Risk factors for intracerebral hemorrhage

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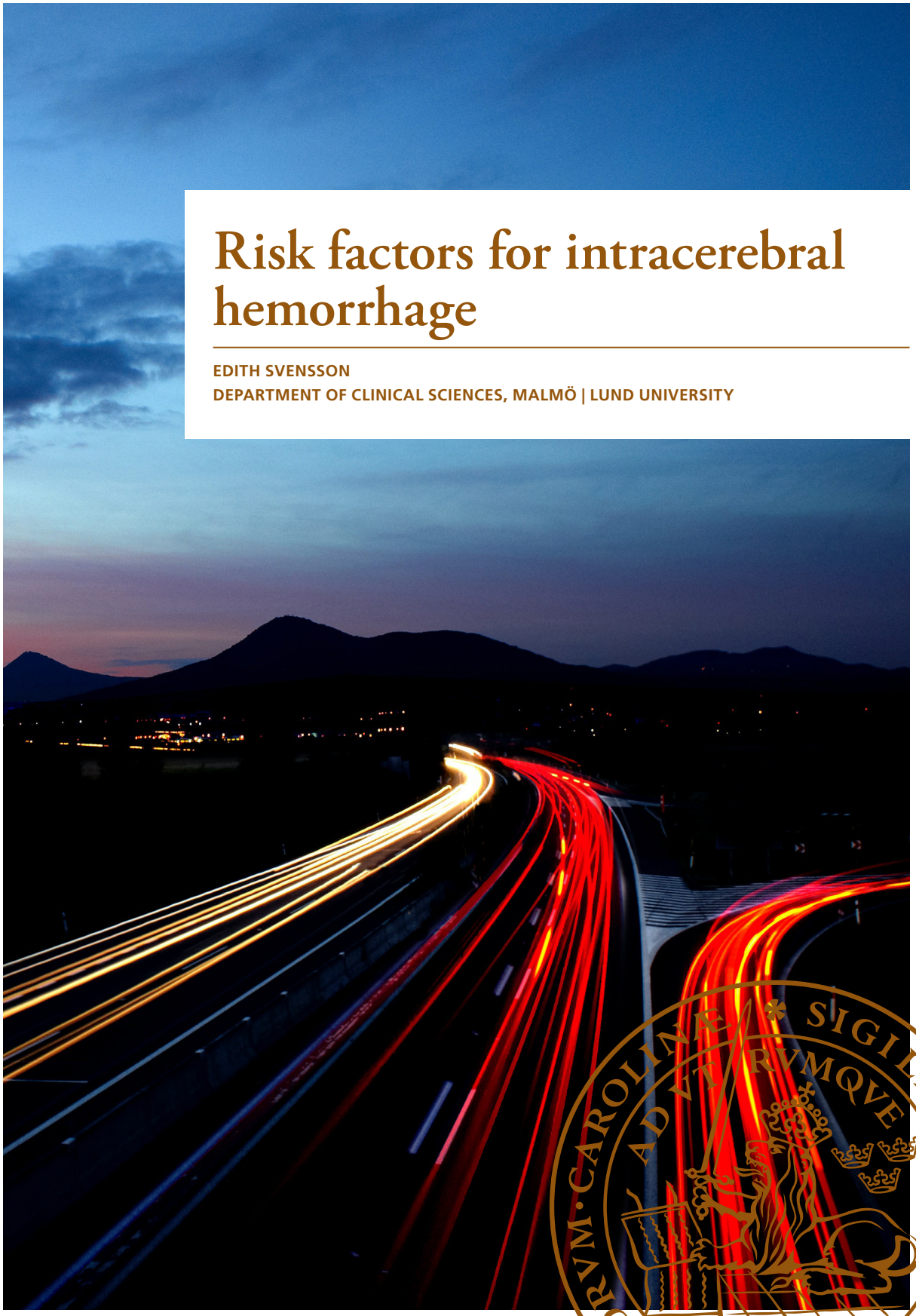

Risk factors for intracerebral hemorrhage

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Edith Svensson

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Intracerebral hemorrhage (ICH) has a high fatality rate and often poor functional outcome. As treatment options are limited, a better understanding of ICH risk factors is crucial to reduce the burden of this devastating form of stroke. Few previous studies have investigated the relationships between circulating biomarkers and ICH, or separately analyzed risk factors for ICH with different locations.

The present thesis investigates the associations of ICH with inflammatory biomarkers, detailed lipid metabolites and factors related to lifestyle and health. In the population-based Malmö Diet and Cancer Study (n=28,449), 333 ICH cases were identified up to 2014 using the Malmö Stroke Register and national registers. One cohort study and three nested case-control studies were performed. Subgroup analyses were performed for ICH with lobar and non-lobar bleeding location, fatal outcome within 28 days, poor functional outcome at three months and large volume.

Important findings include associations of ICH with tumor necrosis factor receptors 1 and 2, fibroblast growth factor 23 and low-density lipoprotein diameter, as well as inverse associations with subfraction traits of very low-density lipoprotein. Diabetes mellitus, whose relationship with ICH has been unclear, was associated only with non-lobar ICH, while smoking was associated with lobar ICH. ICH was also associated with hypertension, use of oral anticoagulants, living alone and lower apolipoprotein B.

This thesis introduces some novel findings, in particular new associations with some circulating inflammatory biomarkers and lipoprotein traits, and differences in risk factors for lobar and non-lobar ICH. It also adds clarity to some suspected risk factors where previous studies have had conflicting results. Unlike in many previous studies, information on all potential risk factors in the present thesis was collected prior to the ICH event. In future research, evaluating causality and the potential usefulness of biomarkers in clinical risk prediction for ICH would be valuable

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To my family

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List of papers

This doctoral thesis is based on the following original papers. The papers are published open access and referred to in the text by their roman numerals.

Paper I: Svensson EH, Abul-Kasim K, Söderholm M, Engström G. Tumor Necrosis Factor Receptor 1 and 2 Are Associated With Risk of Intracerebral Hemorrhage. *Stroke*. 2017; 48:2710-2715.

Paper II: Svensson EH, Abul-Kasim K, Engström G, Söderholm M. Risk factors for intracerebral haemorrhage - Results from a prospective population-based study. *Eur Stroke J*. 2020;5:278-285.

Paper III: Svensson EH, Söderholm M. Fibroblast growth factor 23 is associated with risk of intracerebral hemorrhage. *Eur J Neurol*. 2022;29:114-120.

Paper IV: Svensson EH, Björkbacka H, Engström G, Söderholm M. Lipids and lipoprotein subfractions in relation to intracerebral hemorrhage and subarachnoid hemorrhage. *Manuscript*.

Populärvetenskaplig sammanfattning

Stroke kan delas in i två kategorier: ischemisk stroke, där blodflödet till en del av hjärnan blockeras, och hemorragisk stroke, blödningsstroke, som uppstår när blodkärl i hjärnan brister. Blödning inne i hjärnvävnaden kallas intracerebral blödning och utgör ungefär tio procent av alla strokefall i Sverige. Intracerebral blödning har dålig prognos och är mycket svårbehandlat. För att minska lidande och död till följd av denna allvarliga strokeform är det helt avgörande att arbeta förebyggande. En förutsättning för detta är att bättre förstå varför intracerebral blödning uppstår.

Det finns en hel del studier som pekar ut olika livsstilsfaktorer och sjukdomar som riskfaktorer för att insjukna i intracerebral blödning. Resultaten skiljer dock mycket mellan olika studier, och det är exempelvis fortfarande oklart huruvida rökning, alkohol och diabetes påverkar risken. Ännu har man bara identifierat ett fåtal så kallade biomarkörer, ämnen som kan mätas i blodet, som påverkar risken att drabbas. Högt blodtryck och blodförtunnande läkemedel är väletablerade riskfaktorer.

Syftet med den här avhandlingen var att undersöka riskfaktorer för intracerebral blödning i befolkningen. Vi har analyserat både ”traditionella” riskfaktorer för hjärt-kärlsjukdom och nya lovande biomarkörer.

Drygt 28 000 män och kvinnor rekryterades till studien Malmö Kost Cancer mellan 1991 och 1996. De fyllde i ett frågeformulär, undersöktes av en sjuksköterska och lämnade blodprover som sparades för senare analys. Med hjälp av register har man kunnat ta reda på vilka deltagare som drabbats av intracerebral blödning. Därefter har vi med statistiska metoder räknat ut om olika misstänkta riskfaktorer faktiskt har ett statistiskt säkerställt samband med risken att insjukna. Vi har också kunnat titta separat på blödningar med ytlig respektive djup lokalisering i hjärnan, och blödningar som hade stor volym, hade dödlig utgång eller ledde omfattande funktionsnedsättning.

Vi har visat att de inflammatoriska biomarkörerna TNFR1, TNFR2 och FGF23 är associerade med risk för intracerebral blödning. Diabetes visade sig vara en stark riskfaktor för djupa blödningar, men var inte alls en riskfaktor för ytliga blödningar. Rökning var en riskfaktor för ytliga blödningar. Att leva ensam ökade också risken för intracerebral blödning. Högt blodtryck och blodförtunnande läkemedel var, som i tidigare studier, starka riskfaktorer. Vi studerade även mycket detaljerade egenskaper hos olika blodfetter och konstaterade att risken för intracerebral blödning är högre vid låga nivåer olika fettmolekyler i lipoproteinet med lägst densitet, VLDL, och vid stor diameter på partiklar av ”det onda kolesterolet”, LDL. Detta är helt nya fynd.

Ytterligare studier behövs för att utreda om dessa faktorer verkligen leder till uppkomsten av intracerebral blödning. Förhoppningsvis kommer vidare kunskaper om riskfaktorer att leda till att vi i framtiden blir bättre på att förutspå och förebygga intracerebral blödning.

Abbreviations

ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
CAA	Cerebral amyloid angiopathy
CSVD	Cerebral small vessel disease
CT	Computed tomography
CTP	Computed tomography-based planimetry
FGF23	Fibroblast growth factor 23
LDL	Low-density lipoprotein
ICH	Intracerebral hemorrhage
MDCS	Malmö Diet and Cancer Study
mRS	modified Rankin Scale
NMR	Nuclear magnetic resonance (spectroscopy)
SAH	Subarachnoid hemorrhage
VLDL	Very low-density lipoprotein
TNF	Tumor necrosis factor
TNFR1	Tumor necrosis factor receptor 1
TNFR2	Tumor necrosis factor receptor 2

Introduction

Epidemiology

With origins in ancient Greece, epidemiology – from the Greek "epi", upon, "demos", people, and "logos", study of – is the study of the distribution and determinants of disease frequency.[1] During the 20th century, epidemiology developed as a scientific discipline and became a cornerstone of public health.[1] Epidemiological studies strive to approximate the perfect comparison; following an exposed incarnation and an unexposed incarnation of the same individual through time simultaneously.

