% CI) for SAH associated with drug exposure. All analyses were performed with reference to no use. Factors that changed the effect estimate of OCP use with more than 5 percent were considered confounders.

To examine the impact of alternative definitions of the exposure window, sensitivity analyses were conducted that considered various time intervals for current use (use up to 30, 60, 90, 120, and 150 days).

To avoid presentation of unstable estimates, association measures for products with exposure in less than 3 cases or 3 controls are not reported.

Results

The PHARMO study population comprised of 218 cases with a SAH and 2,179 matched controls. Mean age of the cases was 42 years (standard deviation [SD]: 6 years). Past use of antihypertensives was associated with an increased risk of SAH (table 1). NSAIDs, coxibs, and benzodiazepines increased the risk. However,

exclusion of recent starters weakened the effect, which points to protopathic bias (data not shown), similarly to what was demonstrated in a previous paper [56]. Use of antibiotics or systemic corticosteroids increased the risk of SAH. Odds ratios were 2.17 (95% CI: 1.16-1.54) and 3.17 (95% CI: 1.13-8.92), respectively. Most of the covariates had a very low prevalence in this young female population and none was found to be a confounding factor.

Use of OCP was associated with an increased SAH risk. The odds ratio was 1.79 (95% CI: 1.22-2.64). Subdivision according to progestin content of the OCP showed increased risk in current users of levonorgestrel or desogestrel containing OCP. The odds ratio for levonorgestrel use up to 90 days before index date was 1.88 (95% CI: 1.24-2.85), for desogestrel 1.99 (95% CI: 1.11-3.54) (table 2). Use of OCP containing gestodene or cyproteron was not statistically significantly associated with risk of SAH. Other progestins (lynestrenol, norethisterone, norgestimate, drospirenone, norelgestromin) could not be studied separately due to insufficient numbers.

In table 3 we present the odds ratios for current use of oral contraceptive pills while considering current exposure up to 30, 60, 90, 120, and 150 days before the date of SAH; the estimates were quite stable.

The IPCI study population comprised of 26 cases with an aneurysmal SAH and 46,667 controls. Mean age was 40 years (SD: 7 years); slightly younger than the SAH cases in PHARMO. Most covariates had very low prevalence in these young females and we could not estimate the associations reliably (table 1). Use of NSAIDs and benzodiazepines were strongly associated with aSAH risk, with ORs of 5.01 (95% CI: 1.60-15.7) and 7.65 (95% CI: 2.69-21.7), respectively. These figures are likely caused by protopathic bias.

OCP use was associated with an increased SAH risk, although not statistically significant. The odds ratio was 2.11 (95% CI: 0.82-5.42). Subdivision by progestin content showed a strong association between aSAH and levonorgestrel use (OR=3.19 [95% CI: 1.20-8.48]). Other progestins could not be studied due to small sample size (table 2).

The strength of the effect estimate for overall OCP use did not vary a lot when the risk window was changed below or beyond 90 days (table 3).

Discussion

In our population based studies we found that OCP use was associated with an increased risk of SAH in women less than 50 years of age. Sensitivity analyses showed stable point estimates for different exposure windows that were included in the current user group. The effect was most pronounced in levonorgestrel and desogestrel containing OCP. For the first progestin the increased risk was confirmed for aneurysmal SAH in the IPCI database.

Table 1. Patient characteristics in PHARMO (SAH) and IPCI (aSAH).

	PHARMO						IPCI					
	SAH Cases		*Controls*		*OR*	*95% CI*	*aSAH Cases*		*Controls*		*OR*	*95% CI*
	N	**%**	**N**	**%**			**N**	**%**	**N**	**%**		
Total	218	100	2179	100			26	100	46,667	100		
Mean age (SD) (years)	42 (6)		42 (6)		Matched		40 (7)		40 (7)		Matched	
Diabetes mellitus	4	1.8	39	1.8	1.03	0.48-3.14	0	0	535	1.1		
Anti-hypertensive drug use												
Current use	11	5	71	3.3	1.64	0.85-3.14	0	0	186	0.4		
Past use	16	7.3	52	2.4	**3.25**	**1.83-5.78**	1	3.8	388	0.1		
Current use of other drugs												
Antithrombotics	6	2.8	47	2.2	1.28	0.65-3.23	0	0	302	0.6		
SSRI	6	2.8	122	5.6	0.48	0.21-1.10	2	7.7	1336	2.9		
NSAID	32	14.7	138	6.3	**2.45**	**1.53-3.94**	4	15.4	1764	3.8	5.01	1.60-15.7
COX-2 selective inhibitors	3	1.4	7	0.3	**4.52**	**1.12-18.3**	0	0	88	0.2		
TCA	4	1.8	29	1.3	1.42	0.50-4.07	0	0	357	0.8		
Benzodiazepines	8	3.7	43	2.0	1.97	0.91-4.25	5	19.2	1690	3.6	**7.65**	**2.69-21.7**
Antibiotics	15	6.9	76	3.5	**2.17**	**1.16-1.54**	0	0	1223	2.6		
Anti-migraine	6	2.8	25	1.1	**2.63**	**1.06-6.54**	1	3.8	416	0.9		
Systemic Corticosteroids	5	2.3	17	0.8	**3.17**	**1.13-8.92**	0	0	175	0.4		
Statins	3	1.4	41	1.9	0.73	0.22-2.38	0	0	298	0.6		

Table 2. Odds ratios for use of OCP and risk of SAH (PHARMO) and aSAH (IPCI). OCPs are subdivided according to their progestin content.

	PHARMO							IPCI						
	SAH Cases	*%*	*Controls*	*%*	*OR*	CI_{min}	CI_{max}	*aSAH Cases*	*%*	*Controls*	*%*	*OR*	CI_{min}	CI_{max}
	N		**N**					**N**		**N**				
Current use OCP	93	42.7	684	31.4	**1.79**	**1.22**	**2.64**	11	42.3	13498	28.9	2.11	0.82	5.42
levonorgestrel	59	27.1	412	18.9	**1.88**	**1.24**	**2.85**	9	34.6	7151	15.3	**3.19**	**1.20**	**8.48**
desogestrel	19	8.7	126	5.8	**1.99**	**1.11**	**3.54**	0	0	2869	6.1	NA		
gestodene	7	3.2	65	3.0	1.46	0.62	3.42	1	3.8	1524	3.3	NA		
cyproterone	5	2.3	45	2.1	1.47	0.55	3.91	0	0	902	1.9	NA		
other[a]	3	1.4	36	1.7	1.09	0.32	3.68	1	3.8	1052	2.3	NA		
Past use OCP	60	27.5	731	33.5	1.07	0.71	1.61	5	19.2	10895	23.3	1.08	0.34	3.42
Current use other G03	4	1.8	41	1.9	1.22	0.42	3.57	1	3.8	1023	2.2	NA		
Past use other G03	15	6.9	141	6.5	1.36	0.73	2.52	1	3.8	2229	4.8	NA		
No use of any G03	46	21.1	582	26.7	ref			8	30.8	19022	40.8	ref		

[a] contains: lynestrenol, norethisterone, norgestimate, drosperinone, and norelgestromin.

Table 3. Odds ratios for OCP and SAH risk with current use up to 30, 60, 90, 120, and 150 days before index date.

	PHARMO				IPCI			
Days	*n*	*OR*	CI_{min}	CI_{max}	*n*	*OR*	CI_{min}	CI_{max}
30	75	1.59	1.07	2.36	10	2.08	0.80	5.43
60	86	1.70	1.15	2.51	10	1.99	0.76	5.20
90	93	1.79	1.22	2.64	11	2.11	0.82	5.42
120	93	1.75	1.19	2.57	11	2.03	0.79	5.22
150	96	1.75	1.20	2.57	12	2.16	0.85	5.45

The exact mechanism for the association between OCP use and (aneurysmal) SAH is not known, but several explanations for a relation between OCP use and physiological changes leading to increased vascular risk have been postulated [31, 103]. Endothelium-dependent vasodilatation was found to be reduced in users of OCP containing levonorgestrel [103]. Others concluded that OCP use promotes endothelial dysfunction [31]. Furthermore, exposure to exogenous sex hormones leads to alterations in large arterial wall stiffness, due to changes in elastin and collagen deposition [104, 105]. In large vessels, these changes increase the risk of deformations and dissection or rupture. Analogously, in cerebral arteries this mechanism might lead to higher susceptibility for SAH [106].

Another important potential pathway is increased blood pressure which is a known risk factor for (aneurysmal) SAH. OCP use has been shown to increase blood pressure [107, 108]. In particular levonorgestrel containing OCP is mentioned in relation with increased blood pressure [109, 110]. Increased blood pressure could act as an intermediate factor in the association between OCP and (aneurysmal) SAH. A more than ten-fold increase in haemorrhagic stroke risk has been described in women with a history of hypertension who used OCP [102].

Strengths and limitations

The main strength of our study was that we used two population based databases which had detailed information on medication (brand name, amount, strength, indication, prescribed daily dose and the Anatomical Therapeutic Chemical classification code) both for cases and controls. In addition, our study had a case-control design, in which cases and controls were selected from dynamic cohorts. This implies that selection bias is unlikely as all cases and controls came from the same source population. Because data were collected prospectively and without knowledge of the research hypothesis to be studied, information bias is also highly unlikely. We also consider age matching as an important tool to compare

women of similar age to escape problems concerning cohort effects and attrition of susceptibles.

Despite these strengths, the results of this study should be seen in the light of its limitations which are inherent to any observational study with non-random treatment assignment. In PHARMO, misclassification of the outcome was a concern as there is no information on the overall positive predictive value (PPV) of the discharge diagnosis. In a previous study we showed that the PPV was reasonable, which would lead to only small misclassification [56]. In IPCI, misclassification of the outcome is minimal as we manually validated all cases and only analyzed definite aneurysmal SAH cases.

There are several known risk factors that we could not fully capture by the data provided by the healthcare databases. Important factors are: menopause, alcohol intake, hypertension, and smoking. Confounding by menopause was limited through age restriction (< 50 years) and matching on age. Alcohol use and smoking could not be accurately obtained from the databases, but it is unlikely that alcohol intake is related to prescription of OCPs and the type of progestin. Smoking is a relative contra-indication for OCP use, because of the thrombotic risk. Residual confounding by smoking may result in an underestimation of the risk.

Odds ratios of about 2 for a rare event have limited clinical importance or public health significance, but the fact that the progestin type may impact and is variable among OCPs may be taken into account when prescribing OCPs, especially when women are known to have an intracranial aneurysm [111].

In conclusion, this study shows that OCP use is associated with SAH, but that the risk varies by progestin content. Women with known intracranial aneurysms should consider avoiding OCPs.

3.4 Genome-wide association study of intracranial aneurysm identifies three new risk loci

Saccular intracranial aneurysms are balloon-like dilations of the intracranial arterial wall; their hemorrhage commonly results in severe neurologic impairment and death. We report a second genome-wide association study with discovery and replication cohorts from Europe and Japan comprising 5,891 cases and 14,181 controls with ~832,000 genotyped and imputed SNPs across discovery cohorts. We identified three new loci showing strong evidence for association with intracranial aneurysms in the combined dataset, including intervals near RBBP8 on 18q11.2 (odds ratio (OR) = 1.22, $P = 1.1 \times 10^{-12}$), STARD13-KL on 13q13.1 (OR = 1.20, $P = 2.5 \times 10^{-9}$) and a gene-rich region on 10q24.32 (OR = 1.29, $P = 1.2 \times 10^{-9}$). We also confirmed prior associations near SOX17 (8q11.23-q12.1; OR = 1.28, $P = 1.3 \times 10^{-12}$) and CDKN2A-CDKN2B (9p21.3; OR = 1.31, $P = 1.5 \times 10^{-22}$). It is noteworthy that several putative risk genes play a role in cell-cycle progression, potentially affecting the proliferation and senescence of progenitor-cell populations that are responsible for vascular formation and repair.

Intracranial aneurysms affect approximately 2% of the general population and arise from the action of multiple genetic and environmental risk factors [12]. We previously reported the first genome-wide association study (GWAS) of intracranial aneurysms [34] that identified three risk loci on chromosomes 8q11.23-q12.1, 9p21.3 and 2q33.1 with $P < 5 \times 10^{-8}$. This previous study had limited power to detect loci imparting genotypic relative risk (GRR) < 1.35.

To increase the power to detect additional loci of similar or smaller effect, we ascertained and whole-genome genotyped two new European case cohorts (n = 1,616) and collected genotyping data from five additional European control cohorts. We also increased the size of the original Japanese replication cohort and added a new one (2,282 affected individuals (cases) and 905 controls) (Table 1). The new combined cohort had nearly threefold more cases than the original cohort and increased our power to detect variants with modest effect sizes. For example, this study had 89% and 64% average power to detect common variants (minor allele frequencies (MAF) ≥ 10%) with GRR of 1.25 and 1.20, respectively.

All subjects were genotyped using the Illumina platform. The new as well as the previously analyzed genotyping data were subjected to well-established quality-control measures. We sought to eliminate potential confounding due to population stratification and gender [12, 112] by matching cases and controls of the same gender based on inferred genetic ancestry. As previous studies demonstrated that the Finnish population forms an ancestry cluster distinct from other European populations similar to those included in this study [113, 114], we analyzed our Finnish cohort independently from the others. To maximize opportunities for genetic matching and analytic power, we analyzed all subjects in the remaining European cohorts together. The resulting matched case-control data consisted

of 808 cases and 4,393 controls in the Finnish cohort and 1,972 cases and 8,122 controls in the rest of the combined European cohort. We used the genotype data that passed quality-control filters and phased chromosomes from the HapMap CEU sample to impute missing genotypes [115]. We based our further analyses on 831,534 SNPs that passed the quality-control filters both in the Finnish and European samples (Table 1).

Table 1. Overview of the study cohorts.

	Cohort	*Case (n)*	*Control (n)*	**Quality control-passed** *SNPs (n)*	*GIF*
Discovery	Finland (FI)	808	4,393	1,303,876	1.074
	Combined European (CE)	1,972	8,122	905,906	1.094
	total discovery	2,780	12,515	831,532	1.007
CE subcohorts	NL	708	3,954	905,906	1.108
	DE	789	2,228	905,906	1.059
	AN	475	1,940	905,906	1.057
Replication	Japan 1 (JP1)	829	761	12	
	Japan 2 (JP2)	2,282	905	13	
	total replication	3,111	1,666	12	
	Total	5,891	14,181	12	

Combined European cohort consisted of all European subjects who were not ascertained in Finland. Subcohorts of the European cohort were defined on the basis of case series; NL, cases from The Netherlands with matched controls; DE, German cases with matched controls; AN, @neurIST cases with matched controls. NL, DE and AN were exclusive subsets of the European cohort (see also Supplementary Table 3). AN cases consisted of subjects from Germany, Great Britain, Hungary, The Netherlands, Switzerland and Spain. JP1 and JP2 were two independent Japanese case-control cohorts. Genomic inflation factors of the Finnish and European cohorts (as well as NL, DE and AN) were calculated for 1,303,876 and 905,906 SNPs, respectively. The genomic inflation factor of the discovery cohort (total discovery) was based on the meta-analysis result for 831,532 SNPs after correcting each cohort for genomic control. The discovery data (combined Finnish and European cohorts) was not corrected for genomic control. GIF, genomic inflation factor.

We tested for association of each quality control–passed SNP with intracranial aneurysms using conditional logistic regression, assuming a log-additive effect of allele dosage. We corrected each cohort for residual overdispersion (Table 1) using genomic control [116] and combined the results from the Finnish and European cohorts to obtain P values, ORs and CIs for the discovery cohort of 2,780 cases and 12,515 controls using a fixed-effects model.

To evaluate the strength of association, in addition to obtaining P values, we employed a Bayesian approach [117]. We used the Bayes factor that represents the fold-change of the odds of association before and after observing the data [118] and the posterior probability of association (PPA), calculated through the Bayes

factor, that provides a simple probabilistic measure of the evidence of association [117, 119]. For every SNP, we assumed a uniform prior probability of association of 1/10,000 and set the prior of the logarithm of the per-allele OR as a normal distribution with a 95% probability for the OR to be between 0.67 and 1.5, with larger weights for smaller effect sizes [118, 120].

From the discovery results, we eliminated two imputed SNPs that showed PPAs of 0.97 and 0.94 because their association signals were not supported by surrounding genotyped SNPs and because their genotypes were not confirmed by direct genotyping results (data not shown). This resulted in 831,532 SNPs that passed quality control.

We observed three regions that showed very high PPAs (>0.995; Figure 1a) and also a substantial excess of SNPs with $P < 1 \times 10^{-3}$ (1,295 SNPs versus 831 SNPs expected by chance) even after excluding those within previously identified associated regions [34] (Figure 1b). Moreover, we observed a strong correlation between the P values and Bayes factors for the upper tail of the distribution (Figure 1c).

