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Biomarkers and risk of intracerebral hemorrhage
– Population-based studies in northern Sweden

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To my family,

“If you can dream it, you can do it”

Tom Fitzgerald

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Abstract

Background

Intracerebral hemorrhage (ICH) is a disease associated with a high morbidity and mortality and treatment options for the condition are limited. Even though an ICH event usually comes as a surprise to the affected individual, pathogenetic processes often have occurred before the sudden ICH event and may have preceded disease onset by years. It is possible that individuals at increased risk of ICH could be identified using biomarkers, for example markers of hemostasis and fibrinolysis. Even if these biomarkers are not part of the causal chain, they could be used as risk indicators to better define high-risk groups. Another approach could be to measure already established risk markers for ICH, such as self-reported alcohol consumption, using a blood biomarker. That could increase measurement reliability and consequently the accuracy of the estimates of ICH risk.

Aims

The aim of this thesis was to investigate potential biomarkers and risk of ICH. Specific aims were to evaluate the associations between factor XII, D-dimer, von Willebrand factor (VWF), ABO blood groups with focus on blood group O, phosphatidylethanol (PEth), and risk of ICH.

Methods

In our first study, aiming to investigate the association between factor XII and risk of hemorrhagic stroke, we followed participants of the health examination northern Sweden MONItoring trends and determinants in CArdiovascular disease (MONICA) performed in 1994 as a cohort until 2011. Factor XII concentrations were measured in blood samples drawn at the baseline health examination where the participants also answered a questionnaire regarding lifestyle factors and medical history. Diagnosis codes from the National Patient Register and the Swedish Cause of Death Register were used to find cases of hemorrhagic stroke, defined as ICH or subarachnoid hemorrhage.

In the subsequent studies, the associations between biomarkers (factor XII, D-dimer, VWF, ABO blood groups, and PEth) and risk of ICH were investigated using a matched, nested case-referent design including individuals that had participated in the Västerbotten Intervention Programme, the MONICA and the Mammography Screening Project in 1985–2007. The participants donated blood samples at baseline for future research which were stored at -80 degrees C until biomarker analyses. The majority of the participants also underwent a baseline health examination including a questionnaire. First-ever ICH diagnoses during the study period 1985–2007 were validated using medical records and autopsy reports. To each case, two referents were matched for age, sex, geographical region, health examination date and health examination setting.

Results

In the cohort study of the association between factor XII concentrations and risk of hemorrhagic stroke, 1,852 participants were included among which 30 experienced a hemorrhagic stroke event. There was an association between high factor XII and risk of hemorrhagic stroke in a multivariable model (hazard ratio 1.51; 95% confidence interval [CI] 1.03–2.21 per standard deviation [SD] of factor XII). In the case-referent study of the association between factor XII and risk of ICH, 70 cases with ICH and 137 matched referents were included. We found no association between factor XII and risk of ICH in a multivariable model (odds ratio [OR] 1.06; 95% CI 0.57–1.97 per SD of factor XII).

The study of the association between D-dimer and risk of ICH included 141 cases and 255 matched referents. We found an association between D-dimer and risk of ICH in a multivariable model (OR 1.36; 95% CI 1.05–1.77 per SD of D-dimer). When stratifying the analysis for time between blood sampling and ICH event in tertiles, the association remained significant in the cases with the shortest time between blood sampling and ICH event in a multivariable model (OR 1.78; 95% CI 1.05–3.05 per SD of D-dimer).

The study investigating the association between VWF and risk of ICH included 139 cases and 276 referents. We found no association between VWF and risk of ICH in a multivariable model (OR 0.85; 95% CI 0.54–1.34 per SD of VWF). In the analysis investigating the associations between ABO blood groups and risk of ICH, 162 cases and 317 referents were included. We found no association between blood group O compared to non-O blood groups and risk of ICH (OR 0.96; 95% CI 0.65–1.42).

In the study of the association between PEth concentrations and risk of ICH, 97 cases and 180 referents were included. There was an association between PEth concentrations > 0.30 μmol/L compared to < 0.01 μmol/L and risk of ICH in a multivariable model (OR 4.64; 95% CI 1.49–14.40).