In 1970, the World Health Organization defined stroke as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than of vascular origin".[2] About 80 % of strokes are ischemic and 20 % hemorrhagic. Of hemorrhagic strokes, 2/3 are intracerebral hemorrhage (ICH) and 1/3 are subarachnoid hemorrhage (SAH). Confusingly, the term hemorrhagic stroke is often used synonymously with ICH, and in many studies it is unclear if cases of SAH are included.

Stroke is the world's second largest contributor to death and disease burden in adults, measured in disability-adjusted life years.[3] In 2019, 86 % of all stroke-related deaths and 89 % of stroke-related disability-adjusted life years occurred in lower- and middle-income countries, and stroke burden in developing countries is likely to increase substantially due to demographic changes.[3] In the last decade in Sweden, ICH incidence has been stable at around 2,800 cases per year, and mean age has consistently been around 74 years.[4] A recent study in the Lund Stroke Register compared stroke incidence in 2001–2002 with 2015–2016, and found a decreased incidence rate for ischemic stroke but not for ICH or SAH.[5]

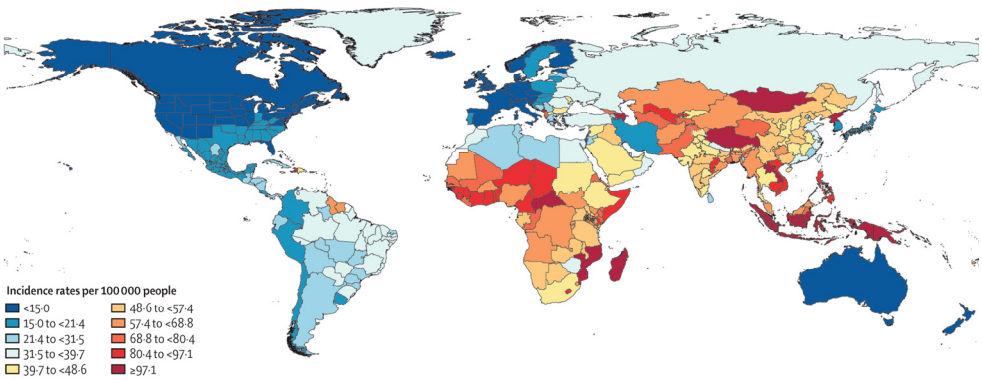

Figure 1. Age-standardized incidence rates of intracerebral hemorrhage in 2019. Reproduced from GBD 2019 Stroke Collaborators. Lancet Neurol. 2021;20:795-820 with permission from Elsevier.

Anatomy

The blood supply of the brain

Branches from the internal carotid artery form the anterior cerebral circulation, which supplies a large portion of the forebrain and parts of the diencephalon and internal capsule.[6] The posterior cerebral circulation is made up of the vertebral arteries that anastomose to form the basilar artery, branches of which supply much of the brainstem and the posterior portions of the brain.[6] Two sources of arterial small vessels converge in a watershed area deep in the subcortical white matter: terminal vessels of medium-sized arteries from the superficial circulation, and small arterial perforators stemming directly from large vessels at the base of the brain.[7]

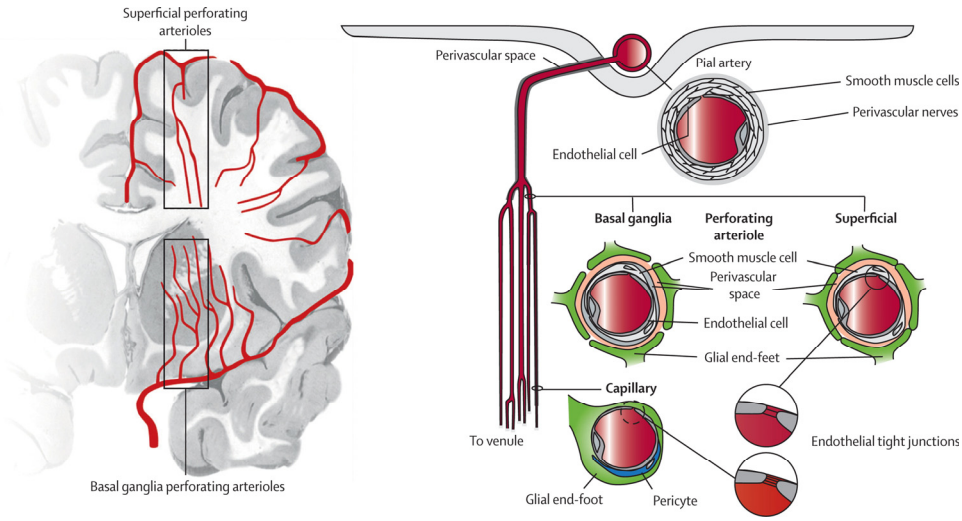

Figure 2. Schematic diagram of key features of the arteriolar and capillary wall. Reproduced from Wardlaw JM et al. Lancet Neurol. 2013:483-97 with permission from Elsevier.

ICH location

ICH is often categorized by location as lobar or non-lobar. Non-lobar ICH is sometimes divided further.[8] In the present thesis, lobar is defined as cortical or subcortical white matter, while non-lobar is defined as basal ganglia, periventricular white matter, internal capsule, cerebellum and brainstem. Review of all verified ICH cases in the Malmö Stroke Register 1993–2000 by a neurologist and a neuroradiologist concluded that 40.6 % were lobar.[9]

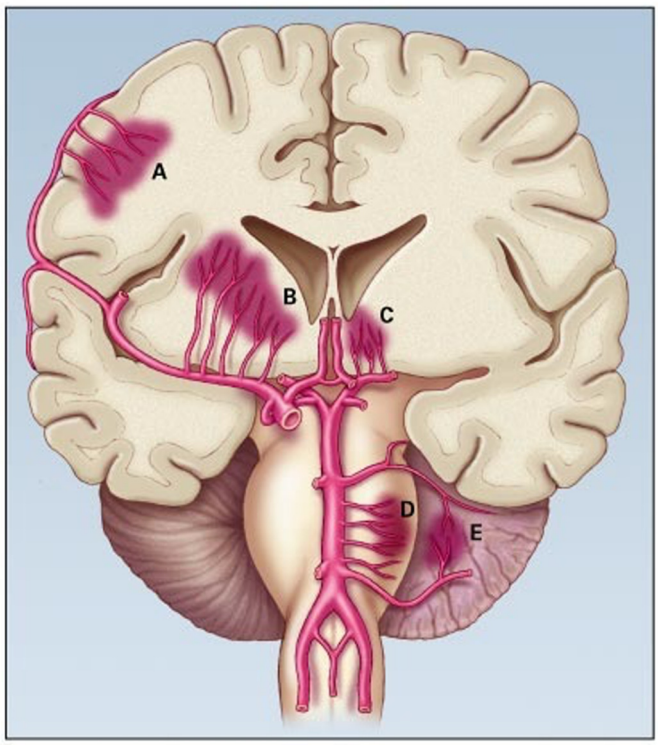

Figure 3. Most common sites and sources of intracerebral hemorrhage. A) Lobes. B) Basal ganglia. C) Thalamus. D) Pons. E) Cerebellum. Reproduced from Qureshi AI et al. N Engl J Med. 2001;344:1450-1460 with permission from Massachusetts Medical Society.

Underlying pathophysiology

Spontaneous ICH occurs due to non-traumatic rupture of small intracerebral vessels that have been damaged over time. It is an acute manifestation of cerebral small vessel disease (CSVD), an umbrella term for various vascular pathologies including hypertensive angiopathy, cerebral amyloid angiopathy (CAA), and rare genetic conditions.[10]

CAA is characterized by beta-amyloid deposition in cortical and meningeal small vessels, leading to brittle and obstructed capillaries prone to microbleeds and small ischemic lesions.[11] CAA is strongly related to age and is contained to lobar regions, favoring the occipital lobes.[10] In population-based autopsy studies, CAA were detected in two thirds of individuals with dementia and one third of individuals without dementia.[12] An aging population with higher prevalence of CAA may lead to an increasing proportion of lobar ICH.

It has been suggested that "hypertensive" ICH and lacunar strokes have a shared underlying pathology.[13] Fisher's "lacunar hypothesis", formed in his autopsy studies in the 1960s, is still widely supported. He coined the term "lipohyalinosis" to describe fibrinoid vessel wall necrosis, where structures in the degenerating walls of small vessels are replaced by connective tissue, narrowing the vessel lumen.[14] Hypertensive CSVD is also characterized by hyaline arteriolosclerosis: degeneration of smooth muscle cells, collagenous hypertrophy and fibro-hyaline deposits.[13] Predominantly, the small perforating arteries of deep brain regions are affected. Hypertensive small vessel disease is closely related to hypertension and diabetes and also occurs elsewhere in the body, notably in the kidney.[10]

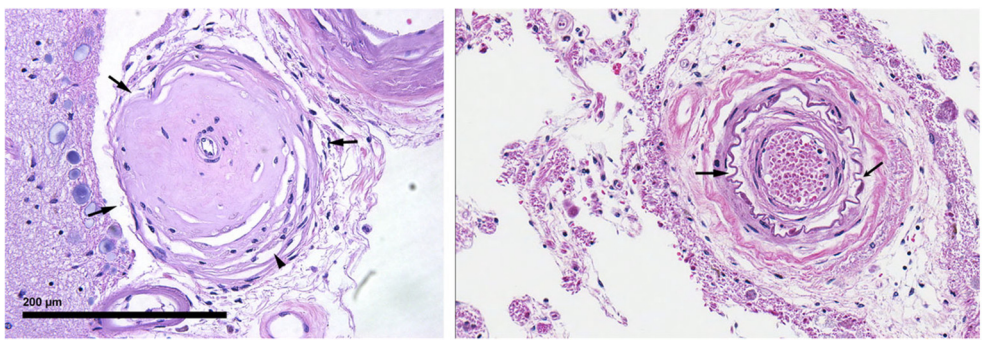

Figure 4. Arteriolosclerosis and lipohyalinosis. Reproduced from Vinters HV et al. Neuropathol Appl Neurobiol. 2018;44:247–66 with permission from John Wiley and Sons.

CSVD can be visualized using magnetic resonance imaging and computed tomography (CT). White matter hyperintensities – disseminated periventricular and subcortical white matter changes caused by increased blood-brain barrier permeability – are found in 20 % of individuals in their 60s and up to 94 % of octogenarians.[10] Cerebral microbleeds are perivascular deposits of extravasated blood products that occur in about 18 % of individuals in their 60s and 38 % of individuals over 80, and in 47–87 % of ICH patients.[10] A causative role of smoking, diet and hypertension has been found for microbleeds, while an inverse association may exist with diabetes.[10] In the Rotterdam study, microbleeds were associated with all stroke types, while specifically lobar microbleeds (characteristic for CAA) were associated with a significantly higher risk of ICH.[10] It should be noted that even with a strictly lobar or non-lobar ICH location, the underlying CSVD may be more widespread.[15–17] While CAA mainly causes lobar ICH, hypertensive CSVD contributes to both non-lobar and lobar ICH.[17] CAA and hypertensive CSVD co-exist and have a poorly understood circular interplay, where small vessel damage impedes clearance of beta-amyloid, which in turn has detrimental effects on vascular autoregulation and blood brain barrier integrity.[12,18]

Risk factors for ICH

Many studies on risk factors for stroke do not separate ischemic and hemorrhagic stroke, and hemorrhagic stroke is not always separated into ICH and SAH. Table 1 summarizes findings from some papers that investigate multiple risk factors for ICH.