We focused on five genomic regions (Figure 1a) that contained at least one SNP with PPA > 0.5 for which the hypothesis of association with intracranial aneurysm was more likely than the null hypothesis of no association. The PPAs of the most highly associated SNPs in these intervals ranged from 0.6621 to > 0.9999 and the P values ranged from 7.9×10^{-7} to 2.2×10^{-16}. The five chromosomal segments included three newly identified SNP clusters on 10q24.32, 13q13.1 and 18q11.2. The remaining two regions were previously identified loci on 8q11.23–q12.1 and 9p21.3 (Figure 2; ref. 2). The third locus identified in our previous study, on 2q33, did not contain any SNPs with PPA > 0.5. Furthermore, consistent with our previous results [34], detailed analysis of the 8q11.23–q12.1 region detected two independent association signals within the < 100-kb interval that spans the SOX17 locus (Fig. 2); these two signals are hereafter referred to as 5′-SOX17 and 3′-SOX17. Thus, the five chromosomal segments comprised six independent association signals for follow-up.

We performed replication genotyping in two Japanese cohorts including 3,111 cases and 1,666 controls (JP1 and JP2, see Table 1). For each independent signal, we selected for replication the genotyped SNP with the highest PPA and added up to two additional SNPs per locus. For the 5′-SOX17 region, we selected two SNPs analyzed previously, as they tag the most significant SNP in the current study.

All but one of the SNPs (rs12411886 on 10q24.32 in JP1) were successfully genotyped and passed quality-control filters. We tested for association of each SNP with intracranial aneurysm using logistic regression stratified by gender, specifying the same model as for the discovery cohort. We combined results from JP1 and JP2 using a fixed-effects model (Table 2). We considered an association to be replicated if the Bayes factor increased the odds of association more than tenfold after the replication data was observed.

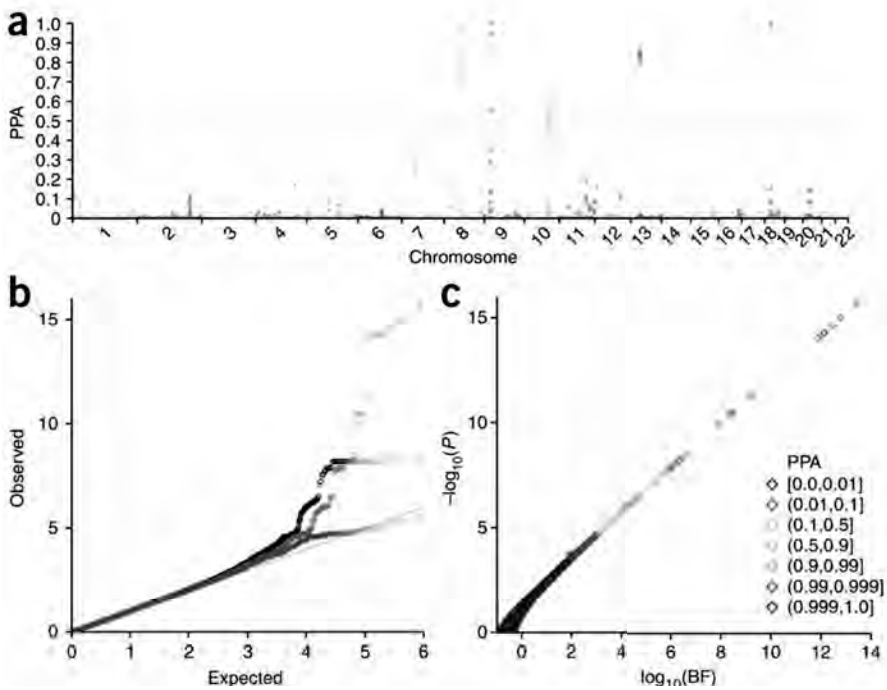

Figure 1. **Genome-wide association analysis results in the discovery cohort.** (**a**) The PPAs for 831,532 quality control–passed SNPs that were analyzed specifying a prior probability of association of 1/10,000 are plotted against genomic locations of SNPs. A gray horizontal line at PPA = 0.5 indicates the cutoff value for follow-up genotyping. (**b**) Quantile-quantile plots of *P* values ($-\log_{10}$ scale) are shown for all the SNPs analyzed (black; *n* = 831,532); for SNPs after excluding those within previously identified regions (red; *n* = 830,907); and for SNPs after excluding all within the final associated intervals (blue; *n* = 830,158). (**c**) A scatter plot of $-\log_{10}$ *P* versus $\log_{10}$ Bayes factors is shown with color for each point indicating the range of PPA values. There are very close relationships among the *P* values for association, the Bayes factor and the PPA value. Note that, given a uniform prior probability of association, the PPA increases as the Bayes factor increases. A vertical line indicates the minimum PPA threshold at 0.5 (Bayes factor = 1.0×10^4) for follow-up.

Of the six candidate loci, all but the 5′-SOX17 interval were replicated, with replication P values ranging from 0.0019 to 1.0×10^{-7}, and the odds of association with intracranial aneurysm increasing by 22.9-fold to 1.5×10^5-fold, yielding robust evidence for replication for each interval (Table 2).

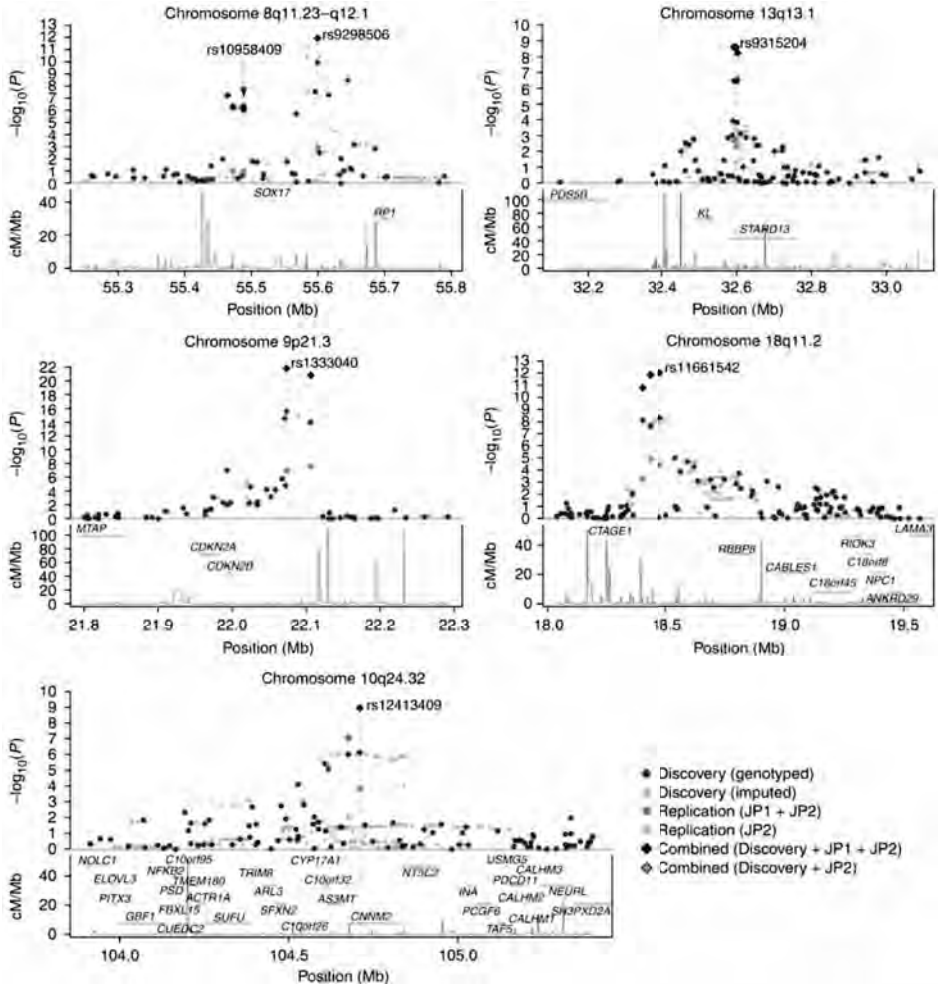

Figure 2. **Regional plots for associated regions.** For each chromosomal interval, $-\log_{10} P$ values for association are plotted against the genomic coordinates (NCBI build 36) in the panel above; the recombination rates obtained from the HapMap database and the RefSeq genes (hg18) within the regions are shown in the panel below. Above, rs identifiers of SNPs listed in table 2 are shown and their positions are indicated by gray vertical lines. Gray dashed lines indicate locations of other SNPs genotyped in the replication cohorts. Dark blue and light blue dots represent results of genotyped and imputed SNPs for the discovery cohort, respectively; orange and light orange squares represent association results for the replication cohort using JP1 combined with JP2 and also JP2-only, respectively; combined results for SNPs genotyped both in the discovery and the replication cohort using JP1 plus JP2 and JP2-only are shown by red and light red diamonds, respectively.

Table 2. Representative SNPs analyzed both in the discovery and replication cohorts.

locus	SNP	position	genes	risk allele	cohort	P value	$\log_{10}$ (Bayes)	PPA	per-allele OR (95% CI)	control RAF	case RAF
8q11.23	rs10958409	55,489,644	*SOX17*	A	Discovery	4.2×10^{-7}	4.64	0.8128	1.24 (1.14-1.35)	0.15, 0.19	0.18, 0.22
					Replication	0.12	-0.11		1.08 (0.98-1.20)	0.28	0.29
					Combined	9.0×10^{-7}	4.30	0.6685	1.17 (1.10-1.25)		
8q12.1	rs9298506	55,600,077	*SOX17*	A	Discovery	1.2×10^{-10}	7.94	0.9999	1.33 (1.22-1.45)	0.81, 0.76	0.85, 0.81
					Replication	0.0012	1.56		1.21 (1.08-1.36)	0.79	0.81
					Combined	1.3×10^{-12}	9.85	$1.0\text{-}1.4 \times 10^{-6}$	1.28 (1.20-1.38)		
9p21.3	rs1333040	22,073,404	*CDKN2A, CDKN2B*	T	Discovery	2.5×10^{-16}	13.41	$1.0\text{-}3.9 \times 10^{-10}$	1.32 (1.24-1.41)	0.56, 0.45	0.63, 0.53
					Replication	1.0×10^{-7}	5.18		1.31 (1.19-1.45)	0.66	0.72
					Combined	1.5×10^{-22}	19.48	$1.0\text{–}3.3 \times 10^{-16}$	1.32 (1.25-1.39)		
10q24.32	rs12413409	104,709,086	*CNNM2*	G	Discovery	7.9×10^{-7}	4.29	0.6621	1.38 (1.22-1.57)	0.91, 0.91	0.94, 0.93
					Replication	0.00014	2.34		1.23 (1.10-1.37)	0.74	0.77
					Combined	1.2×10^{-9}	7.00	0.999	1.29 (1.19-1.40)		
13q13.1	rs9315204	32,591,837	*KL, STARD13*	T	Discovery	3.3×10^{-7}	4.73	0.8443	1.21 (1.13-1.31)	0.21, 0.33	0.24, 0.39
					Replication	0.0019	1.36		1.18 (1.06-1.31)	0.24	0.27
					Combined	2.5×10^{-9}	6.72	0.9981	1.20 (1.13-1.28)		
18q11.2	rs11661542	18,477,693	*RBBP8*	C	Discovery	5.6×10^{-9}	6.39	0.9959	1.21 (1.14-1.30)	0.49, 0.44	0.54, 0.47
					Replication	4.5×10^{-5}	2.79		1.22 (1.11-1.34)	0.61	0.65
					Combined	1.1×10^{-12}	9.92	$1.0\text{-}1.2 \times 10^{-6}$	1.22 (1.15-1.28)		

Genomic locations for SNPs are based on NCBI build 36, and risk alleles are aligned to the forward strand of the reference sequence. Control and case risk allele frequencies (RAFs) for the discovery cohort are shown in the form: RAF of European cohort, RAF of Finnish cohort. Log_{10} (Bayes) indicates the logarithm of the Bayes factor in favor of association. PPA, posterior probability of association. Genes closest to the listed SNPs within the same LD regions are shown.

We combined the discovery and replication results using a fixed-effects model. All of the five loci that replicated in the Japanese cohort surpassed the conventional threshold for genome-wide significance ($P < 5 \times 10^{-8}$), with P values ranging from 2.5 × 10-9 to 1.5×10^{-22}, and all also had PPAs ≥ 0.998 (Table 2).

In order to determine each cohort's contribution to the observed association and to assess the consistency of the effect size across groups, we analyzed each of them separately (Table 1) and then combined the results from the six cohorts using a random-effects model. The association results remained highly significant (Figure 3). For the five loci that were replicated in the Japanese cohorts, we found no evidence of significant heterogeneity (P > 0.1). Every cohort had the same risk allele and provided support for association with the exception of the JP1 sample for the 3'-SOX17 locus, consistent with our previous study [34] (Figure 3).

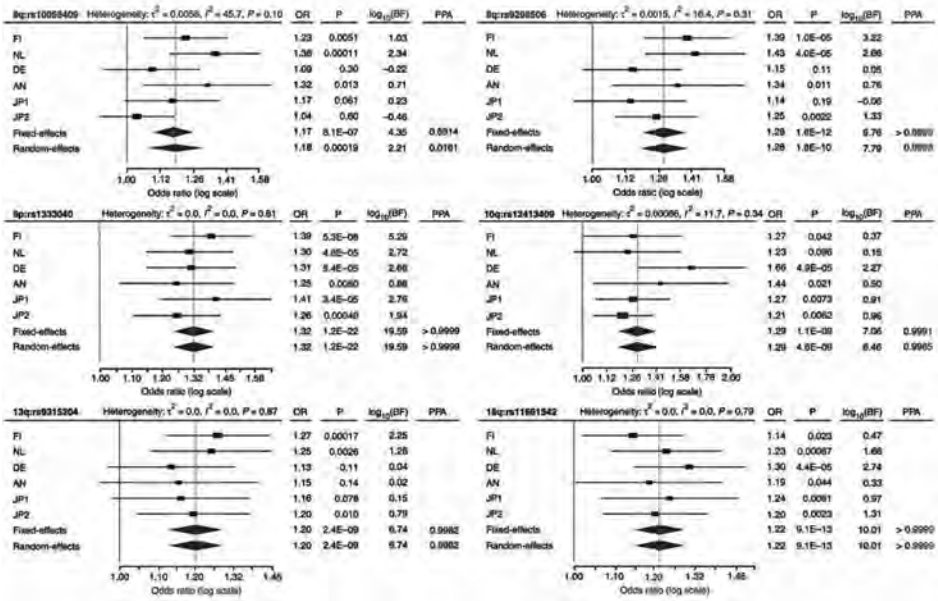

Figure 3. **Consistency of association across cohorts.** Forest plots are shown for meta-analysis of the SNPs listed in table 2. Squares and horizontal segments represent estimated per-allele ORs and 95% CIs for individual cohorts. Diamonds represent the summary OR estimates and 95% CIs for the meta-analyses of six cohorts (using fixed- and random-effects models). Log_{10} (Bayes factor) > 0 supports association with intracranial aneurysm, whereas log_{10} (Bayes factor) < 0 supports no association with intracranial aneurysm. Analyzing the results here as six distinct cohorts rather than four cohorts (as in the primary analysis) resulted in only minor differences due to different weights given to sub-cohorts of the combined European cohort.

The most significant association was detected in the previously reported [34] 9p21.3 region near CDKN2A and CDKN2B with $P = 1.5 \times 10^{-22}$ (OR = 1.32, PPA > 0.9999). All of the newly studied cohorts strongly supported this association with intracranial aneurysm (Figure 3). The same allele is associated with coronary artery disease but not with type 2 diabetes [121]. Similarly, the previously reported 8q11.23-q12.1 region showed significant association. The 3′-SOX17 interval (rs92986506) showed robust association with $P = 1.3 \times 10^{-12}$ (OR = 1.28, PPA > 0.9999) and all new cohorts supported the association of this SNP with intracranial aneurysm (Figure 3). For the 5′-SOX17 region (rs10958409), the new cohorts introduced a substantial heterogeneity, lowering the PPA to 0.016 (Figure 3).

Among the newly identified loci, the strongest association was found at rs11661542 on 18q11.2 (OR = 1.22, $P = 1.1 \times 10^{-12}$, PPA > 0.9999). A cluster of SNPs that is associated with intracranial aneurysm spans the interval between 18.400 Mb and 18.509 Mb and is strongly correlated with rs11661542 (Figure 2). A single gene, RBBP8 (encoding the retinoblastoma binding protein 8), is located within an extended linkage disequilibrium interval (Figure 2).

T he second strongest new association was at rs12413409 on 10q24.32 (OR = 1.29, $P = 1.2 \times 10^{-9}$, PPA = 0.9990), which maps to intron 1 of CNNM2 (encoding cyclin M2) (Fiurre 2). A cluster of SNPs that are strongly correlated with rs12413409 and are located within a ~247-kb interval in the same linkage disequilibrium block supported the association (Figure 2).