Conclusions

High concentrations of D-dimer and PEth are associated with an increased risk of ICH. Our conclusion of the two studies investigating the association between factor XII and risk of hemorrhagic stroke and ICH respectively is that there is no association between factor XII and risk of ICH. We found no association between VWF or blood group O and risk of ICH.

Sammanfattning på svenska

Stroke är en sjukdom som kan bero på antingen en blodpropp eller en hjärnblödning. Vid en stroke dör hjärnceller till följd av syrebrist och personen som drabbas kan få symptom som till exempel påverkan på tal, syn eller svårigheter att röra arm eller ben. Hjärnblödning sker på grund av att ett blodkärl i hjärnan brister. Genom att öka vår kunskap om vad som händer i kroppen under tiden *innan* en person insjuknar i hjärnblödning kan vi bli bättre på att hitta personer med hög risk för att få sjukdomen. Förhoppningsvis leder detta till att vi i framtiden kan bli bättre på att förebygga sjukdomen.

Ett sätt att undersöka detta är att använda biomarkörer. Biomarkörer kan till exempel vara proteiner och andra ämnen man mäter i blodprover. De kan användas till att uppskatta hur hög risk en person har att drabbas av en sjukdom. Det finns idag inga etablerade biomarkörer för att uppskatta risken att insjukna i hjärnblödning.

I den här avhandlingen har vi studerat fem olika biomarkörer som mäts i blodprover. Fyra av biomarkörerna (koagulationsfaktor XII, D-dimer, von Willebrandfaktor och blodgrupp O) tänker vi skulle kunna spegla processer i blodkärlen som sker före en hjärnblödning eller ha att göra med blodets levringsförmåga och förmåga att lösa upp blodproppar. Tidigare forskning har visat att personer som dricker mycket alkohol tycks ha en högre risk att få hjärnblödning. Den femte biomarkören vi har studerat, fosfatidyletanol, visar hur mycket alkohol en person har druckit veckorna innan blodprovet tas. Syftet med den här avhandlingen var att undersöka om de fem biomarkörena skulle kunna användas för att uppskatta en persons risk för hjärnblödning.

Vi har studerat personer som deltagit i hälsoundersökningar i Västerbotten och Norrbotten mellan 1985 och 2007. Hälsoundersökningarna heter MONICA, Västerbottens hälsoundersökning och mammografiscreeningsprojektet. Vid hälsoundersökningarna har personerna lämnat blodprover som skulle få användas för framtida forskning. Blodproverna frystes ned i samband med hälsoundersökningen. Vi har sedan tinat dem och analyserat nivåerna av de olika biomarkörerna. Majoriteten av personerna som är med i våra studier har i samband med att de lämnade blodproverna också svarat på en enkät om tidigare sjukdomar och levnadsvanor samt mätt längd, vikt, blodtryck, blodsocker och blodfetter.

Vi har sedan jämfört nivåerna av olika biomarkörer i blodproven mellan personer som fick en hjärnblödning under studieperioden och en grupp personer som inte fick någon hjärnblödning. Det finns en del faktorer, till exempel högt blodtryck och hög ålder som vi sedan tidigare vet att har samband med ökad risk att få en hjärnblödning. Sådana kända faktorer tog vi hänsyn till i vår jämförelse.

I vår första och andra studie undersökte vi biomarkören koagulationsfaktor XII. I den första studien såg vi ett möjligt samband mellan höga nivåer av

koagulationsfaktor XII och ökad risk för hjärnblödning. När vi undersökte sambandet mellan koagulationsfaktor XII och risk för hjärnblödning i en ytterligare studie där fler personer med hjärnblödning deltog kunde vi inte se något sådant samband. Vi fann ett samband mellan höga nivåer av D-dimer och ökad risk för att få en hjärnblödning. Sambandet var tydligast hos personer för vilka det inte gått så lång tid mellan att blodproverna togs och att hjärnblödningen inträffade. Vi fann inget samband mellan von Willebrandfaktor eller blodgrupp O och risk för hjärnblödning.

Vår sista studie visade att personer med förhöjda nivåer av fosfatidyletanol, en biomarkör för alkoholintag, hade en ökad risk att få en hjärnblödning. Personer med ett fosfatidyletanol-värde som motsvarar ett intag av ungefär fyra standardglas alkoholhaltig dryck per dag hade en nästan fem gånger ökad risk att drabbas av hjärnblödning jämfört med personer med inget eller mycket lågt alkoholintag.