Table 1. Results from some previous studies on multiple ICH risk factors

	Ariesen et al., meta-analysis, (2003, 3,000 cases)[19]	**O'Donnell et al., INTERSTROKE (2016, 3,059 cases)**[20]	**Zia et al., Malmö Preventive Project (2006, 146 cases)**[21]	**Martini et al. (2012, 597 cases)**[22]	**Sturgeon et al. (2007, 135 cases)**[23]	**Sallinen et al. (2020, 250 cases)**[24]
Hypertension	Yes	Yes	Yes	Yes	Yes	Yes (only in patients <70 years)
Lipids	Possibly (low cholesterol)	No (ApoB/ApoA1 ratio)	No (cholesterol) Yes (high triglycerides)	Yes (low cholesterol, only non-lobar ICH)	Yes (low LDL and triglycerides)	–
Smoking	No	No	Yes (only lobar ICH)	No	No	No
Alcohol	Yes	Yes	Yes	Possibly (higher risk in moderate intake, lower risk in high intake)	No	No
Diabetes	No	No	Yes (all ICH, non-lobar ICH)	–	No	–
Oral anticoagulants	–	–	–	Yes	–	–
Low physical activity	No	Yes	No	–	–	No
Overweight	–	Yes, waist-to-hip ratio	Yes, body mass index	–	No	–
Psychosocial factors	–	Yes (stress, life events, depression)	Yes (living alone, psychiatric morbidity, only non-lobar ICH)	Yes (low education)	–	Yes (low education, fatigue) No (depression)

Hypertension

Hypertension is widely recognized as the most important risk factor for ICH, particularly for non-lobar ICH.[25,26] Globally, it has been estimated that as many as 56 % of ICH cases are caused by hypertension.[20]

Smoking

Smoking is an important risk factor for many cardiovascular diseases, including ischemic stroke. Although smoking is often considered to be a risk factor for ICH, there is limited support for this in the literature.[20,23,27–29]

Alcohol

Several, but far from all, studies have found associations of high alcohol intake with ICH.[20–23,30,31] In two previous studies, modest alcohol intake (≤28 grams of alcohol per day) was associated with a lower ICH risk compared to no alcohol intake.[22,31]

Diabetes

A recent meta-analysis concluded that diabetes seems to be associated with ICH, although several studies have found no association.[32] A few studies have found associations of diabetes and high fasting glucose with non-lobar ICH.[21,33,34]

Figure 5. Factors potentially related to ICH risk.

Oral anticoagulants

Warfarin treatment is a risk factor for ICH.[35] Warfarin-associated ICH cases have higher baseline hemorrhage volume, increased rate of hemorrhage expansion and higher fatality rate.[36] Out of all cases of first-time ICH registered in Malmö Stroke Register 1993–2000, nine % had ongoing treatment with oral anticoagulants.[9] 26 % of ICH patients registered in the Swedish national register for acute stroke care, Riks-Stroke, in 2020 had ongoing treatment with oral anticoagulants (29 % warfarin, 71 % new oral anticoagulants).[4] Out of the ICH patients with warfarin treatment, half were within the therapeutic range and 16 % were above it.[4] Compared to warfarin, new oral direct thrombin and factor Xa inhibitors seem to be weaker risk factors for ICH, possibly because of lesser impairment of thrombin generation.[36]

Overweight

There is little agreement between studies on whether the association between body mass index and ICH is positive, negative or non-existing.[23,37,38] Waist-to-hip ratio has been associated with ICH, while Sturgeon et al. found no significant association between waist circumference and risk of ICH.[20,23]

Low physical activity

In the INTERSTROKE study, regular physical activity was associated with lower risk of ICH, in line with a 2003 meta-analysis.[20,39] However, in the Malmö Preventive Project and the 2003 meta-analysis by Ariesen et al., physical activity was not associated with ICH.[19,21] A possible explanation for divergent results is that physical activity is recorded and categorized differently in different studies.

Psychosocial and socioeconomic factors

It is well known that psychosocial and socioeconomic factors are associated with cardiovascular risk, but the association with ICH is less studied.[40] In the INTERSTROKE study, psychosocial factors (a combination of stress, life events, depression) were associated with risk of ICH.[20] In the Malmö Preventive Project, living alone and history of psychiatric morbidity were significantly associated with non-lobar, but not lobar, ICH.[21] It is uncertain whether educational level is associated with ICH risk.[22,23]

Lipids

High total cholesterol and low-density lipoprotein (LDL) cholesterol are risk factors for ischemic stroke, but have consistently been reported to have an inverse association with ICH risk.[41] Apolipoprotein B (apoB), the primary apolipoprotein of LDL, intermediate-density lipoprotein and very low-density lipoprotein (VLDL) particles, has in prospective studies been suggested to be a better predictor of cardiovascular disease risk than LDL itself.[42] In the few studies that have investigated apoB in relation to ICH risk, some have found an inverse association.[43] The relationship between ICH and triglycerides is less clear.[43] VLDL has been implicated as an important risk factor for cardiovascular disease independently from LDL, but is rarely studied as it is more difficult to measure.[44]

Two individuals can have the same LDL cholesterol concentration but different LDL particle concentration, and the individual with denser LDL is believed to be at higher cardiovascular risk.[45] Several lipid metabolites have been identified as cardiovascular risk factors independently of total cholesterol and LDL.[46] However, only a handful of studies have analyzed detailed lipid subfractions traits in relation to stroke, and neither of the two studies on ICH and hemorrhagic stroke found any significant associations.[47,48]

Circulating inflammatory biomarkers

Few biomarkers are known to be associated with incident ICH.

The inflammatory biomarker favored in the clinical setting, C-reactive protein, is strongly associated with cardiovascular disease in general and has been found to be associated with ischemic stroke, but does not seem to be associated with ICH risk.[49–51] It has previously been shown in the MDCS that leukocyte count is not significantly associated with incident ICH.[52]

We have recently shown that the cytokine growth differentiation factor 15, which has antithrombotic properties and promotes oxidative stress and inflammation, is a risk factor for ICH.[53] (Paper not included in the present thesis.)

Tumor necrosis factor receptor 1 and 2 (TNFR1 and TNFR2) are the major receptors for the proinflammatory cytokine tumor necrosis factor (TNF). The TNF receptors are associated with diabetes, arterial stiffness and hypertension.[54–56] In the Framingham study, TNFR2 was associated with incident ischemic stroke and higher prevalence of cerebral microbleeds.[51,57] To our knowledge, TNFR1 and TNFR2 have not previously been studied in relation to ICH.

The phosphate-regulating hormone fibroblast growth factor 23 (FGF23), known for its association with chronic kidney disease, is emerging as a risk factor for different cardiovascular diseases.[58] A 2018 meta-analysis concluded that FGF23 is associated with incident all-cause stroke and hemorrhagic stroke, but not ischemic stroke.[59]

The only previous study that explicitly studied ICH as a separate endpoint found a significant association, but only had 26 ICH cases.[60] One study found that hemorrhagic stroke, defined as ICH or SAH, was associated with FGF23. The other study on hemorrhagic stroke, which may or may not have included SAH in the outcome, found no association. In the MDCS, FGF23 was associated with incident SAH.[61]

Risk factors for ischemic stroke and subarachnoid hemorrhage

The INTERSTROKE study, a case-control study on stroke risk factors in 32 countries, estimated that the following risk factors account for 91 % of population attributable risk for all stroke: Hypertension, physical activity, apoB/apoA1 ratio, diet, waist-to-hip ratio, psychosocial factors (a combination of stress, life events and depression), current smoking, cardiac causes (a combination of atrial fibrillation, previous myocardial infarction, rheumatic valve disease, and prosthetic heart valves), alcohol intake and diabetes.[20] Hypertension had a stronger association with ICH compared to ischemic stroke (in case-case analyses, formal comparison), while smoking, diabetes and apoB/apoA1 ratio were only associated with ischemic stroke. The INTERSTROKE study is frequently cited, including in the present thesis, but it should be noted that risk factor data was collected after the stroke event, and that methodology and data quality may vary between the participating countries.

A 2021 study in the Malmö Diet and Cancer Study (MDCS) with 2,270 cases of incident ischemic stroke found significant associations of IS with the following factors: older age, male sex, current smoking, larger waist circumference, higher systolic blood pressure, diabetes mellitus, lower apoA1, higher apoB and higher leukocyte count.[62]

A study with pooled data from five European prospective cohorts concluded that there may be important differences in risk factor profiles between subtypes of ischemic stroke.[63] Lacunar stroke cases were less likely than non-lacunar ischemic stroke cases to have ischemic heart disease and carotid stenosis. There was also a higher tendency for lacunar stroke cases to be smokers and have high alcohol intake. Hypertension and diabetes were equally prevalent in lacunar and non-lacunar ischemic stroke. As mentioned previously, lacunar stroke may have a shared underlying pathology with “hypertensive” ICH.[13]

A comparison of consecutive ICH and SAH patients in Finland in 1996 concluded that ICH patients were more likely than SAH patients to have hypertension, diabetes, high alcohol intake and anticoagulant treatment, while SAH patients were

more likely to smoke.[64] ICH patients were also older, and a higher percentage was male.

High lipid levels, including high total cholesterol and LDL, is a well-established risk factor for ischemic stroke, and there may be a similar relationship with SAH.[65] Paper IV included analyses of the association of both ICH and SAH with lipid subfraction traits, but only ICH will be presented in the present thesis.