The third new locus is defined by rs9315204 at 13q13.1 (OR = 1.20, $P = 2.5 \times 10^{-9}$, PPA = 0.9981) in intron 7 of STARD13 (encoding the StAR-related lipid transfer (START) domain containing 13) (Figure 2). Two SNPs, rs1980781 and rs3742321, that are strongly correlated with rs9315204 ($r^2 > 0.9$) also showed significant association with intracranial aneurysm (Figure 2). These two SNPs are missense (lysine to arginine) and synonymous coding variants of STARD13, respectively. Another gene that has been implicated in aging phenotypes, KL (encoding klotho), is located nearby [122].

A search of the gene-expression database (eQTL browser, see URLs) for all the intracranial aneurysm-risk loci did not reveal any consistent pattern of association of intracranial aneurysm SNPs with variation in gene expression levels.

In this second GWAS of intracranial aneurysm, which included nearly three times as many cases as the initial study, we detected three new risk loci and obtained strong independent evidence for association of two previously identified loci. The evidence that these are bona fide risk loci for intracranial aneurysm is very strong from both Bayesian measures and conventional P values.

Given our power (~90%) to detect variants that confer risk of intracranial aneurysm with GRR = 1.25 and MAFs ≥ 10%, we expect that we have identified most of these variants, limited principally by potential gaps in SNP coverage. Indeed, across the rest of the genome, there was no locus with PPA > 0.22 and

MAF ≥ 10%, whereas there were 14 loci with PPAs between 0.1 and 0.22 and ORs between 1.16 and 1.25 (data not shown). We expect that a fraction of these loci are genuine intracranial aneurysm risk loci, as suggested by the excess of SNPs with $P < 1 \times 10^{-3}$ (Figure 1b); exploring this possibility will require analysis of larger intracranial aneurysm cohorts and/or genotyping of alleles with lower MAFs.

Based on the results of the first GWAS of intracranial aneurysm and the role of the implicated gene products, Sox17 and $p15^{INK4b}$-$p16^{INK4a}$, we previously hypothesized [34] that the genes associated with intracranial aneurysm might play a role in determining cell cycle progression and may affect the proliferation [123] and senescence of progenitor-cell populations and/or the balance between production of progenitor cells versus cells committed to differentiation. Genes located within the newly identified regions support this idea. The protein encoded by RBBP8, located within the 18q11.2 region, influences progression through the cell cycle by interacting with BRCA1 [124]. Similarly, of the two genes located within the 13q13.1 interval, STARD13 contains the Rho-GAP and C-terminal STAR-related lipid transfer (START) domains and its overexpression results in suppression of cell proliferation [125]. The other gene implicated here, KL, encodes a transmembrane protein that modulates FGF receptor specificity [126]; mice lacking KL show accelerated aging in diverse organ systems [122].

On the assumption that there is a fourfold increase in the risk of intracranial aneurysm among siblings of cases [127, 128] and that the SNPs combine to increase log-odds of disease in an additive fashion, the five intracranial aneurysm risk loci explain 5.2% (within the Finnish cohort), 4.0% (in the European cohort) and 3.5% (in the combined JP1 and JP2 cohort) of the familial risk of intracranial aneurysm. Under this model, the odds of developing an intracranial aneurysm varies 4.99- to 7.63-fold across the top and bottom 1% of genetic risk profiles at these loci in the populations studied here and 3.61- to 4.64-fold across the 5% extremes. When combined with traditional risk factors such as gender, blood pressure and smoking, these findings form the basis of future work aimed at preclinical identification of individuals who are at high risk of intracranial aneurysm formation and rupture.

Methods

Genotyping

Whole-genome genotyping for the discovery cohort was performed on the Illumina platform according to the manufacturer's protocol (Illumina). Replication genotyping in the JP1 cohort was performed using either Taqman (Applied Biosystems) or MassARRAY (Sequenom) assays. For the JP2 cohort, genotyping for cases was performed using the multiplex PCR-based Invader assay (Third Wave Technologies Inc.); genotyping for controls was performed on an Illumina platform as described previously [129].

Ethics

The study protocol was approved by the Yale Human Investigation Committee (HIC protocol #7680). Institutional review board approval for genetic studies, along with written consent from all study participants, was obtained at all participating institutions.

Data storage and analysis tools

We used PLINK [130] v1.06 and R statistical environment v2.9.0 (in particular, the snpMatrix package [131]) for storage of genotype data and data analysis.

Preprocessing

Prior to the analysis of genotyping data, we excluded SNPs that were located either on mitochondrial DNA or sex chromosomes, SNPs with A/T or C/G alleles, those for which all subjects were assigned as 'no call', and those that were assayed on Hap300v1 or 550v1 but were dropped from newer versions.

Sample quality control

We excluded subjects in the discovery cohort who did not conform to our study design on the basis of genotyping and information quality, cryptic relatedness and population outliers. This filtering process resulted in the inclusion of 835 cases and 6,529 controls in the Finnish cohort and 2,000 cases and 8,722 controls in the rest of the combined European cohort.

Imputation

We performed imputation analysis with the HapMap phase II CEU reference panel (release 24) using the IMPUTE v1 software [115]. The analysis was performed separately for the Finnish and European cohorts. We converted posterior probabilities of three possible genotypes to fractional allele dosage scores (between 0 and 2) and used these scores for association tests in order to take into account the imputation uncertainty [132]. For the quality assessment of imputed SNPs, we also converted the posterior probabilities to the most likely genotypes with the threshold at 0.9.

Case-control matching

Population stratification and independent genotyping of cases and controls are major causes of confounding in GWAS [133]. Because our study consisted of multiple independently ascertained cohorts that were genotyped separately, we performed a stringent analysis to control for these biases by inferring the genetic ancestries of subjects [134, 135]. We used the Laplacian eigenmaps [136] to infer population structure. Following the determination of the number of dimensions (K + 1) using the threshold given in Lee *et al*. [137], we used the K-dimensional

nontrivial generalized eigenvectors [138] to calculate the Euclidean distance between any two subjects.

In the course of this analysis, we excluded 'isolated' subjects who were identified by using the nearest-neighbor distance distributions in any of the two-dimensional sections. After excluding these subjects, we observed 13 dimensions in the Finnish cohort and 5 dimensions in the European cohort. The larger dimensions observed in the Finnish sample could be attributable to the presence of many isolated populations in Finland [113].

Before matching, we stratified data into males and females because female gender is a known risk factor of intracranial aneurysm [12, 112]. We also set the maximum distance between cases and controls to match to be less than 0.028 in the Finnish cohort and 0.009 in the European cohort. These values were determined by examining the distribution of the nearest-neighbor distances in K dimensions (data not shown). We matched cases and controls using the fullmatch function in the R-package optmatch [139, 140].

SNP quality control

For both genotyped and imputed SNPs in the discovery cohort, we applied quality-control filters to individual cohorts and to cases and controls separately on the basis of the missing rate, MAF and the P value of the exact test of Hardy-Weinberg equilibrium [141]. For imputed SNPs, we also assessed imputation quality using the average posterior probability, MAF and allelic R^2 metric [142]. Finally, we assessed differential missingness between cases and controls.

Any genotyped SNP that passed the quality-control filters both in the European and Finnish cohorts was referred to as a 'genotyped SNP', and any one for which we used the quality control–passed imputation data either in one or both of the cohorts was classified as an 'imputed SNP'.

For genotyping data of the replication cohorts, we excluded SNPs if any of the following three conditions were met in either cases or controls: (i) missing rate > 0.05; (ii) P value of the exact test of Hardy-Weinberg equilibrium < 0.001; or (iii) MAF < 0.01.

Statistical analysis

Cohort-wise association analysis

We tested for association between each quality control-passed SNP and intracranial aneurysm using conditional and unconditional logistic regression for the discovery and replication cohorts, respectively [143]. For the discovery cohort, we used the matched strata to correct for potential confounding due to population stratification and gender, and for the replication cohorts we adjusted for gender. We assumed the log-additive effect of allele dosage on disease risk. We obtained P values from

the score test (two-sided) and estimated the logarithm of per-allele ORs with standard errors by maximizing the conditional or unconditional likelihood. Both the test statistic and the standard error of the log of the OR were corrected using genomic control [116]. We performed the association analysis for the Finnish and European cohorts, as well as subcohorts of the European group that consisted of NL cases, DE cases or @neurIST cases and their matched controls (Table 1). We used the following R functions to perform the association analysis: clogit, glm and snp.rhs.tests [131].

Meta-analysis

We combined the cohort-wise per-allele ORs in the Finnish and European cohorts using a fixed-effects model of meta-analysis for 831,534 quality control–passed SNPs to obtain the discovery results. For SNPs analyzed both in the discovery and replication cohorts, we combined JP1 and JP2 to obtain replication results and all four cohorts to obtain combined results. Our primary analysis was based on the fixed-effects model [132]. To assess the heterogeneity of the effect size between cohorts, we first divided the European cohort into three groups as described above, aiming to analyze the data without averaging effect sizes over the combined European cohorts and then combined our six cohorts using the random-effects model. We employed the restricted maximum likelihood procedure to estimate the between-cohort heterogeneity variance (τ^2) using the R function MiMa [144] (see URLs). From this estimate, we calculated the Cochran's Q statistic and the I^2 statistic (the percentage of variation across studies that is due to heterogeneity rather than chance) [145].

Bayesian evaluation of the strength of association

To evaluate the strength of association with intracranial aneurysm, we used a Bayesian approach [118, 146]. A limitation of the use of P values alone is that variability in factors such as effect size, MAF and sample size can result in identical statistics that might correspond to markedly different levels of evidence regarding the strength of association [119]. The Bayes factor provides an alternative that compares the probabilities of the data under the alternative hypothesis of association versus the null hypothesis of no association. For computational simplicity, we approximated the Bayes factor as described by Wakefield [117]. For all SNPs, we assumed the same prior distribution for the log-OR: a normal distribution with a mean of 0 and a standard deviation of $\log(1.5)/\Phi^{-1}(0.975)$, where Φ is the normal distribution function [118].

The PPA [119] provides a simple probabilistic measure of evidence by introducing the prior probability of association, π_1. We assumed a uniform prior, $\pi_1 = 1/10{,}000$, for all the SNPs [120]. For Bayes factor > 10^6, changing π_1 to a more conservative value of 1/100,000 would result in little change in the PPA.

To combine the results from multiple cohorts, we extended the formula [147] to be applicable to multiple (> 2) cohorts.

Conditional analysis

For each region that contained a SNP with PPA > 0.5, we examined the number of independent association signals by testing for association of every genotyped SNP with intracranial aneurysm by adjusting for the effect of a specified SNP.

Two-locus interaction analysis

We tested for deviation from a linear model, which assumes that two SNPs combine to increase the log-odds of disease in an additive fashion, using conditional (in the Finnish and European cohorts) or unconditional (in JP1 plus JP2, stratified by cohorts and gender) logistic regression. There was no significant deviation from the linear model (data not shown).

Cumulative effect

We evaluated potential clinical implications of the genetic profiles of the five intracranial aneurysm risk loci following the approach described by Clayton [148]. We fitted a five-locus conditional (Finnish and European cohorts) or unconditional (Japanese cohorts) logistic regression model including the additive and dominance-deviation terms for each locus. Using the estimated effect sizes and each individual's genotype, we calculated the risk scores for every individual. The receiver-operating characteristic curve for each ethnic cohort (Finnish, European and Japanese) was depicted using the risk score.

We also calculated the ratio of the exponential of the mean of the risk scores for control subjects within the top versus bottom 5% or 1% tails of distribution of risk scores in each cohort to obtain approximated odds ratios of disease between these classes.

The sibling recurrence risk was estimated by assuming the polygenic model that fits well to our data [148]. A fraction of the sibling recurrence risk attributable to all of the five loci was calculated by taking the ratio of the logarithm of this value and epidemiologically estimated value of 4 [127, 128].

URLs. eQTL browser, http://eqtl.uchicago.edu/.

Chapter 4

Prediction

4.1 Prediction of 60-day case-fatality after aneurysmal subarachnoid haemorrhage

Background and Purpose: Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating event with substantial case-fatality. Our purpose was to examine which clinical and neuro-imaging characteristics, available on admission, predict 60-day case-fatality in aSAH and to evaluate performance of our prediction model.
Methods: We performed a secondary analysis of patients enrolled in the International Subarachnoid Aneurysm Trial (ISAT), a randomised multicentre trial to compare coiling with clipping in aSAH patients. Multivariable logistic regression analysis was used to develop a prognostic model to estimate the risk of dying within 60 days from aSAH based on clinical and neuro-imaging characteristics. The model was internally validated with bootstrapping techniques.
Results: The study population comprised of 2,128 patients who had been randomised to either endovascular coiling or neurosurgical clipping. In this population 153 patients (7.2%) died within 60 days. World Federation of Neurosurgical Societies (WFNS) grade was the most important predictor of case-fatality, followed by age, lumen size of the aneurysm and Fisher grade. The model discriminated reasonably between those who died within 60 days and those who survived (*c* statistic = 0.73), with minor optimism according to bootstrap re-sampling (optimism corrected *c* statistic = 0.70).
Conclusion: Several strong predictors are available to predict 60 day case-fatality in aSAH patients who survived the early stage up till a treatment decision; after external validation these predictors could eventually be used in clinical decision making.

Introduction

Subarachnoid haemorrhage (SAH) is a devastating event, which is marked by sudden onset of severe headache, causing substantial case-fatality. In 85% of the patients, the SAH is caused by rupture of an aneurysm (aSAH) [1, 40]. From those who survive the first month, approximately one third remains dependent with respect to daily activities during their remaining lifetime [40]. Amongst patients who regain independency, quality of life remains reduced [149].

Early prediction of short term outcome in terms of case-fatality may support clinical decision making and may provide realistic and evidence based expectations to patients and relatives. Predictions may also be used to classify patients according to prognostic risk, which may be useful to compare outcome between different patient series, to study treatment results over time, or to stratify patients for randomised clinical trials (RCT).

Several other prognostic studies on outcome after aSAH have been performed, but most included relatively small numbers. Two included substantial numbers allowing analysis of the effects of multiple independent factors [150, 151]. However, these studies did not examine case-fatality, but arbitrarily dichotomized ordinal outcome scales (modified Rankin Scale or Glasgow Outcome Scale). Moreover, due to their design, these studies were unable to examine the effect of treatment on prediction of outcome.

Our aim was to develop a prognostic model for 60-day case-fatality, based on clinical features and neuro-imaging, regularly readily available on admission to a neurological or neurosurgical unit. These data were obtained from a large RCT conducted in mainly European countries.

Methods

Patients

Data were collected prospectively by the Medical Research Council funded International Subarachnoid Aneurysm Trial (ISAT) (International Standard Randomised Controlled Trial, number ISRCTN49866681). Full details of the ISAT study are available elsewhere [152]. The aim of the trial was to determine whether treatment using endovascular coiling reduced the risk of patients being dependent or dead at one year by 25 percent when compared with neurosurgical treatment (clipping).

Predictors and outcome

We considered all patient characteristics that can be collected easily and reliably within the first hours after hospital admission and that were also present in the ISAT database. These included age, gender, previous occurrence of SAH, CT scan

Fisher grading, lumbar puncture, World Federation of Neurosurgical Societies (WFNS) grading, number of intracranial aneurysms, location of the aneurysm, maximum lumen size of the aneurysm, vasospasm on angiography, and intended treatment by randomization. Fisher grading of blood visible on a plain CT scan runs from grade 1 ("no blood visible") up to grade 4 ("intraventricular or intraparenchymal blood"). Lumbar puncture was not performed in all participants. If it was performed it was graded 1 ("no blood in cerebrospinal fluid") or 2 ("xanthochromia or blood"); 0 otherwise ("no lumbar puncture"). WFNS scale runs from grade 1 ("Glasgow Coma Scale (GCS) 15 and no motor deficit") to grade 5 ("GCS 3-6 with or without motor deficit"). One category additional to the standard WFNS scale was created in ISAT for those in whom WFNS could not be assessed; 'grade 6'. The number of aneurysms was categorized in 1, 2, and 3 or more intracranial aneurysms. We discerned four aneurysm locations: Anterior Cerebral Artery (ACA), Internal Carotid Artery (ICA), Middle Cerebral Artery (MCA), and Posterior Circulation (PC). The maximum lumen size of the aneurysm was expressed in millimetres. Vasospasm was examined on angiography and categorized as 'none', 'mild', 'moderate', or 'severe'. Treatment was either neurosurgical clipping or endovascular coiling; we used treatment as allocated by the randomization procedure. We developed the model based on cases with a complete set of data. The outcome was 60-day case-fatality.

Model

We used univariate logistic regression analysis to estimate the association between single predictors and outcome, expressed as an odds ratio (OR). Predictors have a statistically significant effect if the 95 percent confidence interval (95% CI) does not include the value one. The prediction model was developed with multivariable logistic regression with backward stepwise selection. All potential predictors were entered into the model and those that met Akaike's Information Criterion (AIC) were selected into the model. AIC compares models based on how well they fit the data, but penalizes for the complexity of the model. AIC requires that the increase in model χ^2 when entering a new predictor has to be larger than two times the degrees of freedom: $\chi^2 > 2\ df$. When considering a predictor with 1 *df*, such as gender, this implies that χ^2 has to exceed 2, equivalent to $p < 0.157$. When considering a predictor with 2 *df*, $\chi^2 > 4$, or $p<0.135$; and in case of 4 *df*, $\chi^2 > 8$, or $p < 0.092$ [153].