Vår tolkning av resultaten av våra studier är att D-dimer och fosfatidyletanol i framtiden kanske skulle kunna användas som biomarkörer för att hitta personer med hög risk för hjärnblödning. Innan detta kan bli verklighet krävs dock mer forskning.

Abbreviations

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13

AUDIT, Alcohol Use Disorders Identification Test

BMI, body mass index

CI, confidence interval

CT, computerized tomography

EDTA, ethylenediaminetetraacetic acid

FXII, factor XII

H-ATOMIC, hypertension, cerebral amyloid angiopathy, tumor, oral anticoagulants, vascular malformation, infrequent causes, cryptogenic

ICD, International Classification of Diseases

ICH, intracerebral hemorrhage

IU, international units

MONICA, MONItoring trends and determinants in CArdiovascular disease

mRS, modified Rankin Scale

NSHDS, Northern Sweden Health and Disease Study

OGTT, oral glucose tolerance test

OR, odds ratio

PEth, phosphatidylethanol

SD, standard deviation

SMASH-U, structural vascular lesion, medication, amyloid angiopathy, systemic diseases, hypertension, and undetermined

VIP, Västerbotten Intervention Programme

VWF, von Willebrand factor

Original papers

This thesis is based on the following papers:

I. Johansson K. Jansson J-H, Johansson L, Bylesjö I, Nilsson TK, Eliasson M, Söderberg S, Lind M. Factor XII as a Risk Marker for Hemorrhagic Stroke: A Prospective Cohort Study. Cerebrovasc Dis Extra. 2017;7(1):84–94.

II. Johansson K, Johansson L, Nilsson TK, Lind MM. Factor XII Concentrations and Risk of Intracerebral Haemorrhage. A Prospective Case-Referent Study. J Stroke Cerebrovasc Dis 2021 Jan 4;30(3):105565. doi: 10.1016/j.jstrokecerebrovasdis. 2020.105565. [Epub ahead of print]

III. Johansson K, Jansson J-H, Johansson L, Wiklund P-G, Nilsson TK, Lind M. D-dimer Is Associated With First-Ever Intracerebral Hemorrhage. Stroke. 2018;49 (9):2034–2039.

IV. Johansson K, Jansson J-H, Johansson L, Ekblom K, Lind MM. Von Willebrand factor, ABO blood group, and risk of first-ever intracerebral hemorrhage: A prospective nested case-control study. Thromb Res. 2020;195:77–80.

V. Johansson K, Johansson L, Pennlert J, Söderberg S, Jansson J-H, Lind MM. Phosphatidylethanol Levels, As a Marker of Alcohol Consumption, Are Associated With Risk of Intracerebral Hemorrhage. Stroke. 2020;51:2148–2152.

Introduction

The introduction to this thesis has four main sections: (1) an overview of intracerebral hemorrhage (ICH) including definitions, epidemiology and pathogenesis, as well as treatment and prognosis, (2) a summary of the associations between selected traditional cardiovascular risk markers and risk of ICH, (3) the concepts of biomarkers and biobanks as well as an introduction to the potential new biomarkers of ICH that were investigated in the papers of this thesis, and (4) a brief introduction to a few important epidemiological concepts.

Intracerebral hemorrhage

Definitions

Definitions related to stroke and intracranial bleedings are shown in Table 1. According to the World Health Organization, stroke is defined as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" (1). Stroke can be divided into ischemic stroke and hemorrhagic stroke where ischemic stroke constitutes the majority of the stroke cases, 81% of stroke cases in Sweden (2). Hemorrhagic stroke can be further classified into ICH and subarachnoid hemorrhage (3). A stroke caused by an ICH can be defined as "rapidly developing clinical signs of neurological dysfunction attributable to a focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma" (4). Subarachnoid hemorrhage is a bleeding located in the subarachnoid space of the brain. Intracranial hemorrhage is an umbrella term for all bleedings within the cranial vault whether traumatic or non-traumatic (5).