Treatment and outcome of ICH

It takes only 30 seconds of interrupted blood flow to alter brain metabolism, and within minutes brain tissue starts to infarct.[2] Extravasated blood may quickly lead to both increased intracranial pressure and local mechanical compression.[66,67] Neurotoxic blood components contribute to edema formation and inflammation.[66] Within 24 hours, 1/3 of ICH patients experience hematoma expansion, both from the initial location and from consequent rupture of peripheral vessels.[36] Acute ICH presents with non-focal symptoms related to increased intracranial pressure (decreased level of consciousness, headache, nausea, vomiting) and focal symptoms that depend on the ICH location.[36]

While surgical hematoma evacuation theoretically may reduce mass effect and toxic effects, there is no compelling evidence of a benefit over conservative treatment in supratentorial hemorrhage.[67] In large or compressive cerebellar hemorrhage, surgery may be beneficial.67 In Sweden in 2020, 8 % of ICH cases received neurosurgical treatment (13 % of cerebellar hemorrhages).[4] There is limited evidence of improved fatality and morbidity by lowering blood pressure, lowering intracranial pressure, treating seizures or normalizing body temperature.[67,68] High INR due to treatment with oral anticoagulants should be reversed.[67] Swedish ICH patients that received stroke unit care had a lower risk of death or institutional living after 3 months, compared to other types of hospital wards.[69]

In all first-time ICH cases in Malmö Stroke Register in 1993–2000 (n=474), mean volume was 26.6 mL.[9] 26 % died within 28 days. 3-year mortality was 49 %, and was associated with male sex, older age, intraventricular hemorrhage, large volume, brainstem hemorrhage and lower consciousness level. In 2020, 35% of ICH cases in Riks-Stroke were fatal within three months, compared to 17 % of ischemic stroke cases.[4] 25 % of ICH survivors in Sweden that had previously been functionally independent required assistance with activities of daily living three months after the ICH event.[4] In one study, 40 % of patients had at least one serious adverse event in the first 90 days after ICH, the most common being pneumonia, aspiration, respiratory failure, pulmonary embolism, and sepsis.[70]

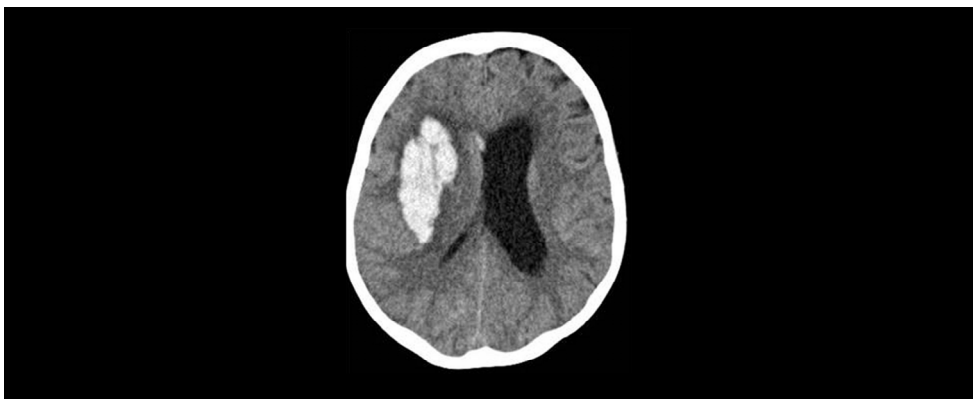

Figure 6. CT scan of intracerebral hemorrhage. Blood appears as white. Reprinted from Taylor CJ. Anaesth. Intensive Care Med. 2020;21:8–12 with permission from Elsevier.

Aims

The overall aim of this thesis was to investigate risk factors for incident intracerebral hemorrhage in the general population. The specific aims of the included papers are as follows:

I. To study the association of tumor necrosis factor receptors 1 and 2 with incident ICH in the general population.

II. To study the association of lifestyle- and health-related factors with incident lobar and non-lobar ICH in the general population.

III. To study the association of fibroblast growth factor 23 with incident ICH in the general population.

IV. To study the association of lipids and lipoprotein subfraction traits with incident ICH (and SAH) in the general population.

Methods

Study population

The Malmö Diet and Cancer Study

The MDCS is a population-based cohort study intended to study the association between diet and lifestyle factors and health outcomes. Between 1991 and 1996, all men born 1923–1945 and all women born 1923–1950, residing in the city of Malmö, Sweden were invited by mail and advertisements in newspapers and public places. Of the 74,138 individuals in the invited birth cohorts, 68,905 were eligible to participate and 28,449 completed the baseline examination, corresponding to 41 % of the eligible population.[71,72] Mean age was lower for women than men (57 vs 59 years) because additional female participants were invited in 1995 to optimize investigation of breast cancer.[73]

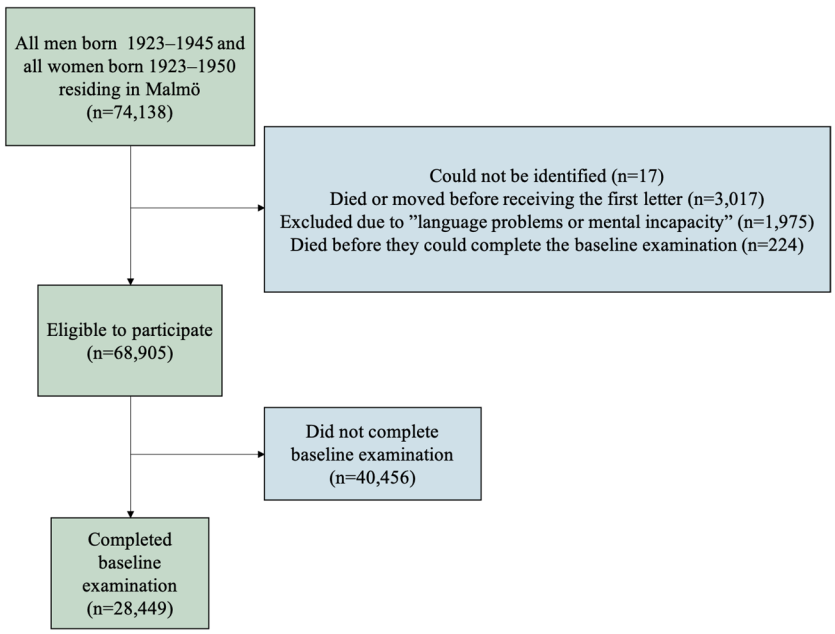

Figure 7. The Malmö Diet and Cancer Study

Assessment of exposure

Baseline examination

The baseline examination consisted of a physical examination performed by a nurse, blood samples and a self-administered questionnaire. Blood pressure was measured using a mercury-column sphygmomanometer in the supine position after 10 minutes of rest. Body mass index was calculated from weight and height. Waist circumference was measured, divided into quartiles with sex-specific cut-offs, and combined into a joint variable. Plasma was separated and frozen to –80 degrees C within 1 hour. The questionnaire was returned after two weeks and checked for completeness by trained study nurses. Participants were classified as diabetic if they reported in the questionnaire that they had a diabetes diagnosis or used anti-diabetic drugs, or if they had been diagnosed with diabetes according to national or local registers. Current smoking was defined as regular or occasional smoking. High alcohol intake was defined as >40 grams/day for men and >30 grams/day for women. A physical activity score was calculated based on time spent on 18 different activities, based on the Minnesota Leisure Time Physical Activity Instrument, and subjects in the lowest quartile were considered to have low physical activity in the present thesis.[74] Here, educational level is condensed into primary (≤9 school years), secondary (10–12 school years) or university level (>12 school years).

Figure 8. Baseline examination

Biomarker quantification

Levels of protein biomarkers, including TNFR1, TNFR2 and FGF23 were measured at SciLifeLab (Uppsala, Sweden) using the Proseek Multiplex CVD I assay from Olink. Before the analysis was performed in 2015, the samples were aliquoted to 96-well plates. The proximity extended assay technique uses probes consisting of antibody pairs linked to oligonucleotides. Upon pairwise binding to target

molecules, a single DNA strand is created through polymerization reactions, and the DNA strands are then quantified by real time polymerase chain reaction.[75]

ApoA1 and apoB concentrations used in paper II were analyzed by Quest Diagnostics (San Juan Capistraon, CA, USA) in 2013 using an immunonephelometric assay that was run on a Siemens BNII (Siemens, Newark, DE, USA).[76]

^{1}H nuclear magnetic resonance (NMR) spectroscopy detects molecule-specific signals, providing qualitative and quantitative lipoprotein measurements: their cholesterol and triglyceride content, and number and size of subfraction particles.[77] NMR analyses for paper IV were performed at the Nightingale lab (Helsinki, Finland) in 2018.

Ascertainment of outcome

Between 1989 and 2010, Malmö Stroke Register continuously registered cases of stroke at the Malmö University Hospital, which was the only hospital serving the city of Malmö.[78]A specialized research nurse performed a broad, systematic search among patients with neurological symptoms in the neurological wards, other relevant wards and the emergency department. ICH cases that occurred after initial hospitalization or other reasons were also included. Supported by a senior neurologist, the research nurse validated all stroke cases by review of patient records and in some cases patient interviews. To identify cases of ICH in the MDCS that occurred after 2010, and in individuals treated for ICH in hospitals outside of Malmö, the national hospital discharge register and causes of death register were used (International Classification of Diseases 9th edition code 431 and 10th edition code I61.0–9). Together, these registers covered all hospitalizations and deaths during the follow-up period.

ICH was considered when CT, magnetic resonance imaging or autopsy showed blood in the brain parenchyma. CT frequency in Malmö Stroke Register increased dramatically in the early 1990s and had reached 98.3 % by 2001.[78] The majority of ICH cases identified in the MDCS were further validated by review of medical charts and images by stroke physicians. A few cases that occurred outside of Malmö (n=10) could not be validated but were still included in the present thesis. Senior neuroradiologists assisted in measurement of hemorrhage volume and classification of hemorrhages by location as lobar or non-lobar. Volume was determined using the formula ABC/2, the most commonly used method for hemorrhage measurement at the time. A is the greatest hemorrhage diameter, B is the diameter 90 degrees to A, and C is the approximate number of CT slices with hemorrhage multiplied by the slice thickness.[79] If available workup did not indicate secondary causes (trauma,

tumor, vascular malformations, hemorrhagic infarction, thrombolysis), cases were considered spontaneous ICH and were included in this thesis.

The modified Rankin Scale (mRS) is the primary outcome scale for most acute stroke trials.[80] It has seven levels with clinically relevant difference, ranging from no symptoms to dead. Riks-Stroke uses a follow-up questionnaire after stroke that includes questions about functional level three months after the stroke event. Self-reported functional outcome was translated into mRS scores using an algorithm that has been shown to have a high level of agreement with actual mRS scores.[81]

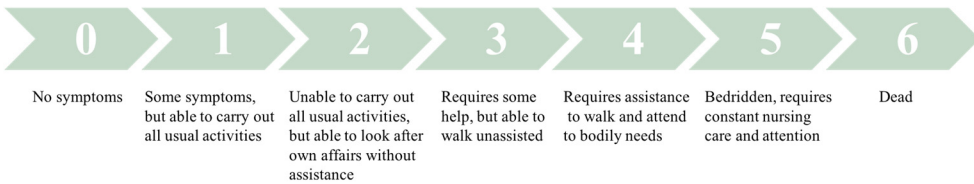

Figure 9. The modified Rankin Scale

Statistical analyses

Cohort studies

A cohort is defined as "any designated group of individuals who are followed or traced over a period of time". Survival analysis is the most efficient method for calculating risks when follow-up duration varies between subjects.[82] The incidence rate in the event-free part of the cohort at a given time t is called hazard. The hazard ratio, given from dividing the hazard in the exposed group with the hazard in the unexposed group, represents instantaneous risk at each point in time. Each individual contributes time-at-risk until the end of follow-up, until experiencing the outcome or until being censored (due to competing event or loss to follow-up). In paper II, we use Cox's proportional hazards regression, a very common technique in survival analysis that was introduced in 1972.[82] We used age as the underlying time scale.