Performance

The performance of the model was assessed with respect to calibration and discrimination. Calibration is the ability of the model to produce unbiased estimates of the probability of the outcome. Calibration was examined with a goodness of fit test, which assesses agreement between predicted and observed risks over the full range of predicted probabilities [154].

Discrimination is the model's ability to separate patients with different outcomes. To quantify the discrimination, we used the concordance (*c*) statistic. For binary outcomes, *c* is identical to the area under the receiver operating characteristic curve [153]. The *c* statistic evaluates whether those with higher predicted risk are more likely to die within 60 days among all possible pairs of patients with different outcomes. A model with a *c* statistic of 0.5 has no discriminative power at all, for example a coin flip. A *c* statistic of 1.0 reflects perfect discrimination.

Model validation

The performance of a prediction model is generally worse in new patients than initially expected. This 'optimism' can be studied with internal validation techniques [153]. Internal validity of our model was assessed with standard bootstrapping procedures [153]. Bootstrapping involves drawing samples of patients with replacement from the study population. Each sample can be considered as if one is repeating the data collection with the same number of patients and under identical circumstances as the original. The multivariable logistic regression coefficients were re-estimated in 300 bootstrap samples. Each of these 300 models was evaluated on the original sample. The average difference in the *c* statistic was determined to indicate the optimism in the initially estimated discriminative ability [153]. A shrinkage factor was estimated from the bootstrap validation procedure and we shrunk the regression coefficients to provide better predictions for future patients [153].

All statistical analyses were performed using R software, version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 2,143 patients were recruited into the ISAT trial by 43 neurosurgical centres, mainly in Europe. CT scans of 14 patients were not performed or available, and in one patient no information on vasospasm was available. We excluded these cases from our analysis. Data on the outcome were available for all patients. Thus, we performed complete case analysis on 2,128 patients (99.3%) of whom 153 (7.2%) died within 60 days.

The distribution of patient characteristics of the study population is presented in Table 1. For reasons of small numbers in the 'severe' category of vasospasm, we aggregated data from the 'moderate' and 'severe' categories into one category. Univariate analysis showed a statistically significant relation with 60-day case-fatality for age, lumen size, Fisher grade, lumbar puncture, WFNS grade, and vasospasm. Sex, location and number of aneurysms and intended treatment were not significantly associated with 60-day case-fatality (Table 1). In the multivariable model with stepwise backward selection age, lumen size, Fisher grade, and WFNS

grade met AIC and were included in the final model. In Table 2 the chi square statistics with corresponding p-values are presented as well as the point estimate of the OR. Age and WFNS grade were the most important predictors.

Table 1. Population characteristics and univariate association with 60-day case fatality.

Predictor	**alive**	**n=1975**	**death**	**n=153**					
	median	**IQR**	**median**	**IQR**	**beta**	**SE**	**OR**	**CI_{min}**	**CI_{max}**
age [10years]	5.2	4.3-5.9	5.6	5.0-6.5	0.36	0.08	1.43	1.23	1.66
lumensize [mm]	5	4-7	6	4-8	0.10	0.02	1.10	1.05	1.15
	n	**%**	**n**	**%**					
Sex									
female	1236	63	103	67			1		
male	739	37	50	33	-0.21	0.18	0.81	0.57	1.15
previous SAH									
yes	129	7	6	4	-0.54	0.43	0.58	0.25	1.35
no	1846	93	147	96			1		
Fisher grade									
1	112	6	2	1			1		
2	350	18	10	7	0.47	0.78	1.60	0.35	7.41
3	840	43	62	41	1.42	0.73	4.13	1.00	17.13
4	673	34	79	52	1.88	0.72	6.57	1.59	27.13
Lumbar puncture									
xantochromia or blood	217	11	7	5	-0.95	0.39	0.39	0.18	0.84
no blood	5	0	0	0	-5.19	20.8	0.01	0.00	∞
no puncture	1753	89	146	95			1		
WFNS grade									
1	1270	64	54	35			1		
2	495	25	51	33	0.89	0.20	2.42	1.63	3.60
3	120	6	13	8	0.94	0.32	2.55	1.35	4.80
4	55	3	19	12	2.09	0.30	8.12	4.51	14.63
5	13	1	7	5	2.54	0.49	12.66	4.86	33.02
6 (not assessable)	22	1	9	6	2.26	0.42	9.62	4.23	21.89
n of aneurysms detected									
1	1555	79	116	76			1		
2	314	16	29	19	0.21	0.22	1.24	0.81	1.89
>=3	106	5	8	5	0.10	0.38	1.10	0.52	2.32

Predictor	alive	n=1975	death	n=153					
	median	IQR	median	IQR	beta	SE	OR	CI_{min}	CI_{max}
Location									
ACA	1008	51	71	46	-0.16	0.19	0.85	0.59	1.23
ICA	638	32	53	35			1		
MCA	277	14	23	15	0.00	0.26	1.00	0.60	1.66
PC	52	3	6	4	0.33	0.45	1.39	0.57	3.38
vasospasm									
none	1575	80	109	71			1		
mild	218	11	24	16	0.46	0.24	1.59	1.00	2.53
moderate/severe	182	9	20	13	0.77	0.26	2.15	1.30	3.55
intended treatment									
clip	983	50	83	54			1		
coil	992	50	70	46	-0.18	0.17	0.84	0.60	1.16

IQR = inter quartile range; beta = regression coefficient in the logistic regression model; SE = standard error; OR = odds ratio; CI_{min} = lower limit of the 95% confidence interval; CI_{max} = upper limit of the 95% confidence interval.

Table 2. Statistical parameters of the final model.

factor	X^2	*df*	p-value	OR	95% CI[a]
WFNS grade	51	5	8×10^{-10}	grade 1 = ref.	
2				1.87	1.23-2.83
3				1.70	0.87-3.32
4				4.87	2.60-9.14
5				7.00	2.54-19.28
6				5.75	2.41-13.73
age [10 yrs]	17	1	4×10^{-5}	1.32	1.13-1.55
lumen size [mm]	12	1	4×10^{-4}	1.08	1.03-1.13
Fisher grade	8	3	0.04	grade 1 = ref.	
2				1.43	0.27-7.65
3				2.67	0.53-13.51
4				2.76	0.54-14.14

X^2 is the chi squared test statistic for the predictor in the final model; *df* = degrees of freedom; [a] 95% CI was calculated based on the S.E. of the estimates of the coefficients in the full model to avoid underestimation of uncertainty.

The goodness of fit test yielded a p-value of 0.86, suggesting that the model fitted the data in which it was developed well. The *c* statistic of the original model was 0.73, meaning that the model discriminates reasonably between patients who die within 60 days from onset of the SAH and those who survive this period.

Validation by means of 300 bootstrap samples resulted in a shrinkage factor of 0.85, which was applied to the betas of the model. The *c* statistic of the internally validated model was 0.70. Details of the final prognostic model for 60-day case-fatality are described in the appendix.

Discussion

We developed a prognostic model to predict the risk of 60 day case-fatality in individual patients after aSAH. Predictions were based on characteristics that are regularly readily available on admission to a neurological or neurosurgical unit and which were collected in a large clinical trial. The full model yielded a *c* statistic of 0.73.

Previously, several models to estimate the probability of unfavourable outcome after aSAH have been developed. Our model was similar to those; we included roughly the same predictors: age, clinical status, and lumen size [150, 151, 155]. However, our study is of added value because of the substantial size and the inclusion of both clipped and coiled patients. The studies by Hoh *et al.* [150] (n = 515) and Mocco *et al.* [155] (n = 148) contained relatively few patients. The small numbers of coiled patients (79 and 35, respectively) and the design of the study did not allow for taking the effect of treatment in consideration. The study of Rosengart *et al.* [151] (n = 3667) was not able to do that either, since patients treated with Guglielmi or other detachable coils were excluded. All three studies used a dichotomized ordinal scale as an outcome, for which the cut off can be (arbitrarily) chosen in different studies. In a sense, examining case-fatality is also a dichotomization of an ordinal scale, though less arbitrary. Therefore, we are convinced that logistic regression is well suited for an outcome that is by its nature dichotomous, whereas for an ordinal outcome we would prefer specific modelling techniques.

Several limitations of this study should be acknowledged. This study used data from one large trial on a selected population of patients who survived the early stage up till a treatment decision and who were in equipoise regarding that decision on treatment with either endovascular coiling or neurosurgical clipping, which may limit external validity. The model may perform well in this development sample, but worse when applied to other groups of patients, for example, a less strictly selected population. Nonetheless, according to a recently published paper, the ISAT population proved to be quite similar to the population admitted with an aSAH to neurosurgical units in the United Kingdom [156]. Although in ISAT, a

lower proportion of poor grade patients were enrolled. Validation of a prognostic model in independent patient series is therefore considered an essential next step [157]. However, since large samples of systematically collected data on aSAH are sparse, assessment of external validity is difficult. For now the external validity of our model remains to be established. This will be a topic of future research.

Although our model represents knowledge obtained from 2,128 SAH patients in equipoise regarding treatment, statistical models can never replace the clinician with regard to decision making; they can only assist with this task. A prediction for an individual aSAH patient a particular situation is always subject to uncertainty. The model makes certain structural assumptions and statistical interaction terms were not included. It is hence possible that specific patterns of risk factors are inadequately reflected in the model predictions. Therefore, predictions should be regarded with care and not directly be applied for treatment limiting decisions.

Although the performance of the presented model was satisfactory, it might potentially be improved by including neuro-imaging biomarkers other than lumen size, location, Fisher grade on plain CT scan, and vasospasm on angiography. Biomarkers regarding anatomy and morphology might be considered, as well as aneurysm characteristics obtained from three and four dimensional angiography [158, 159]. Performance may also be improved by inclusion of subsequent information obtained after admission, including temporal course, neuro-imaging at later time points, eventual rebleeding of the aneurysm, delayed ischemic deficit, and other parameters such as hydrocephalus. The objective of the present study, however, was to investigate prognostic models that predict 60-day case-fatality with predictors available on admission.

Statistical testing for calibration has a number of drawbacks. First, the null hypothesis is of good calibration. Hence, if we test calibration in a small study, we have low power and will not reject the null hypothesis unless miscalibration is very severe. On the other hand, even a model with very good, though not perfect, calibration will fail the test in case of a sufficiently large sample. Moreover, reported goodness-of-fit tests are usually non-significant if they reflect apparent validation on the data that were also used to construct the model. Such non-significant results may contribute to the face validity of a model, but have no real scientific meaning [153].

In conclusion, we presented a prognostic model for predicting 60-day case-fatality after aneurysmal SAH. Our model contained age, lumen size, Fisher grade, and WFNS grade as predictors. After calibration and internal validation, our model showed reasonable performance, although external validity of our model remains to be established.

Appendix

Details of the Prognostic Model

The probability of dying within 60 days is calculated according to the logistic formula: $1/(1 + \exp^{-LP})$. The linear predictor (LP) takes the form of LP = intercept + regression coefficients × predictor values. LP for 60-day case-fatality = -5.812 + 0.2762 × age + 0.3572 × [Fisher grade II] + 0.9756 × [Fisher grade III] + 1.008 × [Fisher grade IV] + 0.6216 × [WFNS grade 2] + 0.5261 × [WFNS grade 3] + 1.574 × [WFNS grade 4] + 1.934 × [WFNS grade 5] + 1.738 × [WFNS grade not assessable] + 0.07662 × lumen size of aneurysm.

Coding of the predictors was as follows: age in decades, lumen size in millimetres; all other predictors, 1 if true and 0 if false.

4.2 Prediction of two month modified Rankin Scale with an ordinal prediction model in patients with aneurysmal subarachnoid haemorrhage

Background and Purpose: Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating event with a frequently disabling outcome. Our aim was to develop a prognostic model to predict an ordinal clinical outcome at two months in patients with aSAH.

Methods: We studied patients enrolled in the International Subarachnoid Aneurysm Trial (ISAT), a randomized multicentre trial to compare coiling and clipping in aSAH patients.

Several models were explored to estimate a patient's outcome according to the modified Rankin Scale (mRS) at two months after aSAH. Our final model was validated internally with bootstrapping techniques.

Results: The study population comprised of 2,128 patients of whom 159 patients died within 2 months (7.5%). Multivariable proportional odds analysis identified World Federation of Neurosurgical Societies (WFNS) grade as the most important predictor, followed by age, sex, lumen size of the aneurysm, Fisher grade, vasospasm on angiography, and treatment modality. The model discriminated moderately between those with poor and good mRS scores (*c* statistic = 0.65), with minor optimism according to bootstrap re-sampling (optimism corrected *c* statistic = 0.64).

Conclusion: We presented a calibrated and internally validated ordinal prognostic model to predict two month mRS in aSAH patients who survived the early stage up till a treatment decision. After external validation this model could eventually be used to support clinical decision making.

Introduction

Prediction research typically aims to predict outcome of individual patients after the onset of a certain disease, using prognostic models. These models, preferably based on data directly available at hospital admission, are essential to support clinical decision making, and to facilitate reliable comparison of outcomes between different patient series and variation in results over time. Furthermore, prognostic models have an important role in randomized controlled trials (RCT), for stratification [160] and statistical analyses that explicitly consider prognostic information, such as covariate adjustment [161, 162], and may provide realistic and evidence-based expectations to relatives.

The majority of published prognostic models predict a binary outcome, such as case-fatality using binary logistic regression [163-166]. Also, outcomes at ordinal scales are often considered as a dichotomized variable. However, there are several objections against collapsing an ordinal outcome scale into a binary one. First, the cut off for dichotomisation is arbitrary and may vary over studies in a single medical field [163, 164, 166]. Secondly, from a statistical perspective dichotomisation is a waste of information and reduces statistical power for the analysis of treatment effects or other covariates of interest [167, 168]. Furthermore, from a clinical point of view dichotomisation may lead to less useful models. For example, for a patient with a minor stroke a model predicting survival versus mortality is of limited value since the risk is low, while a prediction of complete recovery versus some remaining symptoms may be very useful. For a patient with a severe stroke, this will be the other way around.

An alternative for dichotomisation is application of a statistical approach that uses the full ordinal outcome scale. This leads to efficient use of the data and clinically relevant predictions. Several of these approaches for modelling ordinal response variables have been proposed, including proportional odds (PO) logistic regression, multinomial (or polytomous) logistic regression, or simple linear regression [153]. Each of these methods has its pros and cons.

Our aim was to develop an ordinal prognostic model to predict clinical outcome at two months in patients with aneurysmal subarachnoid haemorrhage (aSAH), based on clinical features and neuro-imaging which are regularly readily available on admission to a neurological or neurosurgical unit. SAH is a devastating event, causing substantial mortality. In 85% of the patients, the SAH is caused by rupture of an aneurysm (aSAH) [1, 40]. Of those who survive the first month, approximately one third remains dependent with respect to daily activities during the remaining lifetime [40]. Also amongst patients who regain independency, quality of life remains reduced [149]. A frequently used outcome measurement is the modified Rankin Scale (mRS) [169]. This is an ordered scale for measuring motor function and runs from 0 (no symptoms at all) to 6 (dead) (Table 1).

Table 1. Definition of the modified Rankin scale.

Grade	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

Material and methods

Patients

Data were collected prospectively by the Medical Research Council funded International Subarachnoid Aneurysm Trial (ISAT) (International Standard Randomised Controlled Trial, Number ISRCTN49866681). Full details of ISAT are available elsewhere [152]. The aim of the trial was to determine whether treatment using endovascular coiling reduced the risk of patients being dependent or dead at one year by 25 percent (as defined by modified Rankin Scale grade 3-6) when compared with neurosurgical treatment (clipping) for that cohort of patients.

Predictors and outcome

We considered all patient characteristics that could be obtained easily and reliably within the first hours after hospital admission and that were also present in the ISAT database. These included age, gender, previous occurrence of SAH, CT scan Fisher grading, World Federation of Neurosurgical Societies (WFNS) grading, number of intracranial aneurysms, location of the aneurysm, maximum lumen size of the aneurysm, vasospasm on angiography, and intended treatment at randomization. Fisher grading of blood visible on a plain CT scan runs from grade 1 ("no blood visible") up to grade 4 ("intraventricular or intraparenchymal blood"). WFNS scale runs from grade 1 ("Glasgow Coma Scale (GCS) 15 and no motor deficit") to grade 5 ("GCS 3-6 with or without motor deficit"). One additional grade was created in ISAT for those in whom WFNS could not be assessed; 'grade 6'. The number of aneurysms was dichotomized into one or more than one intracranial aneurysms. Four aneurysm locations were distinguished: Anterior Cerebral Artery (ACA), Internal Carotid Artery (ICA), Middle Cerebral Artery (MCA), and Posterior

Circulation (PC). The maximum lumen size of the aneurysm was expressed in millimetres. Vasospasm was examined on angiography and dichotomized into 'absent' or 'present'. Treatment was either neurosurgical clipping or endovascular coiling; we used treatment as allocated by the randomization procedure. The outcome measure in our study was the modified Rankin Scale (mRS) at two months (Table 1) [169].

Model

We started the development of the model discarding patients without information on outcome. The few missing values in predictors were imputed by means of single imputation (SI, in R language: aregImpute, n.impute=1, type='pmm').

A simple approach to analyze an ordinal outcome, such as the mRS, is to dichotomize the outcome variable by one of several possible cut off points, e.g. 01 vs. 23456 [164], 012 vs. 3456 [152, 163], 0123 vs. 456 [166], and 012345 vs. 6 (case-fatality) [165]. We applied binary logistic regression to develop models for these dichotomized responses. Next, we addressed the two main aspects of our ordinal outcome; the fact that it contains order and separate categories. A simple solution for modelling order, while neglecting the categorised nature of our outcome, is to apply linear regression using ordinary least squares. For the opposite – modelling categories, while neglecting order – we used multinomial regression. A more sophisticated approach is to use a proportional odds (PO) model. Such a model takes both order and separate categories into account. The PO logistic model is a rather straightforward extension of binary logistic regression [170]. A common set of regression coefficients is assumed across all levels of the outcome, and intercepts are estimated for each level. The advantage of the PO model is its parsimony in dealing with an ordered outcome. The price we pay is the assumption of proportionality of the odds. This assumption is equivalent to saying that any cut-point on the outcome scale would lead to the same (binary) logistic regression coefficient [153].

We inspected proportionality by studying the univariate odds ratios for each cut off for each predictor. We plotted the score residuals of binary logistic models for each potential predictor separately. The trend of the score components against the levels of the outcome scale should be flat if the proportional odds assumption holds [171]. When the PO assumption is not fulfilled for all potential predictors, we could also investigate a further alternative model: the partial PO model [172].

The association between predictors and outcome is expressed as odds ratios (OR). Predictors have statistically significant effects when the 95% confidence interval does not include the value one.

A multivariable PO model was developed containing predictors that met Akaike's Information Criterion (AIC) in a backward stepwise procedure [173]. AIC compares models based on how well they fit the data, but penalizes for the complexity of the model. AIC requires that the increase in model $\chi 2$ when entering

a new predictor has to be larger than two times the degrees of freedom: $\chi 2 > 2$ df. When considering a predictor with 1 df, such as gender, this implies that $\chi 2$ has to exceed 2, equivalent to $p < 0.157$. When considering a predictor with 2 df, $\chi 2 > 4$, or $p < 0.135$; and in case of 4 df, $\chi 2 > 8$, or $p<0.092$ [153].

Performance

The performance of the final PO model was assessed with respect to calibration and discrimination. Calibration is the ability of the model to produce unbiased estimates of the probability of the outcome. Calibration was tested with a goodness of fit test, which assesses agreement between predicted and observed risks over the full range of predicted probabilities. Discrimination is the model's ability to separate patients with different outcomes. To quantify the discrimination, we used the c statistic. A model with a c statistic of 0.5 has no discriminative power at all, for example a coin flip. A c statistic of 1.0 reflects perfect discrimination.

Model validation

The performance of a prediction model is generally worse in new patients then initially expected. This 'optimism' of the original model can be studied with internal validation techniques [153]. Internal validity of the models was assessed with standard bootstrapping procedures. Bootstrapping involves drawing samples of patients with replacement from the development population. Each sample can be considered as if one is repeating the data collection with the same number of patients and under identical circumstances as the original. Regression models were estimated in 300 bootstrap samples. Each of these 300 models was evaluated on the original sample. The average difference in the c statistic was determined to indicate the optimism in the initially estimated discriminative ability [153]. A shrinkage factor was estimated from the bootstrap validation procedure and we shrunk the regression coefficients to provide better predictions for future patients [153].

All statistical analyses were performed using R software, version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 2,143 patients were recruited to the ISAT trial by 43 neurosurgical centres, mainly in Europe. We excluded 15 patients with missing information on the two month mRS. Fisher grade of 14 patients was not available and in one patient no information on vasospasm was available. We statistically imputed these missing values, leaving 2,128 patients for analysis, of whom 347 were in mRS grade 0 (16%), 583 in mRS grade 1 (27%), 528 in mRS grade 2 (25 %), 296 in mRS grade 3 (14 %), 80 in mRS grade 4 (4 %), 135 in mRS grade 5 (6%) at the two month assessment, and of whom 159 (8 %) died before the two month assessment.

Table 2. Univariate associations for different cut offs for mRS (odds ratios), the univariate PO estimate (odds ratios), and linear regression (linear regression coefficient).

	1	2	3	5	PO	linear
age [10years]	1.19	1.38	1.48	1.49	1.26	1.28
lumensize [mm]	1.05	1.06	1.08	1.10	1.05	1.06
Sex						
Male	0.67	0.71	0.83	0.86	0.69	0.75
previous SAH						
Yes	1.19	1.01	0.61	0.55	1.04	0.93
Fisher grade						
1	1	1	1	1	1	1
2	0.93	1.39	2.29	2.01	0.99	1.09
3	1.29	2.51	4.45	4.32	1.50	1.67
4	2.03	4.47	7.72	7.31	2.49	2.72
WFNS grade						
1	1	1	1	1	1	1
2	1.94	2.47	2.60	2.40	2.08	1.99
3	3.34	4.46	3.67	2.64	3.51	3.18
4	6.74	7.85	12.55	7.84	8.38	7.99
5	6.06	10.83	16.26	12.00	12.43	11.25
(not assessable) 6	5.35	9.92	13.14	9.55	9.97	9.37
number of IA						
>1	1.11	1.21	1.30	1.17	1.15	1.15
location						
ICA	1	1	1	1	1	1
ACA	0.86	0.83	1.04	0.90	0.86	0.90
MCA	0.80	0.95	1.01	0.97	0.84	0.88
PC	0.89	0.83	1.74	1.39	0.97	1.06
vasospasm						
present	1.69	1.65	1.49	1.71	1.67	1.60
intended treatment						
Coil	0.69	0.60	0.81	0.88	0.68	0.73

Univariate analyses in the binary models for different cut offs, the PO model, and the linear regression model are presented in Table 2. The ORs for each cut off were reasonably similar except for previous SAH (fu_prevhaem) and Fisher grade 2 (Fisher=2). This violation of the PO assumption is also noted by statistically significant deviations from the horizontal line in Figure 1. The linear regression

coefficients were surprisingly close to the ORs from the PO model. The multinomial model yielded 108 coefficients, apart from 6 intercepts (not shown). In a partial PO model 6 intercepts were fitted, 6 coefficients for previous SAH, 18 coefficients for Fisher grade, and one for each of the other predictors (not shown).

For the sake of interpretability and clinical usefulness, we chose to accept the violation of the PO assumption in the PO model. Age and WFNS grade were the most important predictors in the multivariable PO model (Table 3). Other statistically significant predictors were sex, lumen size, Fisher grade, vasospasm, and treatment modality.

The goodness of fit test yielded a p-value smaller then 0.05 for all levels of mRS, suggesting that the model poorly fitted the data in which it was developed. In our final model the PO assumption was violated only for Fisher grade 2 (Figure 2). The c statistic of the final model was 0.65 (optimism-corrected: 0.64). Details of the model are described in the appendix.

Table 3. Final PO and linear models – shrunken estimates of OR and regression coefficients.

	PO			Linear		
	OR	***CI$_{min}$*** [a]	***CI$_{max}$*** [a]	***coefficient***	***CI$_{min}$*** [a]	***CI$_{max}$*** [a]
age [10years]	1.18	1.10	1.26	1.20	1.13	1.27
lumensize [mm]	1.04	1.01	1.07	1.04	1.02	1.07
Sex						
Male	0.78	0.66	0.91	0.85	0.73	0.98
Fisher grade						
1	1			1		
2	1.00	0.69	1.43	1.07	0.77	1.49
3	1.28	0.91	1.79	1.37	1.01	1.86
4	1.50	1.06	2.13	1.56	1.13	2.14
WFNS grade						
1	1			1		
2	1.74	1.44	2.10	1.68	1.42	1.98
3	2.43	1.75	3.39	2.27	1.69	3.04
4	6.04	3.90	9.37	5.70	3.89	8.35
5	7.90	3.43	18.17	7.56	3.72	15.35
(not assessable) 6	7.70	3.91	15.16	7.00	3.92	12.50
vasospasm						
present	1.36	1.13	1.64	1.30	1.10	1.54
intended treatment						
Coil	0.69	0.60	0.81	0.75	0.65	0.85

OR = odds ratio; CImin = lower limit of the 95% confidence interval; CImax = upper limit of the 95% confidence interval. [a] 95% CI was calculated based on the standard error of the estimates of the coefficients in the full model to avoid underestimation of uncertainty.

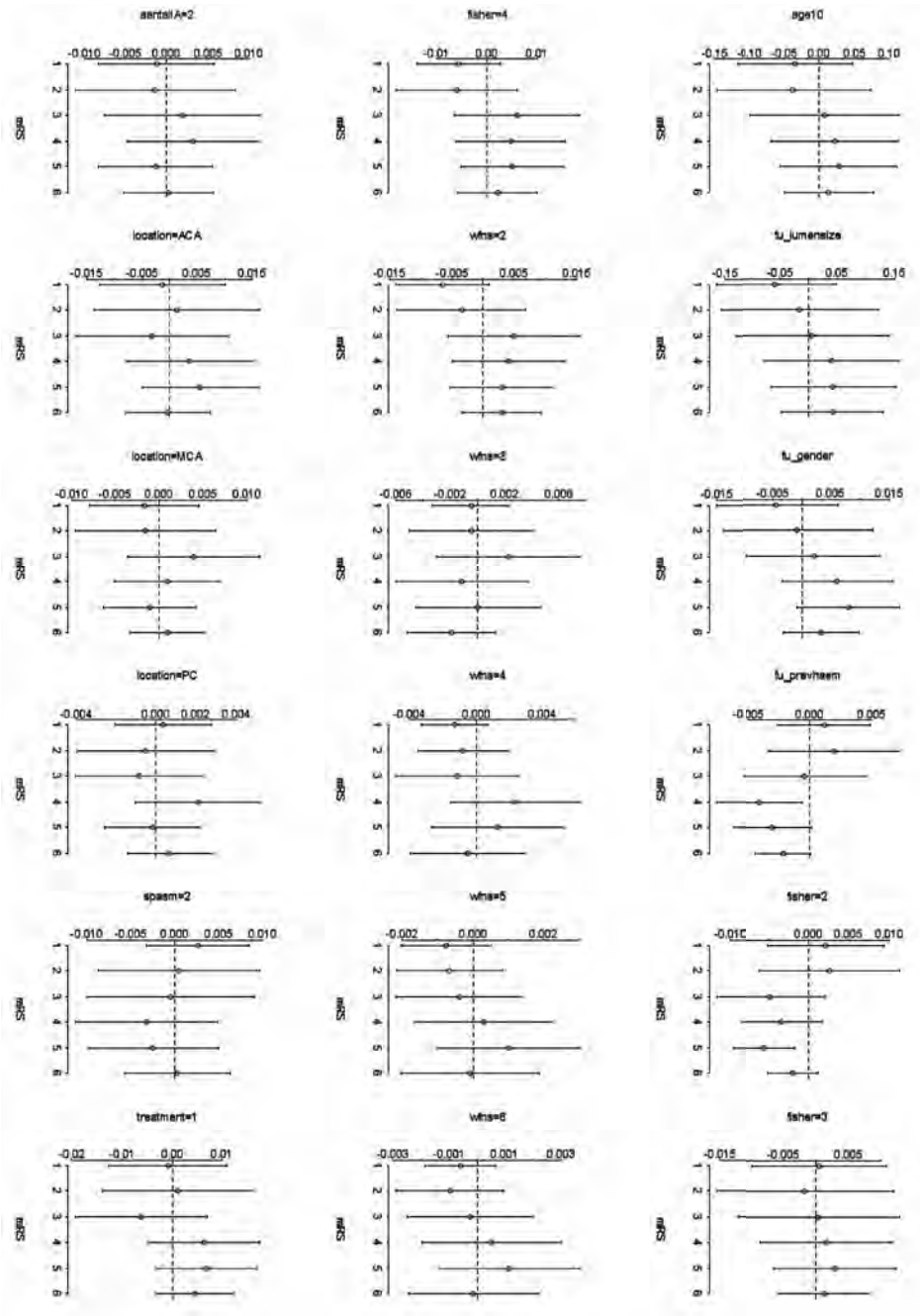

Figure 1. Residual plots (score.binary) of univariate associations of potential predictors to examine deviations from the PO assumption.

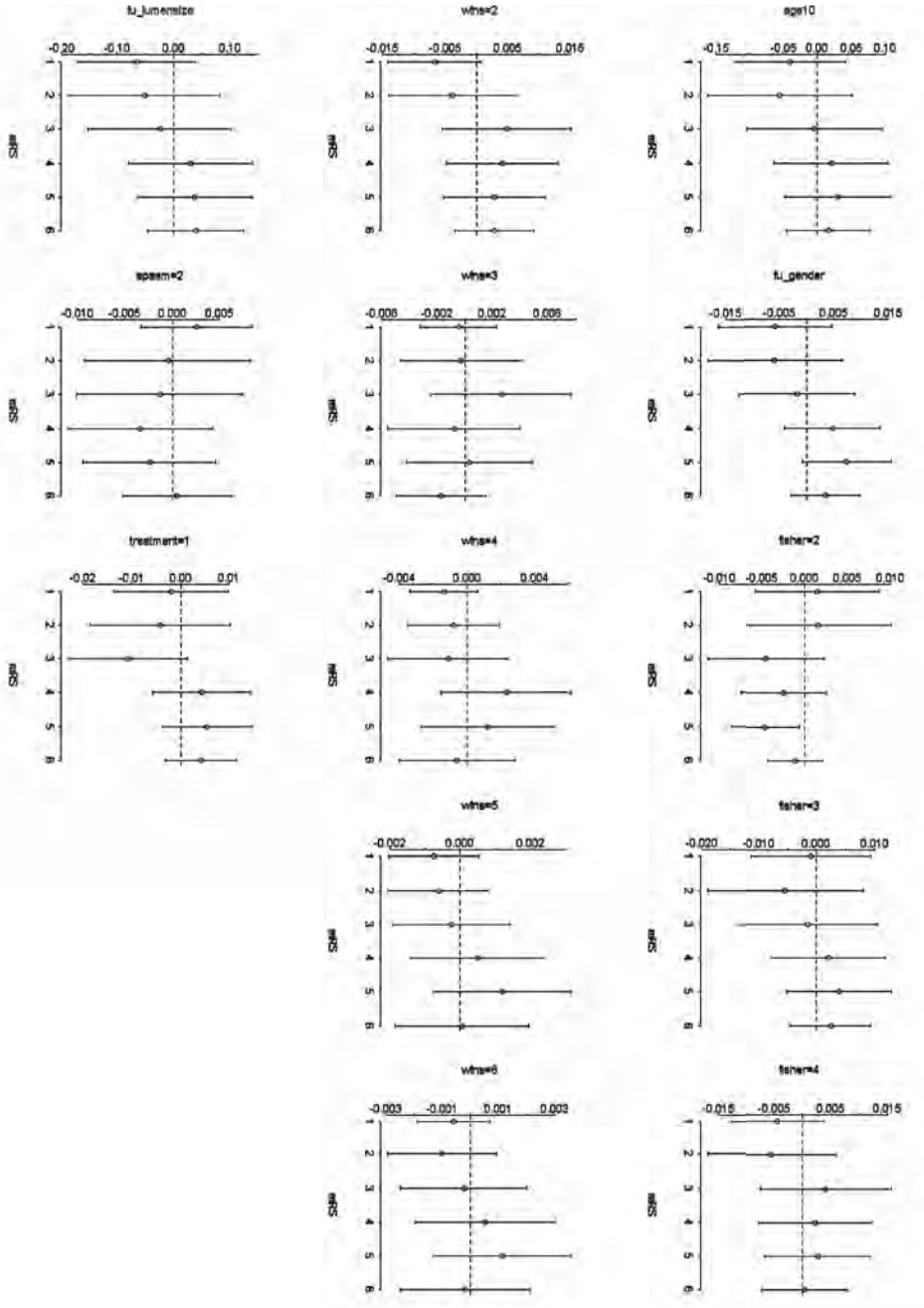

Figure 2. Residual plots (score.binary) of predictors in the selected multivariable model to examine deviations from the PO assumption.

Discussion

We developed and validated a prognostic proportional odds model to predict the risk of two month modified Rankin Scale in individual patients after aneurysmal subarachnoid haemorrhage. Predictions were based on characteristics that were collected in a large clinical trial and that are regularly readily available on admission to a neurological or neurosurgical unit. The c statistic was modest, indicating a mediocre ability to predict clinical outcome at the two month assessment.