In 1995, Lunn and McNeil demonstrated that Cox's proportional hazards regression can be used to analyze competing risks in survival analysis.[83] The dataset is duplicated with two rows per individual, e.g. one row where the failure is lobar ICH and one row where the failure is non-lobar ICH. The Cox regression is stratified on these event types. When all variables remain duplicated, the results are identical to those from regular Cox regression. However, when one variable is un-duplicated,

the effect measure for that specific variable will be the same for both strata. A likelihood ratio test, with one degree of freedom, of the duplicated analysis and the un-duplicated analysis gives a p-value for the difference in effect measures for the covariate. In paper II, we refer to this as the p-value for heterogeneity between the associations of each variable with lobar and non-lobar ICH.

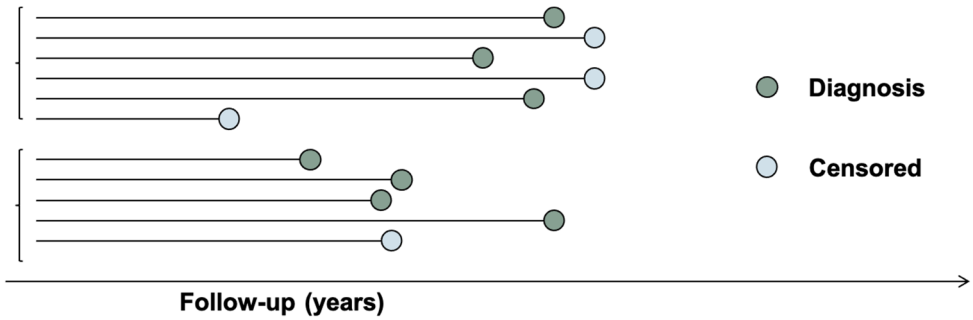

Figure 10. Principals for a cohort study

Nested case-control studies

Within a cohort, nested case-control studies can be performed to limit the number of individuals for whom additional exposure information – e.g. new biomarkers– needs to be obtained. This study design was used in papers I, II and IV. With incidence density sampling, controls with the same length of follow-up as their respective case are selected longitudinally from cohort members at risk just prior to the failure time of each case.[84] Cases may also serve as controls in an earlier risk-set, when they were still at risk of the studied outcome. To estimate the incidence rate ratio in a nested case-control study, as in papers I, II and IV, conditional logistic regression is used so that the analysis is stratified by each case-control set.

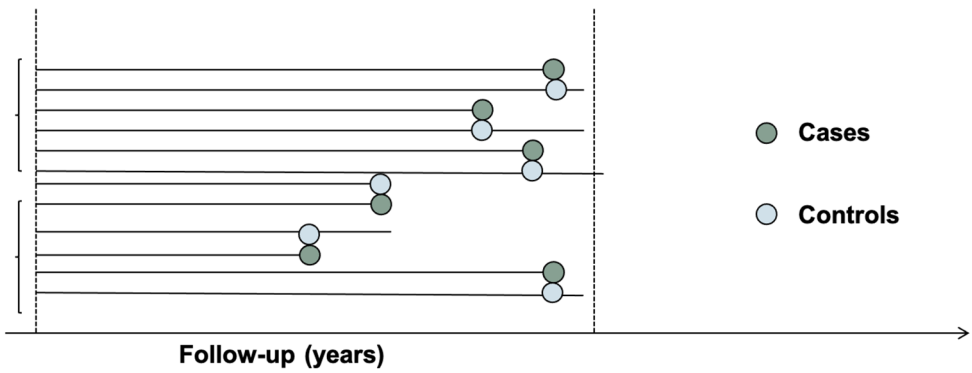

Figure 11. Principals for a nested case-control study

Sensitivity analyses

In paper I, sensitivity analyses were used to evaluate whether associations of TNFR1 and TNFR2 were affected by fasting status or history of stroke or myocardial infarction at baseline. In a sensitivity analysis in paper III, we excluded all individuals with prevalent or incident chronic kidney disease to account for a potential confounding effect of renal function on the association between FGF23 and ICH.

Missing data

For most covariates in this thesis, the amount of missing data was limited, and it was handled using listwise deletion. In short, only complete cases were included in the multivariable-adjusted analyses. An exception is estimated glomerular filtration rate, which was deemed crucial to include in the adjustment model in paper III because of the relationship between FGF23 and kidney function, but which was only available for the MDCS-Cardiovascular sub-cohort. Estimated glomerular filtration rate was therefore estimated using multiple imputation. Multiple imputation generates several plausible options for the missing values based on an imputation model consisting of all relevant variables, including the outcome (ICH). The desired analysis is then performed separately in these parallel datasets, and the final result (in this case, the odds ratio for the association between FGF23 and ICH) is pooled across the imputed datasets.[85]

Principal component analysis

Principle component analysis is a data reduction method used to summarize a multitude of variables in a few uncorrelated components that account for maximum possible variance from the original variables.[86] Principle component analysis is useful for identifying patterns of association across variables, but should only be used in datasets with a high degree of correlation among the original variables. The first component accounts for maximum possible variance. The second component, which is uncorrelated with the first component, captures most information not captured by the first component, and so on.

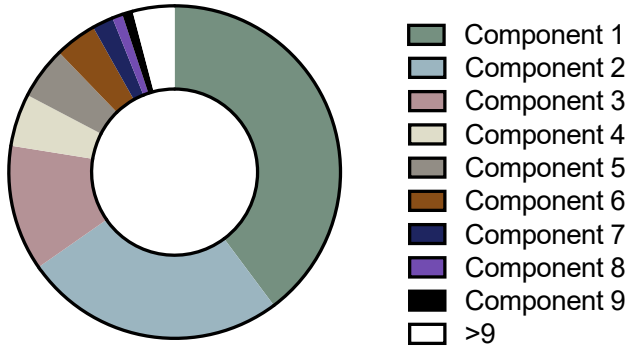

Figure 12. Percent of the variance in the lipid data explained by each principal component

Ethical considerations

Epidemiological research is often considered to have fewer ethical issues than, e.g., clinical trials or laboratory research involving animals. All patients in the MDCS volunteered in a study of risk factors for chronic diseases, including cardiovascular disease, and donated blood samples for research. There was no medical risk associated with the studies in the present thesis. There is, however, still an integrity risk. This risk was minimized by using pseudonymized data in all studies. The potential benefits from the study include new information of factors that increase the risk of ICH, and perhaps identification of new targets for prevention. The potential benefits outweigh the potential risks of the studies.

The MDCS and its sub-projects were approved by the Lund University ethics committee (LU 51/90, 166/2007, 633/2009, 566/2013, 2016/452). Written informed consent was obtained from all participants. Region Skåne approved validation of the ICH cases by review of hospital records and CT scans (KVB000256 2012-10 Dnr 002-15).

Results

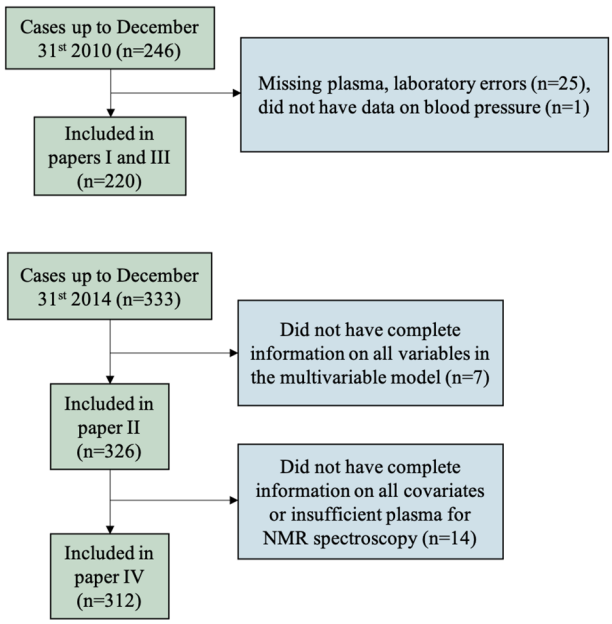

Figure 13. ICH cases included in each paper

Up to 2014, mean follow-up time in the MDCS was 18.4 years. Mean age at the ICH event was 74.8 years (range, 47–91 years), and 47 % of ICH cases were male. The crude incidence rate for ICH in the MDCS was 63.7 (95% confidence interval 57.2–70.9) cases/100,000 person-years. Hemorrhage location was defined for 183 cases up to 2010 (82 lobar, 101 non-lobar) and 268 cases up to 2014 (117 lobar, 151 non-lobar). Up to 2010, data on volume was available for 166 cases out of 220 (median 14 mL, range 0.14–254 mL, interquartile range 4–45 mL), and 41 cases had hemorrhages >40 mL and were classified as high volume. Data from Riks-Stroke on functional outcome three months after the ICH event was available for 171 cases out of 220, and 135 had poor functional outcome defined as mRS 3–6. 68 cases out of 220 were fatal within 28 days.

Baseline characteristics

Compared to the whole cohort, ICH cases were older at baseline, had higher systolic and diastolic blood pressure, and were more likely to be male and live alone. 80 % of ICH cases and 62 % of the whole MDCS were hypertensive, defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 and/or use of blood pressure-lowering drugs at baseline. Less than 1 % of MDCS subjects use oral anticoagulants at baseline, but it was more common in ICH cases (2.4 % of cases). Mean age at baseline was 58.2 years for the whole cohort and 61.8 years for ICH cases. Compared to lobar ICH cases, non-lobar ICH cases had higher blood pressure and were more likely to have diabetes and live alone.

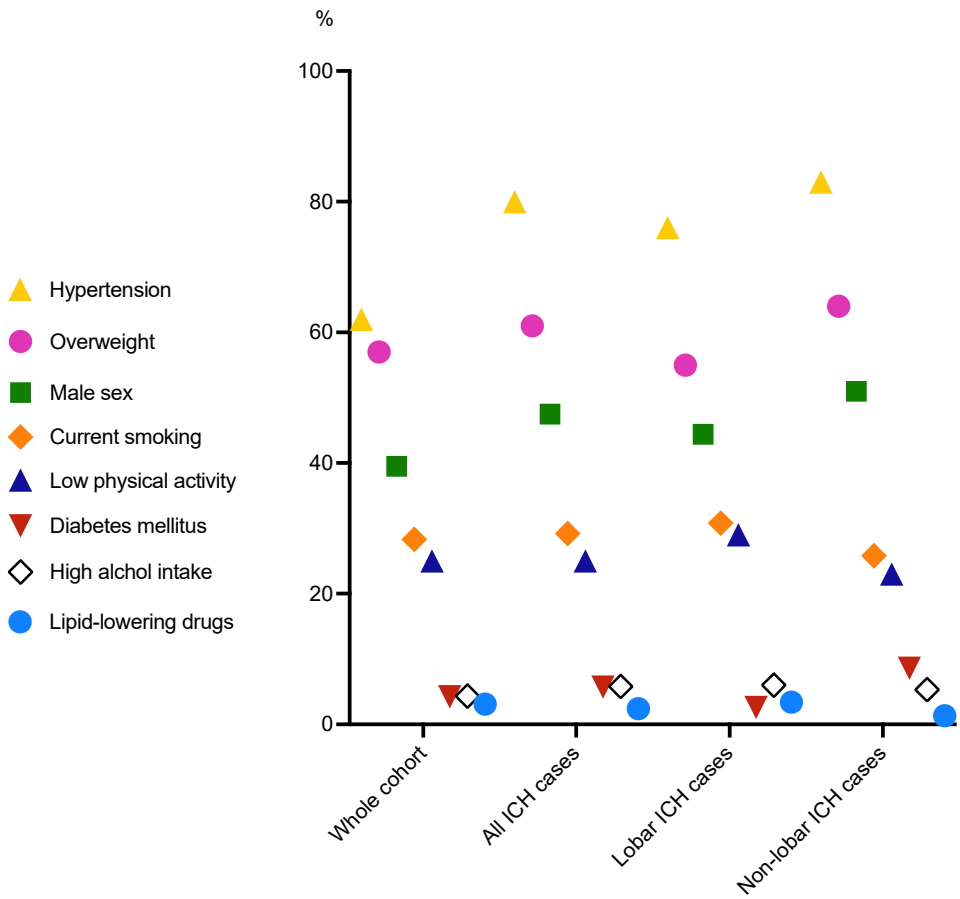

Figure 14. Baseline characteristics in the whole MDCS cohort, all ICH cases, lobar ICH cases and non-lobar ICH cases. Hypertension = systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 and/or use of blood pressure-lowering drugs. Overweight = body mass index ≥25 and/or waist circumference ≥94 for men and ≥80 for women. High alcohol intake = >40 grams/day for men, >30 grams/day for women

Papers I and III

Both paper I and III are nested case-control studies within the MDCS, based on the same 220 ICH cases that had occurred up to 2010, and the same 244 age- and sex-matched controls.

In paper I, we show that higher levels of TNFR1 and TNFR2 are associated with incident ICH. These associations remained significant after multivariable adjustment. The odds ratio was higher for TNFR1. In paper III, higher FGF23 was associated with incident ICH.

In subgroup analyses, the odds ratios tended to be somewhat higher for non-lobar ICH compared to lobar ICH. TNFR1 and TNFR2 were significantly associated with ICH with poor functional outcome at three months and ICH fatal within 28 days. The association with ICH with large volume was equally strong, but not quite significant. FGF23 was significantly associated with ICH in all subgroup analyses.

Odds ratios are reported with 95 % confidence intervals. Associations with p-values below 0.05 are considered statistically significant.

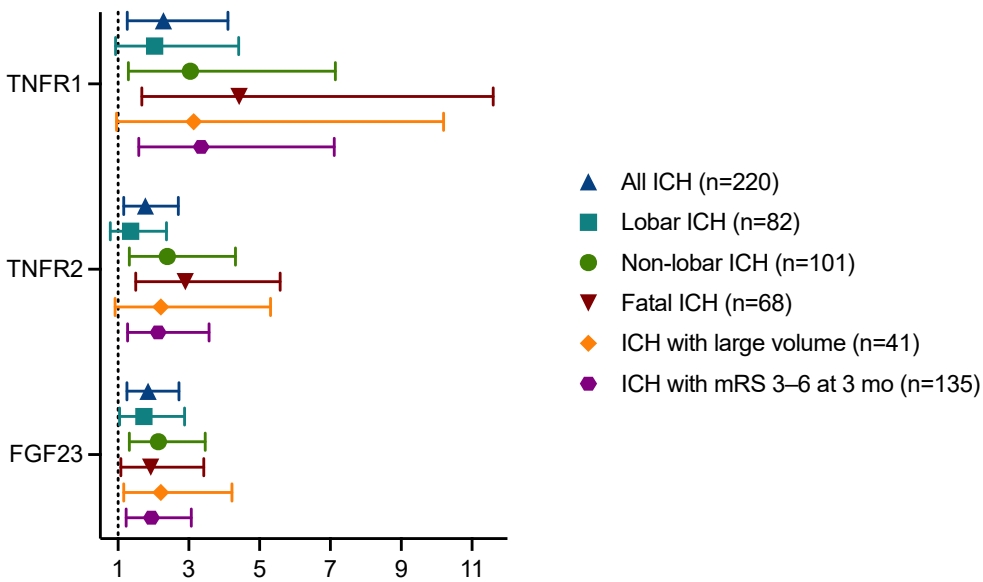

Figure 15. Associations of all ICH and ICH subgroups with TNFR1, TNFR2 and FGF23 with multivariable-adjusted odds ratios with 95 % confidence intervals per 1 unit increase on the log2 scale. N = number of ICH cases in each subgroup.

Paper II

ICH was associated with systolic and diastolic blood pressure. Smoking was associated with all ICH and lobar ICH, but not with non-lobar ICH. Diabetes mellitus had a strong association with non-lobar ICH, but was not significantly associated with lobar ICH or all ICH. Living alone was associated with all ICH and non-lobar ICH, but not lobar ICH. ApoB levels were inversely associated with all ICH, while results were not significant in subgroup analyses. Use of oral anticoagulants was strongly associated with both ICH types, but results were not significant for non-lobar ICH. For diabetes and diastolic blood pressure, there was a significant heterogeneity between the associations with lobar and non-lobar ICH. High alcohol intake was associated with ICH in the age- and sex-adjusted analyses, but results were not significant after multivariable adjustment. Educational level, body mass index, waist circumference and physical activity were not associated with ICH (Table 2 of paper II). Hazard ratios are reported with 95 % confidence intervals. Associations with p-values below 0.05 are considered statistically significant.

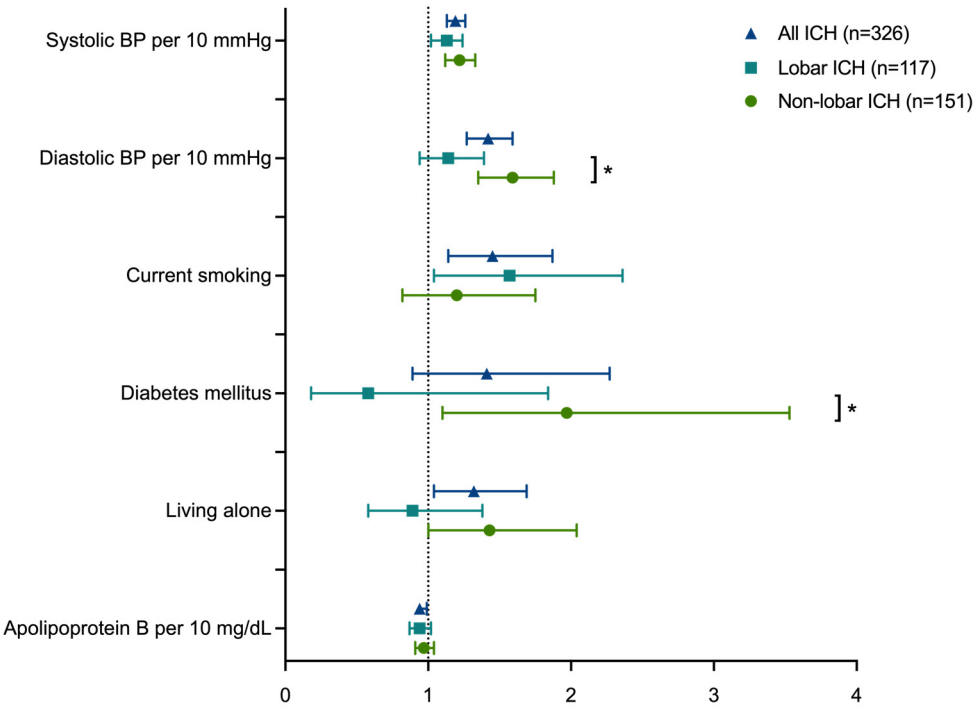

Figure 16. Variables significantly associated with all ICH, lobar ICH and/or non-lobar ICH. Multivariable-adjusted hazard ratios with 95 % confidence intervals. * Statistically significant heterogeneity in the association with lobar and non-lobar ICH.

Paper IV

After correction for multiple testing, significant inverse associations with ICH were found for traits of L-VLDL and M-VLDL (particle concentration, cholesterol concentration, total lipids, total phospholipids, free cholesterol, triglycerides). VLDL triglycerides were inversely associated with ICH. Larger LDL diameter positively associated with ICH. Non-significant inverse associations with ICH were found for total cholesterol, apoB, triglycerides, VLDL cholesterol, LDL cholesterol, and traits of LDL subfractions and IDL. High density lipoprotein was not associated with ICH. Odds ratios are reported with 95 % confidence intervals. Associations with p-values below 0.0056 are considered statistically significant.

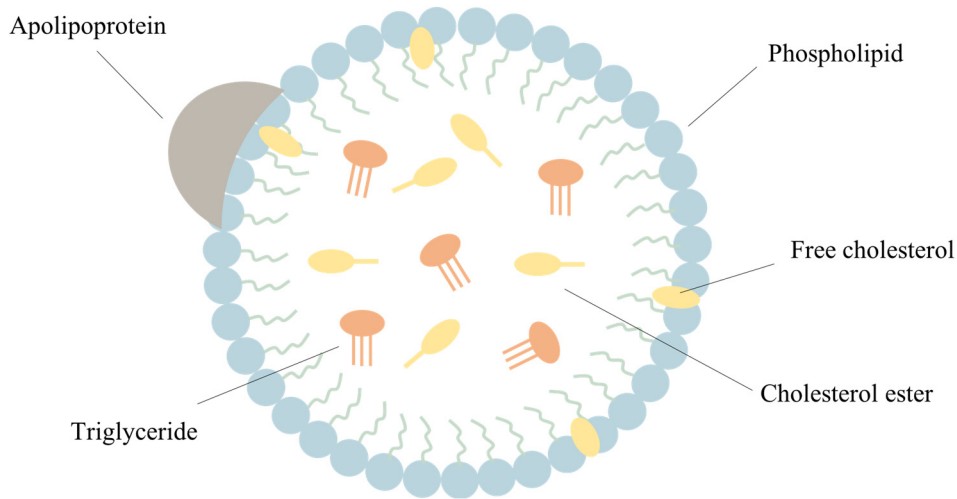

Figure 17. Schematic of a lipoprotein particle.