The dependence of our proportional odds model on the assumption of proportionality should not be overstressed. The potential inaccuracy caused by mild violation of the PO assumption is likely less severe than would be the case in arbitrary dichotomisation of an ordinal outcome. Dichotomisation involves more loss of information [174]. Probably one would prefer a "wrong, but useful" model, despite possibly violating some underlying model assumptions [175]. Moreover, the PO model predicts the probability of being in each mRS level for each individual patient. This makes the model useful for all patients, regardless of severity.

Besides the PO model we explored several other models. The ordinary least squares model seemed to perform quite well (see Table 3). Although the categorical nature of the outcome variable is neglected, the model seems to perform reasonable and may yield estimates of regression coefficients that are quite similar to the PO model. This model might suffice to gain insight in which predictors play an important role in this clinical question. On the contrary, the multinomial model and – to a lesser extent – the partial PO models suffer from highly limited interpretability and therefore usability. A plethora of coefficients is produced by these models. If one is very specifically interested in one outcome grade, the model might be of some use. In most cases, we consider a more pragmatic approach however preferable. There are many more potentially useful modelling techniques for ordinal outcomes [176, 177]. One such technique is the continuation ratio (CR) model, which has been said to be likely to fit ordinal responses when subjects have to 'pass through' one category to get to the next. For a worked example see a tutorial by Harrell *et al*. [171].

Several limitations of our analyses should be acknowledged. This study used data from one large trial on a selected population of patients in equipoise regarding treatment with either endovascular coiling or neurosurgical clipping, which limits generalizability. Nonetheless, according to a recently published paper, the ISAT population proved to be quite similar to the population admitted with an aSAH to neurosurgical units in the United Kingdom [156]. The model may perform well in the development sample, but poorly when applied to other groups of patients, for example, a less strictly selected one. Validation of a prognostic model in independent patient series is considered an essential next

step [157]. However since large samples of systematically collected data on aSAH are sparse, assessment of external validity is difficult. For now the generalizability and overall validity of our model remains to be established. This will be a topic of future research.

Although our model represents knowledge obtained from 2,128 SAH patients, predictions for individual aSAH patients are always subject to uncertainty. The model makes certain structural assumptions and statistical interaction terms were not included. Hence, it is possible that specific patterns of risk factors are inadequately reflected in the model predictions. Therefore, predictions should be regarded with care and not directly be applied for treatment limiting decisions.

The modest performance of the presented model might potentially be improved by including neuro-imaging biomarkers other than lumen size, location, Fisher grade on plain CT scan, and vasospasm on angiography. Biomarkers regarding anatomy and morphology might be considered, as well as aneurysm characteristics obtained from three and four dimensional angiography [158, 159]. Performance may also be improved by inclusion of subsequent information obtained after admission, including temporal course, neuro-imaging at later time points, eventual rebleeding of the aneurysm, delayed ischemic deficit, and other parameters such as hydrocephalus. The objective of the present study, however, was to investigate prognostic models that predict two month mRS with predictors available at admission.

In conclusion, we presented a calibrated and internally validated ordinal prognostic model for predicting two month outcome after aSAH. After external validation this model could eventually be used to support clinical decision making.

Appendix

Details of the Prognostic Model

The probability of an outcome (mRS level) within two months is calculated according to the logistic formula: 1/(1 + exp-LP). The linear predictor (LP) takes the form of LP = intercept + regression coefficients × predictor values. LP for mRS = 0.250 [mRS ≥ 1] – 1.15 [mRS ≥ 2] – 2.25 [mRS ≥ 3] – 3.08 [mRS ≥ 4] – 3.39 [mRS ≥ 5] – 4.11 [mRS≥6] + 0.164 × age – 0.255 × [male] – 0.00445 × [Fisher grade II] + 0.245 × [Fisher grade III] + 0.404 × [Fisher grade IV] + 0.555 × [WFNS grade 2] + 0.889 × [WFNS grade 3] + 1.80 × [WFNS grade 4] + 2.07 × [WFNS grade 5] + 2.04 × [WFNS grade 6, 'not assessable'] + 0.0404 × lumen size of aneurysm + 0.307 × [vasospasm present]– 0.367 [coil].

Coding of the predictors was as follows: age in decades, lumen size in millimetres; all other predictors, 1 if true and 0 if false.

Chapter 5

General discussion

The aim of the studies described in this thesis was to examine certain epidemiological aspects of occurrence, aetiology, and predictors for outcome of subarachnoid haemorrhage (SAH). The studies were performed in the context of the @neurIST project, a European Commission funded project focusing on integration of knowledge on intracranial aneurysms from molecular level up to population level. One of the overall aims of the project was to develop an individualised prediction model that would allow predicting behaviour of an unruptured aneurysm and outcome of eventual rupture of an aneurysm. To reach this final aim, several intermediate questions have to be solved. This additional information was necessary to complement data from literature in populating a decision tree that would allow for prediction of risk in patients with an intracranial aneurysm. To that extent it was necessary to obtain frequency and association measures in as many subgroups (gender, age, main risk factors) as possible since these data are often missing in the literature.

In this chapter, I will first summarize the main findings of our studies. Secondly, I will discuss several general methodological issues related to the studies described in this thesis. Finally, some directions for future research will be suggested.

Main findings

Incidence of subarachnoid haemorrhage

We investigated the incidence of aneurysmal SAH in the general Dutch population (**chapter 2**). We conducted our study in two different databases; the Dutch nationwide hospital registry (Landelijke Medische Registratie, LMR) and the Integrated Primary Care Information (IPCI) database. The overall incidence rate of SAH observed in the LMR database was 7.12 per 100,000 person years (95% CI: 6.94-7.31); those found in IPCI 5.53 (95% CI: 4.56-6.66). These findings represent between 800 and 1200 new cases of SAH each year in the Netherlands. The incidence increased with age from around 1.30 (95% CI: 0.75-2.06) in the age category under 40 years up to 18.05 (95% CI: 16.51-19.69) (LMR) in persons over 80 years of age. Preponderance was observed in the incidence rates in females. The overall incidence rate ratio of SAH for women compared to men was 1.72 (95% CI: 1.63-1.81). This differential risk occurred gradually and was most pronounced in the fourth and fifth decade, appearing from the fourth decade of life onwards.

Risk of subarachnoid haemorrhage due to statins, antithrombotics, and oral contraceptives

Since most risk factors for (aneurysmal) SAH are similar to risk factors for cardiovascular disease, we were interested to investigate whether drugs that may actually impact on cardiovascular disease, through their action on vascular

endothelium, may be associated with SAH. Vascular endothelium, which is affected by statins, is believed to play a role in SAH [55]. Although statins decrease cerebrovascular event rates during use, withdrawal of statins causes a rebound effect leading to increases in vascular event rates beyond that of the absence of prevention alone [27]. This observed rebound effect has been attributed to the inhibition of endothelium nitric oxide synthase (eNOS) mediated pathways by statins [27]. Withdrawal of statins is supposed to impair the eNOS mediated tension regulation, which in turn may lead to short term increased stress on the vessel wall and risk of SAH. It has also been suggested that eNOS has both haemodynamic and structural roles [178]. Experimental data from eNOS knockout mice models demonstrated that luminal remodelling is impaired in the absence of eNOS, suggesting that nitric oxide (NO) derived from eNOS, in addition to its role as a vasodilatator, may have a role in controlling vessel wall architecture [179]. In order to investigate this association, we conducted a population-based case-control study within the PHARMO database (**chapter 3.1**). During the study period 1004 incident cases of SAH were identified. Current use of statins did not significantly decrease the risk of SAH (OR=0.77, 95%CI: 0.55-1.07), although there was a tendency. The odds ratio for recent withdrawal compared to none users was 1.62 (95%CI: 0.96-2.73). Compared to current use, recent withdrawal was associated with an increased risk of SAH (OR=2.34, 95%CI: 1.35-4.05). Interaction analysis showed that the effect of statin withdrawal was highest in patients who had also recently stopped anti-hypertensive drugs (OR=6.77, 95%CI: 2.10-21.8). The excess risk of SAH in this combination of withdrawal of statins during uncontrolled blood pressure is probably due to impaired NO bioavailability when it is most needed. The endothelium usually copes with increases in blood pressure by releasing NO to the smooth muscles around the vessel which subsequently relax [180].

Another drug class of interest for us were the antithrombotic agents (**chapter 3.2**). Use of platelet aggregation inhibitors and oral anticoagulants (vitamin K antagonists) have both been associated with increased risk of intracranial haemorrhage (ICH) [28]. It remains unclear whether and to what extent these antithrombotic agents are associated specifically with SAH, as this has not been separated out [25, 29]. Since these agents are frequently given to prevent (recurrent) cardiovascular events, observed associations are liable to confounding by indication. We used several approaches to study the association and the impact of confounding on these estimates. In our case-control analysis the adjusted odds ratio (OR) for the risk of SAH in current use of platelet aggregation inhibitors was 1.32 (95% CI: 1.02-1.70) and in current use of vitamin K antagonists 1.29 (95% CI: 0.89-1.87) compared to no use. In the case-crossover analysis, which effectively deals with confounding factors that remain stable over time within a person, platelet aggregation inhibitors were not associated with SAH anymore (OR=1.04, 95% CI: 0.56-1.94), but the effect for vitamin K antagonists became much stronger (OR=2.46, 95% CI: 1.04-5.82). To further investigate this change in findings we

examined whether an increase in use of these drugs over time (which may bias a case-crossover analysis) could explain this change. In the case-time-control analysis, which takes this time varying user prevalence into account, the OR for platelet aggregation inhibitors changed to 0.50 (95% CI: 0.26-0.98) and for vitamin K antagonists to 1.98 (95% CI: 0.82-4.76). To be conservative, we concluded that use of platelet aggregation inhibitors was not associated with an increased SAH risk; the modest increase observed in the case-control analysis could be explained by confounding. Use of vitamin K antagonists seemed to be associated with an increased risk of SAH. The increase was most pronounced in the case-crossover analysis and therefore cannot be explained by unmeasured confounding. This is important from a clinical perspective as it might indicate that patients with an unruptured aneurysm should preferentially be treated with platelet aggregation inhibitors. It is unlikely that vitamin K antagonists will *cause* a rupture, but they may aggravate minor leaks.

Based on findings regarding preponderance in SAH incidence in women after the fourth decade of life, we hypothesized that a disbalance in sex hormones may influence SAH risk. Therefore, we investigated the effect of oral contraceptive pills (OCP) on SAH (**chapter 3.3**). We observed that OCP use was associated with an increased risk of SAH of 1.79 (95% CI: 1.22-2.64) in PHARMO. In IPCI the risk was increased as well, but not statistically significant. Subdivision according to progestin content showed that levonorgestrel was associated with an 89 percent increase in SAH risk (OR=1.89, 95% CI: 1.24-2.87). Desogestrel was associated with a 98 percent increase (OR=1.98, 95% CI: 1.11-3.54). In IPCI the association between OCP use and aneurysmal SAH was 2.32 (95% CI: 0.89-6.05). The association between use of levonorgestrel containing OCP and aneurysmal SAH was 4.28 (95% CI: 1.65-11.1). We therefore concluded in two different populations that use of OCP are associated with an increased risk of SAH and aneurysmal SAH.

Genetic risk loci

A previous genome-wide association study (GWAS) of intracranial aneurysms reported that identified three risk loci on chromosomes 8q11.23-q12.1, 9p21.3 and 2q33.1 with $P < 5 \times 10^{-8}$ [34]. That study had limited power to detect loci imparting genotypic relative risk (GRR) < 1.35. To study additional loci of similar or smaller effect a second GWAS was conducted in a larger cohort. The study comprised 5,891 cases and 14,181 controls with ~832,000 genotyped and imputed SNPs across discovery cohorts. Our study had 89% and 64% average power to detect common variants (minor allele frequencies (MAF) ≥ 10%) with GRR of 1.25 and 1.20, respectively. We identified three new loci showing strong evidence for association with intracranial aneurysms in the combined dataset, including intervals near RBBP8 on 18q11.2 (OR = 1.22, $P = 1.1 \times 10^{-12}$), STARD13-KL on 13q13.1 (OR = 1.20, $P = 2.5 \times 10^{-9}$) and a gene-rich region on 10q24.32 (OR = 1.29, $P = 1.2 \times 10^{-9}$). We also confirmed prior associations near SOX17 (8q11.23–q12.1; OR = 1.28, P = 1.3

$\times 10^{-12}$) and CDKN2A-CDKN2B (9p21.3; OR = 1.31, P = 1.5×10^{-22}). Several of the putative risk genes play a role in cell-cycle progression, potentially affecting the proliferation and senescence of progenitor-cell populations that are responsible for vascular formation and repair.

Predicting outcome after aneurysmal subarachnoid haemorrhage

Early prediction of short term outcome in terms of case-fatality may support clinical decision making in patients admitted with an aneurysmal SAH and may provide realistic and evidence based expectations to patients and relatives. Predictions may also be used to classify patients according to prognosis, which may be useful to compare outcome between different patient series, to study treatment results over time, or to stratify patients for randomised clinical trials (RCT). We developed prediction models for outcome after aneurysmal SAH. Data were collected prospectively by the International Subarachnoid Aneurysm Trial (ISAT). The first model focussed on the binary outcome case-fatality within 60 days. The study population comprised of 2,128 patients who had been randomised to either endovascular coiling or neurosurgical clipping (**chapter 4.1**). In this population 153 patients (7.2%) died within 60 days. World Federation of Neurosurgical Societies (WFNS) grade was the most important predictor of case-fatality, followed by age, lumen size of the aneurysm and Fisher grade. The model discriminated reasonably between those who died within 60 days and those who survived (c statistic = 0.73), with minor optimism according to bootstrap re-sampling (optimism corrected c statistic = 0.70). The probability of dying within 60 days is calculated according to the logistic formula: $1/(1 + \exp^{-LP})$. The linear predictor (LP) describes the model represented by an intercept and regression coefficients for the predictor values.

The second model focussed on the ordinal outcome 'modified Rankin Scale' (mRS) at two month assessment (**chapter 4.2**). This is an ordered scale for measuring disability and runs from 0 (no symptoms at all) to 6 (dead). The prediction model included WFNS grade, age, sex, lumen size of the aneurysm, Fisher grade, vasospasm on angiography, and treatment modality. The model discriminated moderately between those with poor and good mRS scores (c statistic = 0.65), with minor optimism according to bootstrap re-sampling (optimism corrected c statistic = 0.64).

Methodological considerations

Population based SAH research

LMR

For our study on the incidence of SAH we use the nationwide hospital registry (Landelijke Medische Registratie, LMR). The LMR database contains discharge diagnoses and admission details of nearly all Dutch general and university

hospitals. Discharge diagnoses and procedures are coded by trained personnel in the hospital. In this database, we identified all patients with the diagnose code for SAH during a certain period. Our main concern in this procedure was misclassification of outcome. A small validation study of discharge diagnoses in our own hospital showed that 10 percent of the cases were false positive. Another major concern was patients who died before reaching medical care; they were not included in the incidence estimate. Previous studies have estimated the percentage of persons dying outside hospitals to be between 11 and 13 percent [41, 42].

The major advantage of the LMR database is its completeness with regard to the Dutch hospitals. Registration of discharge diagnoses was obligatory for all hospitals. The limitations of the LMR data concern covariates and misclassification. Since LMR is a hospital discharge database, covariates can only be assessed in so far they have led to a hospital admission. Only an overall disease score can be assigned to patients [181-183]. Validation of cases to reduce or quantify misclassification is generally not possible in LMR data. It would request de-anonymisation of at least the hospitals from which the cases are obtained. Secondly, permission has to be obtained to go through patient files to verify the diagnoses.

PHARMO

The pharmacoepidemiological studies in this thesis used data from PHARMO Record Linkage System (RLS) [70]. This database, which is representative for the Dutch population, includes drug-dispensing records from community pharmacies and hospital discharge records of more than two million community dwelling inhabitants of 50 demographically defined areas in the Netherlands. For all residents, the computerized drug-dispensing histories contain data concerning the dispensed drug, type of prescriber, dispensing date, dispensed amount, prescribed dosing regimens, and the legend duration of use (prescription length). All drugs are coded according to the Anatomical Therapeutic Chemical (ATC) Classification [76]. The hospital records include detailed information concerning discharge diagnosis, procedures, dates of hospital admission and discharge, and discharge destination or dying in the hospital before discharge. Diagnoses are classified according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). For the studies in this thesis we used cases of SAH.

The advantageous feature of PHARMO is that it links hospital discharge data and pharmacy data.

This database was used to increase the power of the studies on drug and outcome associations performed in IPCI. However, a major limitation in PHARMO is that is not possible to distinguish between SAH in general and aneursymal SAH specifically. Another limitation concerns the impracticality of case validation, which is similar to that in the LMR database.

IPCI

For some studies in this thesis, data was obtained from the Integrated Primary Care Information (IPCI) database which contains anonimized medical records of around one million general practitioner's patients. The database is representative for the Dutch population regarding age and sex. In the Netherlands, general practitioners act as gatekeepers for secondary care [184]. Consequently, their records can be considered to hold most relevant medical information about a patient. No paper records are being kept by general practitioners participating in the project except for specialist letters which can be requested for the case validation process. Given the rarity of the disease under study, using large databases is mandatory to collect enough cases for meaningful results. Since data in the IPCI database was collected prospectively and irrespective of the research question, selection bias plays no role.