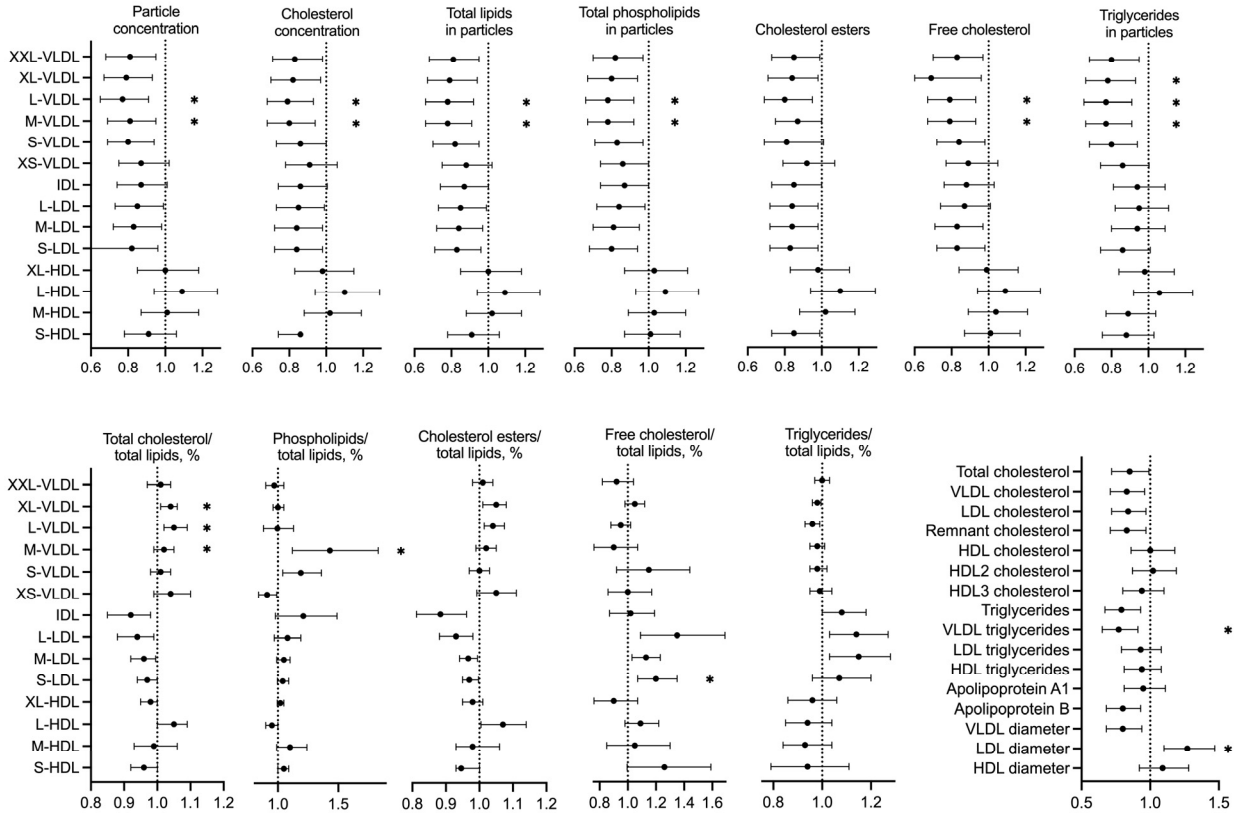

Figure 18. Associations of lipid subfractions and their traits with ICH. Multivariable-adjusted odds ratios per 1 standard deviation increment (per 1 % increment for ratios) with 95 % confidence intervals. Model includes age, sex, systolic blood pressure, body mass index, current smoking, high alcohol intake (>40 grams/day for men and >30 grams/day for women), diabetes mellitus, use of lipid-lowering drugs, use of blood pressure-lowering drugs and use of oral anticoagulants. * p<0.0056

Discussion

General discussion

Medical conditions and drugs

Hypertension is the most important risk factor for ICH, so the association with blood pressure in the present thesis was expected. There are likely to be several underlying mechanisms. Hypertension contributes to development of CSVD, possibly related to inflammation and stimulated growth and proliferation of vascular smooth muscle cells stimulated by angiotensin II.[10,36] Arterial stiffness exposes the fragile vessels in the brain to greater blood pressure fluctuations.[87]

In paper II, we find that diabetes is associated with a doubled risk of non-lobar ICH, while there was no association with all ICH or lobar-ICH. The association of diabetes specifically with non-lobar ICH is in line with results from some recent studies, and may be due to the role of diabetes in hypertensive CSVD.[21,33,34] The heterogeneity in the association of diabetes with ICH with different locations was statistically significant in paper II. If the proportion of lobar and non-lobar ICH varies between studies, it may in part explain why results differ. If diabetes truly is a risk factor for ICH, the high prevalence of diabetes in the general population makes it an important contributor to ICH burden.

Use of oral anticoagulants was a strong risk factor for ICH. However, low prevalence in the MDCS affected results in the subgroup analyses, which should be interpreted with caution. It is likely that use of oral anticoagulants is a risk factor for both lobar and non-lobar ICH.

Lifestyle factors

Smoking has previously been found to be associated with lobar but not non-lobar ICH, and with lobar but not non-lobar microbleeds.[21,88] This is in line with the results from paper II, where smoking was associated with all ICH and lobar ICH, but not non-lobar ICH, although this heterogeneity was non-significant. A potential mechanism behind the association of smoking with ICH is that smoking causes microaneurysms.[21] A complex relationship exists between smoking, smoking cessation, blood pressure and weight. In many studies, smokers have lower weight

and blood pressure than non-smokers, and smoking cessation is associated with increases in weight and blood pressure.[89,90] This could contribute to differences between studies, although adjusting for these variables had limited impact on the association of smoking with ICH in the present thesis.

In the present thesis, high alcohol intake was significantly associated with ICH in age- and sex-adjusted analyses but not in the multivariable-adjusted analyses. Any model including systolic blood pressure gave non-significant results, suggesting that the relationship between alcohol and ICH might be related to hypertension. A recent Swedish study found a strong independent association of incident ICH with the alcohol biomarker phosphatidylethanol, but no association with self-reported alcohol intake, suggesting that misclassification of high-risk individuals leads to underestimation of a true association.[91] Such misclassification might have contributed to the lack of association in our study. Our cut-off, 40 grams/day for men and 30 grams/day for women, was also fairly high. It is also possible that our sample was not large enough to identify a small risk increase, as only four percent of the cohort were classified as having high alcohol intake. It is possible that high alcohol intake is less common in MDCS participants compared to non-participants.

Physical activity was not associated with ICH, in line with results from several studies.[20,21] A possible explanation for divergent results is physical activity is recorded and categorized differently in different studies.

Living alone was associated with ICH. However, it is not possible to determine whether this is a true effect of living alone (e.g. no partner encouraging one to seek medical attention and comply with treatment) or due to residual confounding (e.g. higher alcohol intake and smoking than admitted in the questionnaire, or other variables not included in the analysis). We found no association between education and ICH in paper II, in line with results from Sturgeon et al.[23]

Inflammatory biomarkers

Our finding that the TNF receptors are associated with ICH is in line with a previous study that reporter higher TNFR2 levels in individuals with microbleeds, which can be considered a precursor to ICH.[57] TNF is one of the most important proinflammatory cytokines in the body. TNF receptors are associated with apoptosis and cell proliferation, respectively, and have been noted to have complex interactions with each other.[92–94] Experimental activation of TNF disrupts the blood-brain barrier.[95] Impairment of the blood-brain barrier is, in turn, considered to be an important mechanism of CSVD. The association of TNF receptors with ICH may be related to their previously reported association with diabetes, arterial stiffness and hypertension, although results remained significant after adjustment for blood pressure and diabetes.[54–56]

FGF23 is associated with several cardiovascular outcomes, but it is unclear if it may have a causal role.[96] Volume of white matter hyperintensities – a radiological sign of CSVD – was associated with FGF23 in one study.[97] This presents at potential link between FGF23 and ICH. The reason for the association between FGF23 and ICH is not known. While FGF23 has a positive feedback loop with the renin-angiotensin system, which controls blood pressure, our findings were still significant after adjustment for blood pressure.[98,99] However, FGF23 has been found to be associated with incident hypertension, so the association of FGF23 with ICH may be related to a future increase in blood pressure.[100] Inflammation has been suggested to play an important role in development of CSVD, and FGF23 has a positive feedback loop with inflammatory cytokines and suppresses anti-inflammatory vitamin D.[98,99] In vitro, FGF23 can promote oxidative stress in endothelial cells, and activate fibroblasts, and it drives cardiac remodeling in animals.[98] It is not known if FGF23 targets other cells in the vessel wall, e.g. vascular smooth muscle cells.[98]

Lipids

In paper IV, ICH risk was inversely associated with subfraction traits of VLDL, intermediate density lipoprotein and LDL, as well as with total triglycerides, VLDL triglycerides and apoB. Some of these associations were no longer significant after correction for multiple testing. Larger LDL diameter was associated with higher ICH risk. In paper II, immunonephelometrically measured apoB was inversely associated with all ICH, while results were not significant in subgroup analyses of lobar and non-lobar ICH, likely due to lack of power.

This is one of the first studies to investigate VLDL and its subfractions in relation to ICH, and, to our knowledge, the first to find significant associations. The inverse associations of ICH with apoB, total cholesterol and LDL cholesterol were expected and in line with previous literature. It may be speculated that the association between ICH and apoB, and perhaps even LDL cholesterol, is actually due to VLDL levels. Our results also support an inverse association between ICH and triglycerides, which has been seen in some previous studies but far from all.

Small LDL particles are generally considered to be the most atherogenic.[45] Our finding that larger LDL diameter was associated with higher ICH risk, is contrary to this. Apart from one previous study, which found a non-significant association between larger LDL diameter and higher risk of ICH in subjects with a history of stroke, this has not been seen previously.[101] Just as for the well-established but inexplicable inverse association between ICH and LDL cholesterol, it is unclear why ICH would differ from other types of cardiovascular disease on this matter. It has been theorized that low cholesterol impairs the wall integrity of small vessels, and perhaps a protective effect of small LDL is the higher tendency for small LDL to incorporate into the vessel wall.[45,102]

The novel findings of associations between some lipid metabolites and ICH need to be validated in future studies.

It has previously been suspected that lowering cholesterol using statins may be accompanied by an increased risk of ICH, but a large meta-analysis has found no association between statin therapy and higher risk of ICH.[103] In the MDCS, statin use was very uncommon and was adjusted for in the multivariable model, making it very unlikely to influence our results.