The major advantage of the IPCI database compared with the others is the possibility of case validation. We used the specialist letters to manually validate aneurysmal SAH cases obtained from the IPCI database. The limitation of this database was the relatively small size. Also the fact that SAH is a pre-eminently specialist diagnosis makes the search for these patients in a GP database an indirect one.

Pharmacoepidemiology in subarachnoid haemorrhage; bias and confounding

Studying associations between drugs and SAH in electronic medical record databases is challenging due to a variety of potential biases and mechanisms of confounding. In all observational studies, various types of bias, e.g. selection bias, protopathic bias, information bias and confounding by indication, may influence the study findings. We will discuss these forms of bias in the context of the studies that were performed. The primary study design that was adopted in the pharmacoepidemiological studies in this thesis was the case-control study. Case-control studies are more efficient in studying dose, duration and exposure responses than cohort studies, whereas their validity is the same in case of information from electronic medical records, since data collection is always independent from the study question and recall bias, which plagues field case-control studies, is not an issue.

The potential for selection bias was minimal in our studies as all data were obtained from prospectively collected medical records that are maintained for patient care or health care policy purposes on a population level.

Protopathic bias occurs when a drug is used to treat prodromic symptoms of the study outcome. In statistical analysis it may appear as if the drug is positively associated to the outcome [185]. One could erroneously conclude that the drug *causes* the outcome. In our statin withdrawal study described in chapter 3.1 protopathic bias was observed for pain medication. We found increased odds ratios for NSAIDs, coxibs, and anti-migraine medication, as the prodromes of SAH

are most likely headache and neck pain due to a 'warning leak'. Our suspicion of protopathic bias was supported by the sub analyses in patients using these agents for more than 30 days; in these patients the association could not be found which argues against a causal relation.

Information bias can occur as result of misclassification of either exposure or outcome. To minimize the potential effect of information bias by misclassification of the outcome a three-step validation process was undertaken where feasible. Validation was performed in IPCI cases. First, all potential cases were identified by means of a broad search of coded diagnoses and free text narratives in the electronic medical record of all patients. Second, electronic medical records of all potential cases were manually reviewed and validated by a medical doctor. Finally, specialist letters concerning all probable and definite cases were reviewed by a medical doctor and a neurologist. During the validation process reviewers were blinded to drug exposure. Due to this extensive validation process, false positives are highly unlikely in the IPCI cases. However, false negatives remain a concern in incidence studies as they cannot be ruled out. In case control studies, this is less of a concern due to study design.

As elaborated above, misclassification of outcome was a limitation in the studies performed in the PHARMO and LMR databases, since the opportunities for extensive validation were limited due to the nature of the databases. The performed search was basically a search for International Classification of Diseases, Ninth Revision (ICD-9) code 430; subarachnoid haemorrhage.

Misclassification of exposure was a concern in the pharmocoepidemiological studies in this thesis since we used prescription and dispensing data and had no information on actual use of the medication. Misclassification is likely to be non-differential between cases and controls and would lead to underestimation of the association between drug use and outcome. To deal with misclassification of exposure due to irregular intake, we performed various sensitivity analyses in our studies by varying the exposure window. In our study on statin use, sensitivity analyses on different risk windows of withdrawal showed that the association measure is highest when withdrawal is closest to the index date, i.e. persons who stopped more recently. In the OCP study the association was stronger for the longer exposure windows. In the case-control design of the antithrombotics study the tendency was similar as in the OCP study. In the case-crossover and case-time-control designs of the antithrombotics study, sensitivity analyses did not show changes in point estimates.

One highly important issue that was encountered in our epidemiological studies was confounding by indication. Confounding by indication refers to an extraneous determinant of the outcome parameter that is present if a perceived high risk or poor prognosis is an indication for medical intervention. In our studies the indication is a confounder because it correlates with the drug prescription and is a

risk indicator for the disease under study [186]. Confounding by indication is likely in several of the associations studied, since the choice of cardiovascular drug is often associated with the condition of the patient, which in itself can be a risk factor for SAH. Confounding by indication was addressed in the design and analysis phases of the studies. In the study on statin withdrawal we compared patients who stopped using statins with current users to reduce the impact of indication of use as a confounder. There is a strong potential for confounding by indication, since statins are regularly used in patients with increased cardiovascular risk, which is also a risk factor for SAH [1]. In the antithrombotics study confounding by indication was taken into account by applying a case-crossover design [78]. The indication for platelet aggregation inhibitors is mostly secondary prevention of cardiovascular and cerebrovascular disease. These share many risk factors with SAH and may therefore cause confounding by indication. But in a case-crossover study the patient acts as its own control, so potential, both measured and unmeasured, confounders that are stable over time cancel out. Confounding by indication was less pronounced in the OCP studies since birth control is not associated itself with SAH, apart from transient differences in sexual activity, which may be a risk factor [36].

Due to the use of electronic medical records, we could consider many risk factors in IPCI. In PHARMO, we could only adjust for drug proxies of confounding factors and therefore residual confounding cannot be excluded. We could also either adjust main analyses for potential confounder or match on patient characteristics. However, secondary use of medical record data renders our study vulnerable to potential confounders that are not covered in the database. In the case-control designs in PHARMO we could not adjust for lifestyle factors such as smoking, alcohol abuse, obesity, and actual blood pressure. Nonetheless, in the case of the antithrombotic study we could address this residual confounding by applying a case-crossover design.

Modelling SAH outcome

ISAT

Data for our modelling studies were obtained from the International Subarachnoid Aneurysm Trial (ISAT) [152]. Thus, the modelling studies used data from one large trial on a selected population of patients in equipoise regarding treatment with either endovascular coiling or neurosurgical clipping, which limits generalizability. Nonetheless, according to a recently published paper, the ISAT population proved to be quite similar to the population admitted with an aneurysmal SAH to neurosurgical units in the United Kingdom [156]. Although in the ISAT, a lower proportion of poor grade patients were enrolled. Therefore, results from these modelling studies should be validated externally, i.e. in independent data on survival and outcome in aneurysmal SAH patients. Unless external validation

proves these models valid, they should not be directly used in clinical setting. Let alone be applied for treatment limiting decisions.

Ordinal outcomes

Outcome status in the medical domain is often measured as an ordered categorical scale. So far, it is not evident what method should be applied to model these ordinal outcomes satisfactory. The abundance of proposed models makes it hard to decide where to start and what to aim at. From a theoretical perspective, it is nearly impossible to make a start. From a pragmatic point of view, starting with a straightforward extension of the well known logistic regression model (LRM) is a reasonable first step. The extended version of LRM is the proportional odds (PO) model. The advantage of the PO model is its parsimony in dealing with an ordered outcome. The price we pay is the assumption of proportionality of the odds. This assumption is equivalent to saying that any cut-point on the outcome scale would lead to the same (binary) logistic regression coefficient [153]. However, the dependence of our PO model on the assumption of proportionality should not be overstressed. The potential inaccuracy caused by mild violation of the PO assumption is in all likelihood not more severe than would be the case in arbitrary dichotomisation of an ordinal outcome. Especially since dichotomisation involves loss of information [174]. Probably one would prefer a "wrong, but useful" model, despite possibly violating some underlying model assumptions [175]. Moreover, the PO model predicts the probability of being in each outcome level for each individual patient. This makes the model useful for all patients, regardless of severity.

Suggestions for future research

Pharmacoepidemiology

Studies in this thesis show the potential and pitfalls of use of electronic medical records for assessing associations between drug use and subarachnoid haemorrhage. A need exists to systematically investigate these associations, since they represent potential avoidable risk factors for SAH. New methodologies are warranted and in development to evaluate and quantify the benefit-risk profiles of several drugs [187]. Use of electronic medical record databases seem key in these assessments since they reflect reality rather than artificial trial circumstances and allow for longer follow up. Added to that, not all drugs currently under interest in SAH research have been recently investigated in a trial setting, let alone for the specific purpose of examining cerebrovascular risk profiles. In case of severe and rare clinical outcomes, such as SAH, a single electronic medical record database may not provide sufficient subjects and heterogeneity in exposure to conduct sound pharmocoepidemiologic research in the full range of drugs of interest. For

this reason we combined results from different databases to study the association between oral contraceptive pill use and SAH. Combination of results is a first step towards eventual aggregation of data from different sources. Currently, such an approach is challenging due to differences in languages and medical terminology applied. Added to that, researchers should cope with substantial differences in quality and type of data. This strategy however may provide enough statistical power to study rare diseases and rare drug exposures. Methodologies for analyzing aggregated data from different sources have been proposed in the field of intracranial aneurysms [188]. Similar methodologies in other fields are now being investigated in multidisciplinary research consortia [189-191]. They should be optimalized and made available for other diseases and fields of research in the future.

Modelling

There seems to be reasonable unanimity on modelling binary outcomes. Recently, a very broad yet concise and therefore practically useful book on development, validation and updating clinical prediction models has been published [153]. For medical researchers, the information in the book probably suffices to develop prediction models with binary clinical outcomes. The case for ordered categorical outcomes is not so straightforward. Modelling ordinal outcomes is fairly complicated from a statistical point of view, but very important from a medical one. Often, outcome status in the medical domain is measured as an ordered categorical scale. Many models have been proposed and an abundance of programs in several statistical packages is available [176, 177]. However, for the medical researcher without a background in statistics or mathematics it is nearly impossible to weigh all possibilities and perform sound research. Therefore, future research and sound advice from supportive research disciplines on this important issue would be of great help.

Another direction of future research in prediction modelling in neurovascular disease is how to deal with missing data. Many suggestions have been made on how to impute data [192, 193]. But methods for imputation and modelling in one go will have to be developed and promoted in the field of medical research [194, 195].

Unruptured intracranial aneurysms

Since 85 percent of SAH originates from an intracranial aneurysm it might prove worthwhile to try and target unruptured aneurysms before they rupture. Population prevalence of unruptured aneurysms has been estimated at 2 percent [13] and the estimated yearly rupture rate of unruptured aneurysm is around 1 percent [196]. Future research should focus on identifying risk factors for harbouring, development, or rupture of an unruptured intracranial aneurysm.

Genome wide association studies (GWAS) proved to be able to detect genetic variances that are associated with intracranial aneurysms [35]. More risk loci are to be expected from future GWAS.

Another field of future research should be the development of aneurysms [197, 198]. High risk populations will have to be defined and decision modelling will have to be applied to investigate whether screening proves beneficial and cost effective.

Individualized risk assessment

The ultimate goal of research in the field of SAH and unruptured intracranial aneurysms will be to develop individualised risk prediction models. A reasonable amount of knowledge on rupture risk has been gathered in a recent review of reviews [22]. Added to information on patient and aneurysm characteristics, imaging biomarkers such as with electrocardiographically gated CT angiography (4D CTA) for visualizing pulsation of intracranial aneurysms and morphodynamic analysis of cerebral aneurysm pulsation might prove of great value for this goal [158, 159].

Within the @neurIST consortium considerable efforts have been put in the development of computer-interpretable guideline services and individualised risk prediction models [188, 199]. Future research in medical practice should use the benefits from technologies in which digital data enables predictable outcomes through quantitative models that integrate physical processes across spatial scales down to the molecular level. It should focus on personalized, predictive, integrative and evidence-based approaches to medicine. These will use computational tools to link individual patient data with virtual population databases via the knowledge of biological processes encoded in mathematical models [200].

6.1 Summary

Subarachnoid haemorrhage (SAH) is bleeding into the subarachnoid space – the area between the arachnoid membrane and the pia mater surrounding the brain. SAH originates from arteries localised on the brain's surface. SAH can be caused by traumatic injury of the head or can occur spontaneously. Spontaneous SAH is caused by rupture of an intracranial aneurysm in about 85 percent of cases. Reliable knowledge about the risks of (aneurysmal) SAH in different populations will help in screening and prevention strategies and in predicting the prognosis of individual patients. One of the overall aims of the research presented in this thesis was to provide evidence for the development of an individualised prediction model that would allow predicting behaviour of an unruptured aneurysm and outcome of eventual rupture of an aneurysm. This work was performed as part of the European @neurIST project.

The specific aim of this thesis was to investigate the incidence, aetiology, and outcome of intracranial aneurysms and SAH.

In ***chapter 2*** we report on the incidence, treatment, and case-fatality of (aneurysmal) SAH in the Netherlands. Recent data on the Dutch situation were missing, so we tried to fill the gap by means of a population-based study. We found that the Netherlands belong to low incidence countries, as opposed to high incidence countries such as Finland and Japan, where the incidence is twice as high as in most other countries. The majority of aneurysmal SAH patients was treated by means of a neurosurgical clipping procedure. Case-fatality proved to be high: up to one-third of SAH patients died within one month after onset.

In ***chapter 3.1*** we report on the effect of statin withdrawal on SAH risk. Since nitric oxide (NO) has been suggested to be involved in SAH related vasospasm, and withdrawal of statins is supposed to impair the endothelium NO synthase mediated tension regulation, which in turn may lead to short term increased stress on the vessel wall and risk of SAH, we hypothesized that statins influence the risk of SAH during use and shortly after withdrawal. In this chapter we showed that statins are indeed associated with the risk of SAH. Whereas current use of statins may protect against SAH, withdrawal is associated with a clear increase in risk, especially if patients have also stopped antihypertensive drugs. The observed results support our hypothesis that statins have an effect on vascular endothelium and are associated with SAH risk.

Chapter 3.2 was a quest to investigate the association between certain antithrombotic drugs – platelet aggregation inhibitors and vitamin K antagonists – and SAH. These drugs have been associated with an increased risk of intracranial

haemorrhage. Whether use of these antithrombotic drugs is associated with an increased risk of SAH previously remained unclear, especially since confounding by indication might play a role. We applied three different study designs to approach the problem of elucidating the association, while taking potential confounding by indication into account. We found that use of platelet aggregation inhibitors was not associated with an increased SAH risk, whereas use of vitamin K antagonists was associated with an increased risk of SAH. This implicates that vitamin K antagonists should be prescribed cautiously in patients with known unruptured aneurysms.

In ***chapter 3.3*** we describe a study on the association between use of oral contraceptive pills (OCP) and (aneurysmal) SAH risk. The fact that the age-specific incidence rate of SAH is higher in postmenopausal women than in men suggests an influence of sex hormones. Based on epidemiological findings, evidence that endothelium-dependent arterial vasodilatation increases significantly during the ovulatory phase of the menstrual cycle, when oestrogen levels are highest in naturally cycling young women, and studies showing that some progestins may antagonize the beneficial vascular effects of oestrogen in postmenopausal women, it is logical that the type of progestin used in OCP may determine how different OCPs affect the arterial vasculature in young women. Although the association has been under debate for a long time, previous studies remained inconclusive on the subject. In this study we observed that use of OCP seems to be associated with an increased risk of (aneurysmal) SAH.

In ***chapter 3.4*** a study on genetics of intracranial aneurysms is described. This study has been performed by a large multinational consortium. In this study we report on a genome-wide association study with discovery and replication cohorts from the United States of America, Europe, and Japan comprising nearly six thousand cases and fourteen thousand controls with over eight hundred thousand genotyped and imputed single nucleotide polymorphisms across discovery cohorts. We identified three new loci showing strong evidence for association with intracranial aneurysms. We also confirmed two previously found associations. Several of the putative risk genes play a role in cell-cycle progression, potentially affecting the proliferation and senescence of progenitor-cell populations that are responsible for vascular formation and repair.

In ***chapter 4.1*** we present a prediction model of the outcome in terms of case-fatality within sixty days from the onset of aneurysmal SAH. Our aim was to develop this model based on clinical features and neuro-imaging, regularly readily available on admission to a neurological or neurosurgical unit. Early prediction of short term outcome in terms of case-fatality may support clinical decision making and may provide realistic and evidence based expectations to patients and relatives.

Predictions may also be used to classify patients according to prognostic risk, which may be useful to compare outcome between different patient series, to study treatment results over time, or to stratify patients for randomised clinical trials. In the internally validated final model age, lumen size of the aneurysm, Fisher grade, and World Federation of Neurosurgical Societies (WFNS) grade were included as predictors. The model fitted the data in which it was developed well and the *c* statistic showed that the model discriminates reasonably between patients who die within sixty days from onset of the aneurysmal SAH and those who survive this period.

The study presented in ***chapter 4.2*** is the sequel of this prediction research. A frequently used outcome measurement in stroke research is the modified Rankin Scale. This is an ordered scale for measuring motor function and runs from zero (no symptoms at all) to six (dead). Outcomes at ordinal scales are often dichotomized. However, there are several objections against collapsing an ordinal outcome scale into a binary one. The most important objection being that from a clinical point of view dichotomisation may lead to less useful models. For example, for a patient with a minor stroke a model predicting survival versus mortality is of limited value since the risk is low, while a prediction of complete recovery versus some remaining symptoms may be very useful. For a patient with a severe stroke, this will be the other way around. An alternative for dichotomisation is application of a statistical approach that uses the full ordinal outcome scale. This leads to efficient use of the data and clinically relevant predictions. Our aim was to develop an ordinal prognostic model to predict clinical outcome at two months in patients with aneurysmal SAH. For the sake of interpretability and clinical usefulness, we chose to accept a minor violation of the proportional odds assumption in the proportional odds model we applied. Age and WFNS grade were the most important predictors in the internally validated multivariable proportional odds model. Other statistically significant predictors were sex, lumen size of the aneurysm, Fisher grade, vasospasm, and treatment modality. However, the model poorly fitted the data in which it was developed and the *c* statistic of the final model showed moderate discrimination between patients in different outcome categories.

In ***chapter 5*** the results and conclusions are summarized and interpreted. The scientific consequences of our findings are discussed. Further, we explore some methodological issues and give suggestions for further research.

6.2 Samenvatting

Een subarachnoïdale bloeding (SAB) is een bloeding in de subarachnoïdale ruimte tussen het spinnewebvlies (*arachnoidea mater*) en het zachte hersenvlies (*pia mater*) die om de hersenen liggen. Een SAB ontspringt aan de bloedvaten die rondom de hersenen liggen in de subarachnoïdale ruimte. Een SAB kan veroorzaakt worden door een traumatische verwonding van het hoofd of kan spontaan optreden. Een spontane SAB wordt in ongeveer 85 procent van de gevallen veroorzaakt door ruptuur van een intracranieel aneurysma. Betrouwbare kennis over de risico's op een (aneurysmatische) SAB in verschillende populaties helpt bij beslissingen over screening en preventie en in het voorspellen van de prognose van individuele patiënten. Een van de overkoepelende doelstellingen van het onderzoek, dat in dit proefschrift wordt beschreven, was het leveren van *evidence* voor het ontwikkelen van een geïndividualiseerd predictiemodel dat het gedrag van een niet-geruptureerd aneurysma en de uitkomst van een eventuele ruptuur zou kunnen voorspellen. Het onderzoek werd uitgevoerd als onderdeel van het Europese@ neurIST project. Het specifieke doel van dit proefschrift was het onderzoeken van etiologie en uitkomst van intracraniële aneurysma's en SAB.

In ***hoofdstuk 2*** worden gegevens gerapporteerd betreffende incidentie en behandeling van een SAB en het overlijden ten gevolge van een SAB in Nederland. Aangezien recente Nederlandse gegevens ontbraken, hebben wij geprobeerd het gat te dichten met een populatiegebaseerde studie. We vonden dat Nederland behoort tot landen met een lage incidentie, in tegenstelling tot bijvoorbeeld Finland en Japan, waar de incidentie ongeveer twee keer zo hoog is als in andere landen. De meerderheid van de SAB-patiënten werd behandeld door het neurochirurgisch plaatsen van een clip op het aneurysma. Het aantal patiënten dat overleed ten gevolge van of vlak na een SAB was hoog: tot een derde van de SAB-patiënten overleed binnen een maand na het optreden van de SAB.

In ***hoofdstuk 3.1*** beschrijven we het effect van onttrekking van statinen op het SAB-risico. Aangezien eerder gesuggereerd is dat stikstofmonoxide (NO) betrokken is bij SAB-gerelateerde vasospasmen en onttrekking van statinen verondersteld wordt de endotheel-NO-synthasegemedieerde regulatie van de vaatwandspanning te verslechteren, was onze hypothese dat statinen het risico op SAB beïnvloeden tijdens gebruik en kort na onttrekking. In dit hoofdstuk toonden wij aan dat statinen inderdaad geassocieerd zijn met het risico op een SAB. Gebruik van statinen biedt mogelijk bescherming tegen een SAB, maar onttrekking is geassocieerd met een duidelijk verhoogd risico, vooral bij patiënten die ook gestopt zijn met het nemen van bloeddrukverlagende middelen. De gevonden resultaten

steunen onze hypothese dat statinen een effect hebben op het vasculaire endotheel en geassocieerd zijn met het risico op een SAB.

Hoofdstuk 3.2 was een zoektocht naar de associatie tussen bloedverdunners – in dit geval plaatjesremmers en vitamine K antagonisten – en SAB. Deze middelen zijn eerder geassocieerd met intracraniële bloedingen. Of deze bloedverdunners een verhoogd SAB-risico met zich meebrengen is tot nog toe onduidelijk gebleven, vooral doordat *confounding by indication* een rol zou kunnen spelen. We hebben drie verschillende studie-opzetten toegepast om de mogelijke associatie te bepalen en tegelijkertijd rekening te houden met *confounding by indication*. We vonden dat het gebruik van plaatjesremmers niet geassocieerd was met een verhoogd SAB-risico, maar het gebruik van vitamine K antagonisten wel. Dit impliceert dat er oplettendheid betracht dient te worden bij het voorschrijven van vitamine K antagonisten aan patiënten, van wie bekend is dat zij een niet-geruptereerd intracranieel aneurysma hebben.

In ***hoofdstuk 3.3*** beschrijven we een studie naar de associatie tussen het gebruik van 'de pil' (orale anticonceptie) en een (aneurysmatische) SAB. De bevinding dat leeftijdsspecifieke incidentie van SAB hoger is in postmenopausale vrouwen suggereert dat geslachtshormonen van invloed zijn op het optreden van een SAB. Wanneer we ons baseren op epidemiologische bevindingen, bewijs dat endotheelafhankelijke arteriële vasodilatatie significant toeneemt tijdens de ovulatiefase in de menstruatiecyclus, wanneer oestrogeenspiegels het hoogst zijn in jonge vrouwen met een natuurlijke cyclus en studies die aantonen dat sommige progestagenen het gunstige vasculaire effect van oestrogenen tegenwerken in postmenopausale vrouwen, is het waarschijnlijk dat het type progestageen in de pil bepaalt wat het vasculaire effect van de pil is bij jonge vrouwen. Hoewel deze associatie al geruime tijd onderwerp van discussie is, bleef een definitieve conclusie op basis van eerdere studies uit. In onze studie zagen we dat pilgebruik geassocieerd leek met een verhoogd risico op een (aneurysmatische) SAB.

In ***hoofdstuk 3.4*** wordt een studie beschreven over genetica van intracraniële aneurysmata. Deze studie is uitgevoerd door een groot multinationaal consortium. In deze studie beschrijven we een *genome-wide association study* met ontdek- en replicatiecohorten uit de Verenigde Staten, Europa en Japan die bijna zesduizend *cases* en veertienduizend controles bevatten waarin meer dan achthonderdduizend *single nucleotide polymorphisms* (*SNPs*) zijn onderzocht. Er zijn drie nieuwe *loci* geïdentificeerd met krachtig bewijs voor associatie met intracraniële aneurysmata. Tevens zijn twee eerder gevonden associaties bevestigd. Het is vermeldenswaardig dat de genen, die vermoedelijk door de regio's van de gevonden SNPs gecodeerd worden, een rol spelen in de voortgang van de celcyclus en daardoor mogelijk

een rol spelen in de proliferatie en veroudering van voorlopercelpopulaties die verantwoordelijk zijn voor vorming en reparatie van vaatstructuren.

In ***hoofdstuk 4.1*** presenteren we een predictiemodel voor de uitkomst van een aneurysmatische SAB na zestig dagen in termen van overlijden. Ons doel was om dit model te ontwikkelen gebaseerd op klinische en neuroradiologische kenmerken, die doorgaans beschikbaar zijn bij opname op een neurologische of neurochirurgische afdeling. Tijdige voorspelling van de kortetermijnuitkomst in termen van overlijden zou het maken klinische beslissingen kunnen ondersteunen en zou realistische en *evidence based* verwachtingen voor patiënten en verwanten kunnen opleveren. Predicties kunnen ook worden gebruikt om patiënten te classificeren op basis van hun prognose, wat gebruikt kan worden om uitkomsten te vergelijken tussen verschillende patiëntenseries, om behandelingsresultaten over de tijd te bestuderen of om patiënten te stratificeren voor gerandomiseerde klinische trials. In het intern gevalideerde uiteindelijke model waren leeftijd, grootte van het aneurysma, Fisher gradering en *World Federation of Neurosurgical Societies* (*WFNS*) gradering opgenomen als voorspellers. Het model paste goed op de gegevens waarop het was ontwikkeld en de *c*-waarde toonde dat het model redelijk onderscheid kan maken tussen patiënten die binnen zestig dagen na SAB overlijden en patiënten die deze periode overleven.

De studie die wordt beschreven in ***hoofdstuk 4.2*** is het vervolg op dit predictie-onderzoek. Een veelvuldig gebruikte uitkomstmaat in beroerte-onderzoek is de *modified Rankin Scale*. Dit is een ordinale schaal om motorische functie te meten en loopt van nul (geen symptomen) tot zes (dood). Ordinale uitkomsten worden vaak gedichotomiseerd. Echter, er zijn bezwaren tegen het samenvoegen van ordinale categorieën om een binaire uitkomst te krijgen. Het belangrijkste bezwaar is dat dichotomiseren kan leiden tot een model dat vanuit klinisch oogpunt minder bruikbaar is. Het is bijvoorbeeld niet heel zinvol voor een patiënt met een lichte beroerte de kans op overlijden te voorspellen, aangezien die heel laag is en andere uitkomsten veel relevanter zijn voor deze patiënt. Een alternatief voor dichotomiseren van de uitkomst is het toepassen van een statistische benadering die de volledige ordinale schaal benut. Dat leidt tot efficiënt gebruik van de gegevens en klinisch relevante modellen. Ons doel was het ontwikkelen van een ordinaal predictiemodel om de klinische uitkomst twee maanden na een SAB te voorspellen. Omwille van interpreteerbaarheid en klinische bruikbaarheid hebben we een milde schending van de aanname van proportionaliteit van de kansen geaccepteerd in het model dat we hebben toegepast. Leeftijd en WFNS gradering waren de belangrijkste voorspellers in het intern gevalideerde multivariabele proportionele-kans-model. Andere statistisch significante voorspellers waren geslacht, grootte van het aneurysma, Fisher gradering, vasospasme en behandelingsmodaliteit. Echter, het model paste matig op de gebruikte gegevens. De *c*-waarde toonde een

matig vermogen van het model om patiënten met verschillende uitkomsten te onderscheiden.

In ***hoofdstuk 5*** worden de resultaten en conclusies samengevat en geïnterpreteerd en worden de wetenschappelijke consequenties van onze bevindingen besproken. Voorts wordt in dit hoofdstuk ingegaan op enkele methodologische onderwerpen en worden suggesties gedaan voor verder onderzoek.

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PhD Portfolio

Research skills

Master of Science in Clinical Epidemiology, NIHES, Rotterdam	2006-2007
Course: Introduction to R, Rotterdam	2008
Course: Logistic Regression Modelling, Utrecht	2008

International conference presentations

23rd International Conference on Pharmacoepidemiology, Quebec, Canada; oral presentation 'Hormonal contraceptives and risk of aneurysmal subarachnoid haemorrhage'.	2007
8th Congress of the European Association for Clinical Pharmacology and Therapeutics, Amsterdam, the Netherlands; oral presentation 'Hormonal contraceptives and risk of aneurysmal subarachnoid haemorrhage'.	2007
5th International Congress on Vascular Dementia, Budapest, Hungary; poster presentation 'Homocysteine, white matter lesions and brain atrophy: the SMART-MR Study'.	2007
24th International Conference on Pharmacoepidemiology, Copenhagen, Denmark; oral presentation 'Withdrawal of endothelium affecting drugs and risk of subarachnoid haemorrhage'.	2008
5th UseR! Conference 2009, Rennes, France; oral presentation 'Workflows for Data Mining in Integrated multi-modal Data of Intracranial Aneurysms using KNIME'.	2009
25th International Conference on Pharmacoepidemiology, Rhode Island, USA; poster presentation 'Prediction of 60-day case-fatality after aneurysmal subarachnoid haemorrhage'.	2009

Teaching

Supervising practicals in Evidence Based Medicine at the Faculty of Medicine, Erasmus MC, Rotterdam	2006-2009
Teaching practicals in (pharmaco-)epidemiology at NIHES and Faculty of Medicine, Erasmus MC, Rotterdam	2006-2009

Curriculum vitae

Roelof Risselada was born on June 26th, 1980 in Warmenhuizen, the Netherlands. In 1998 he graduated from the Murmellius Gymnasium in Alkmaar. He started medical school at Utrecht University in 1999. After he passed his MD examinations in 2006, he started working as PhD candidate at the Department of Medical Informatics of the Erasmus MC in Rotterdam. There, he performed the research projects described in this thesis. In 2007 he obtained his Master of Science degree in Clinical Epidemiology at the National Institute of Health Sciences (NIHES) in Rotterdam. In 2009 he obtained his Bachelor of Arts degree in Philosophy at Utrecht University. As of August 2010 he works as resident in psychiatry at GGZ Friesland

List of publications

Manuscripts based on studies described in this thesis

Risselada R, De Vries LM, Dippel DW, Van Kooten F, Van der Lugt A, Niessen WJ, Firouzian A, Stricker BH, Sturkenboom MC. Incidence, treatment, and case-fatality of subarachnoid haemorrhage. *Submitted*

Risselada R, Straatman H, van Kooten F, Dippel DW, van der Lugt A, Niessen WJ, Firouzian A, Herings RM, Sturkenboom MC. Withdrawal of statins and risk of subarachnoid hemorrhage. *Stroke*. 2009 Aug;40(8):2887-92.

Risselada R, Straatman H, Van Kooten F, Dippel DW, Van der Lugt A, Niessen WJ, Firouzian A, Herings RM, Sturkenboom MC. Platelet aggregation inhibitors, vitamin K antagonists, and risk of subarachnoid haemorrhage. *Accepted for publication in the Journal of Thrombosis and Haemostasis.*

Risselada R, Straatman H, Van Kooten F, Dippel DW, Van der Lugt A, Niessen WJ, Firouzian A, Herings RMC, Sturkenboom MC. Oral contraceptives and risk of subarachnoid haemorrhage. *Submitted*

Yasuno K, Bilguvar K, Bijlenga P, Low SK, Krischek B, Auburger G, Simon M, Krex D, Arlier Z, Nayak N, Ruigrok YM, Niemelä M, Tajima A, Von Und Zu Fraunberg M, Dóczi T, Wirjatijasa F, Hata A, Blasco J, Oszvald A, Kasuya H, Zilani G, Schoch B, Singh P, Stüer C, Risselada R, Beck J, Sola T, Ricciardi F, Aromaa A, Illig T, Schreiber S, van Duijn CM, van den Berg LH, Perret C, Proust C, Roder C, Ozturk AK, Gaál E, Berg D, Geisen C, Friedrich CM, Summers P, Frangi AF, State MW, Wichmann HE, Breteler MM, Wijmenga C, Mane S, Peltonen L, Elio V, Sturkenboom MC, Lawford P, Byrne J, Macho J, Sandalcioglu EI, Meyer B, Raabe A, Steinmetz H, Rüfenacht D, Jääskeläinen JE, Hernesniemi J, Rinkel GJ, Zembutsu H, Inoue I, Palotie A, Cambien F, Nakamura Y, Lifton RP, Günel M. Genome-wide association study of intracranial aneurysm identifies three new risk loci. *Nat Genet.* 2010 May;42(5):420-5.

Risselada R, Lingsma HF, Bauer-Mehren A, Friedrich CM, Molyneux AJ, Kerr RS, Yarnold J, Sneade M, Steyerberg EW, Sturkenboom MC. Prediction of 60 day case-fatality after aneurysmal subarachnoid haemorrhage: results from the International Subarachnoid Aneurysm Trial (ISAT). *Eur J Epidemiol*. 2010 Apr;25(4):261-6.

Risselada R, Lingsma HF, Molyneux AJ, Kerr RS, Yarnold J, Sneade M, Steyerberg EW, Sturkenboom MC. Prediction of two month modified Rankin scale with an ordinal prediction model in patients with aneurysmal subarachnoid haemorrhage. *Accepted for publication in BMC Medical Research Methodology*

Other publications

Friedrich CM, Hofmann-Apitius M, Dunlop R, Chronakis I, Sturkenboom MC, Risselada R, Oliva B, Sanz F. Initial Results on Knowledge Discovery and Decision Support for Intracranial Aneurysms. *Proceedings of HEALTHINF 2008 Conference*. INSTICC, 2008, Volume 1, 265-272.

Broekman ML, Risselada R, Engelen-Lee J, Spliet WG, Verweij BH. Glioblastoma multiforme in the posterior cranial fossa in a patient with neurofibromatosis type I. *Case Report Med.* 2009;2009:757898.

Firouzian A, Manniesing R, Flach ZH, Risselada R, Van Kooten F, Sturkenboom MC, Van der Lugt A, Niessen WJ. Intracranial aneurysm segmentation in 3D CT angiography: Method and quantitative validation with and without prior noise filtering. *Eur J Radiol.* 2010 Mar 24.