Methodological considerations

Missing data

Missing data can be due to different mechanisms. Data is considered missing completely at random if all subjects are equally likely to have missing data. If missingness is due to an observed variable, the data is missing at random. If probability of missingness is depends on an unobserved variable, the data is missing not at random. An illustrative example of this by Sterne et al. concerns data on blood pressure.[85] The mechanism behind missingness is different if A) it is due to broken measurement equipment, B) if younger people (an observed variable) both had lower blood pressure and were less likely to have their blood pressure measured, and C) if people with an unobserved risk factor related to hypertension were more or less likely to miss doctor's appointments where blood pressure would be measured. In multiple imputation, it is very important that data is not missing not at random. For estimated glomerular filtration rate in paper III, we believe that we have strong support for this assumption, since participation in the MDCS-Cardiovascular sub-cohort was randomized and the participation rate was high.

Changes during follow-up

All variables in the present thesis were registered at baseline, between 1991 and 1996. During follow-up period, it is likely that some variables have changed. It is likely that many MDCS subjects have quit smoking and been treated for their hypertension, which if anything should bias the results towards null. It is also likely that many started treatment with oral anticoagulants during follow-up.

Data quality and information bias

Information bias occurs when information is measured or recorded inaccurately. The most important type is misclassification bias, which can be non-differential

(cases and controls equally likely to be misclassified, usually bias towards the null) or differential (performance of exposure identification differs for cases and controls, bias away from the null).[104] In the present thesis, where all data was collected before cases occurred, misclassification is expected to be mostly non-differential.

The questionnaire

In the MDCS, a physical activity score was calculated from responses in a questionnaire by multiplying time spent of different activities with an intensity score. A validating study using accelerometers found a relatively low correlation between self-reported and measured physical activity.[105] Other examples of potential misclassification are under-reporting of alcohol intake and smoking.

Functional outcome

The mRS has been criticized for bias towards motor disability and failure to account for cognition, emotion, and psychosocial function.[106] In Riks-Stroke, the reliability of data on the patients' function level may be affected by the fact the questionaries were self-administered, with no clinical assessment.[107] A study comparing "real" mRS scores to the score translated from Riks-Stroke's questionnaire concluded that about two thirds of patients were correctly classified, with a higher risk of a too high score than a too low score.[81] If many patients were wrongly classified as having poor functional outcome, and this misclassifications was non-differential in relation to levels of TNF receptors and FGF23, results from those subgroup analyses in papers I and III would have been attenuated.

Hemorrhage volume

Although more sophisticated than ABC/2, at the time, computed tomography-based planimetry (CTP) was not widely available. There is a good overall agreement between ABC/2 and CTP within 5 mL or 20 % of CTP volume, and 95 % of ABC/2 scans are within 12.5 mL of the CTP.[79] However, as the ABC/2 method is based on the assumption that the hemorrhage has a bipyramidal ellipsoid shape, it tends to overestimate size of hemorrhages with large volume, lobar location, and irregular shape.[108] If anything, an overestimation of hemorrhage size should attenuate the results, since it leads to less extreme cases being included in this subgroup, as long as misclassification is non-differential.

Olink panel

Proximity extension assays have strongly decreased antibody cross-reactivity compared to other immunoassays (e.g. bead-based or planar arrays), since both antibodies in the pair must bind to the target to trigger the PCR-based quantification.[75] For proteins with low concentrations, such as TNF receptors and FGF23, it is crucial to minimize noise from background interactions with more abundant bystander proteins. A downside to using this panel is that results are in

arbitrary units, which does not allow direct comparisons of absolute biomarker levels with other studies. However, it does not affect the validity of the results.

^{1}H nuclear magnetic resonance spectroscopy

NMR spectroscopy has excellent reproducibility, allows precise structure determination and is essentially free of batch effects.[109,110] However, NMR has lower sensitivity than mass spectroscopy.[109] Due to spectral overlap, lipid subclasses at the center of the methyl peak, e.g. LDL, have a higher error than those at the limits (VLDL and high density lipoprotein-3).[45]

Fasting vs non-fasting samples

The original samples from the MDCS were non-fasting. However, food intake has a modest impact on lipid levels, and does not appear to affect associations of lipid metabolites with cardiovascular disease risk.[111] There is no evidence that levels of inflammatory biomarkers, e.g. TNF receptors and FGF23, are affected by fasting status.

Confounding bias, collider stratification bias, selection bias

Confounding bias occurs due to lack of conditioning on a common cause of exposure and outcome.[112] The traditional solution is to adjust analyses using multivariable regression models, or to stratify analyses. Covariates for the multivariable regression analyses in the present thesis were selected a priori based on previous literature. A graphical method called directed acyclic graphs can also be used to identify potential confounders that should be adjusted for, and colliders that should not be adjusted for (see below). However, it can be argued that one should adjust for important ICH risk factors such as hypertension and use of oral anticoagulants regardless of whether the directed acyclic graphs suggested that they were confounders. There is always a risk of residual confounding due to confounders that are unknown, unmeasured or imperfectly measured.

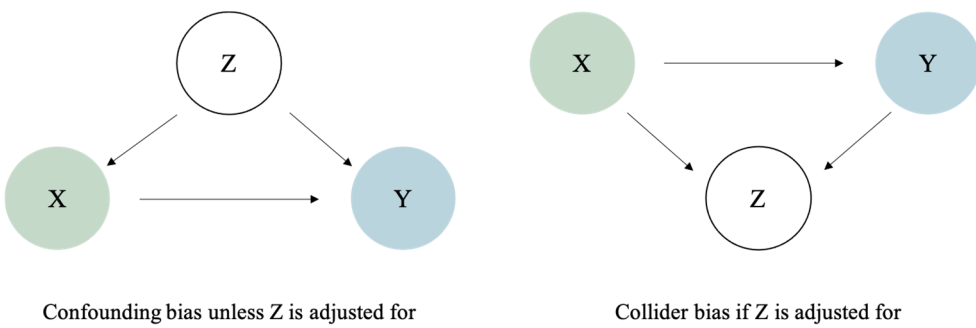

Figure 19. Directed acyclic graphs depicting confounding bias and colliding bias.

In a directed acyclic graph, a collider is a common effect of two parents.[112] If X and Y both cause Z, then adjusting for Z (or an effect of Z) will create a conditional association between X and Y. This may introduce a spurious association, or reverse the true direction of an association, even though both intuitive reasoning and stepwise regression may suggest that Z should be adjusted for to avoid confounding bias.[113] If Z represents participating in the study, and X and Y represent the exposure and outcome of the study, then the collider bias in question may actually be considered a form of selection bias.

Selection bias occurs when the association between exposure and outcome differs between study participants and non-participants due to a systematic error in the selection process.[1] A form of selection bias relevant to cohort studies is non-response bias. Non-response affects internal validity if non-participation is related to both exposure and outcome.[104] The MDCS had a 41 % participation rate, and non-participants had higher incidence of cancer and higher mortality than participants, although prevalence of smoking and obesity were similar.[73] This would have been an important issue if our aim had been to study prevalence of a certain risk factor. However, unless the association between a certain risk factor and ICH risk differs between participants and non-participants, non-participation does not affect the internal validity of our studies. As the decision to participate in the MDCS preceded the ICH event, this is unlikely. If the outcome had been e.g. cancer, people with undetected cancer may have started to feel sick and be less likely to participate.

An important prerequisite in all case-control studies is that controls should be sampled from the same study base as give rise to the cases. In the nested case-control studies in the present thesis, both cases and controls come from the MDCS.

Loss to follow-up introduces selection bias if it is non-random in relation to exposure and outcome.[104] In the present thesis, loss to follow-up is not a large issue since the vast majority of patients with ICH in Sweden are admitted to hospitals and registered, and this should not differ between exposed and non-exposed individuals. Furthermore, the Swedish patient register and causes of death register covered all hospitalizations and deaths during the follow-up.

To match or not to match?

In case-control studies, matching controls to cases may improve statistical power.[105] However, matching is a form of conditioning, and, as discussed previously, conditioning can lead to selection bias. To avoid this, one must not match on variables that are common descendants of exposure and outcome.[105] In the present thesis, matching is restricted to age and sex.

Risk factors or risk markers?

The present study investigated associations between different variables and ICH. A risk marker can be considered a risk factor if a change to this factor also changes ICH risk.[114] In other words, if the relationship is causal. Epidemiological studies, with the exception of Mendelian randomization studies, cannot determine if an association is causal or not.

Conclusions

This thesis investigated risk factors for incident ICH in the general population. Based on the findings of the included papers, the following conclusions were made:

1. Tumor necrosis factor receptor 1 and tumor necrosis factor receptor 2 are associated with incident ICH. The associations were significant in subgroup analyses of lobar ICH, non-lobar ICH, fatal ICH, ICH with large volume and ICH with poor functional outcome. These findings are novel.
2. Diabetes mellitus has a strong association with non-lobar ICH, but is not associated with all ICH or lobar ICH. We found that this heterogeneity was statistically significant, which has not been described previously.
3. Smoking, living alone, high blood pressure, use of oral anticoagulants and lower levels of apolipoprotein B are associated with incident ICH.
4. Fibroblast growth factor 23 is associated with ICH, which adds support the small number of previous studies on FGF23 and hemorrhagic stroke. The significant associations of FGF23 with lobar ICH, non-lobar ICH, fatal ICH, ICH with large volume and ICH with poor functional outcome have not been described previously.
5. Traits of medium-sized and large VLDL subfractions, including particle concentration and cholesterol concentration, are inversely associated with ICH. This is a novel finding.
6. Larger LDL diameter is significantly associated with higher risk of ICH, which has not been seen previously.

Future perspectives

Globally, deaths and disability from ICH are on the rise as the incidence rate increases in low- and middle- income countries. With limited treatment options, preventive measures against this devastating form of stroke are more important than ever. Firstly, we need to better understand the complex pathophysiology behind ICH. Secondly, useful clinical risk prediction models are needed.

An important limitation in most epidemiological studies is they cannot establish causality. A promising solution to this is Mendelian randomization, where genetic variants are used as instrumental variables. It would be of great interest to evaluate whether levels of biomarkers such as TNFR1, TNFR2 and FGF23 are causally related to ICH. If that is the case, perhaps they might even serve as therapeutic targets in the future. Evaluating the potential usefulness of TNF receptors and FGF23 in clinical risk prediction would also be valuable.

There is evidence that supports that lobar and non-lobar ICH have differences in underlying pathophysiology. In the present thesis, diabetes and diastolic blood pressure were only associated with non-lobar ICH. More studies are needed on differences in risk factor profile for ICH with different locations.

The relationships of cohabitation status and psychosocial factors with risk of ICH has only been explored in a few studies, and the reason behind these associations still is unclear.

Using NMR metabolomics platforms to study the association between lipid profile and ICH risk a new and promising method. More studies are needed on VLDL subfractions and lipoprotein particles diameters in relation to ICH risk.

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