Table 1. Definitions related to stroke and intracranial bleedings

Term	Definition
Stroke	"Rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin." (1)
ICH	"A focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma" (4). This definition overlaps with the definition of spontaneous ICH, a term that underlines the non-traumatic nature of the hemorrhage (6).
Stroke caused by an ICH	"Rapidly developing clinical signs of neurological dysfunction attributable to a focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma" (4).
Subarachnoid hemorrhage	"Bleeding into the subarachnoid space (the space between the arachnoid membrane and the pia mater of the brain or spinal cord)" (4).
Hemorrhagic stroke	ICH or subarachnoid hemorrhage (3)
Intracranial hemorrhage	"Encompasses ICH, subdural hematoma, epidural bleeds, and subarachnoid hemorrhage" (5)

ICH can occur in different locations. A lobar ICH is located in the cortical or subcortical regions of the cerebrum whereas a deep ICH is located deep inside the cerebral parenchyma, such as in the basal ganglia or the thalamus. Sometimes, all infratentorial bleedings are defined as deep ICHs (7). Among ICH events, 45–50% are deep, 41% are lobar, 6–8% are cerebellar and 3–5% are located in the brainstem (8, 9).

Epidemiology

In 2017, there were more than three million incident ICH cases globally and in high-income countries, ICH cases constituted 19% of stroke events (10). The proportion of ICH among all stroke varies between 9% and 27% globally (11). In Sweden, ICH constitutes 15% of all stroke (2).

In a multinational systematic review and meta-analysis, the overall incidence of ICH was 25/100.000 person-years (12). The crude incidence of ICH did not decrease between 1980 and 2008 according to the same meta-analysis (12). Similarly, in Sweden, the age- and sex- standardized incidence of ICH in 2015

was 24/100.000 person-years and there was no significant decrease in incidence between 2001–2002 and 2015–2016 (2).

In contrast, according to the National Board of Health and Welfare in Sweden, the number of patients with an International Classification of Diseases (ICD) - 10 in-patient diagnosis of ICH (I.61), has decreased over the years from an incidence of 53 per 100,000 inhabitants aged 15 and older in 1998 to 37 per 100,000 in 2019 as shown in Figure 1 (13).

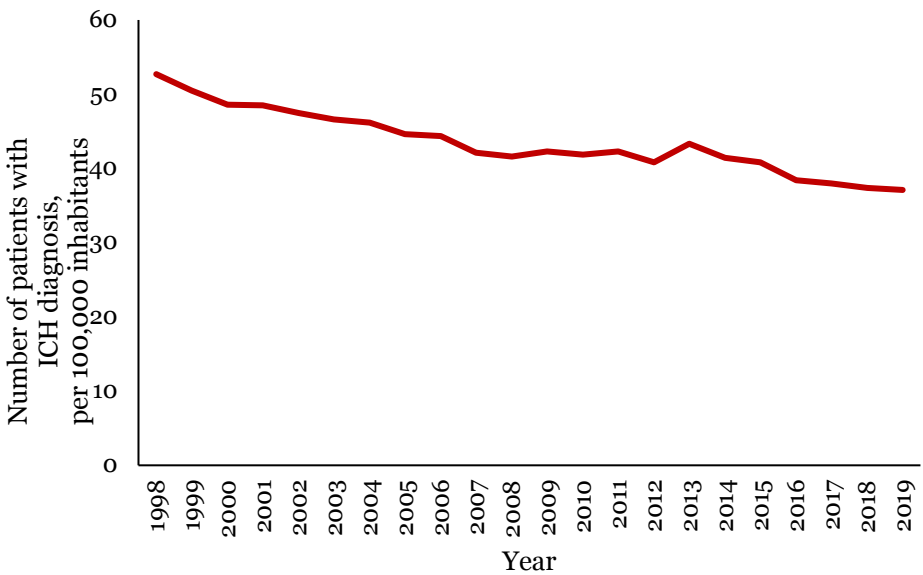

Figure 1. Number of patients per 100,000 inhabitants aged 15 and older with an ICH diagnosis, in Swedish in-patient care between 1998 and 2019 according to the National Board of Health and Welfare, Sweden

The number of Swedish patients in different age categories with an in-patient ICD-10 diagnosis code I.61 in 2019 according to the National Board of Health and Welfare in Sweden is shown in Figure 2 (13).

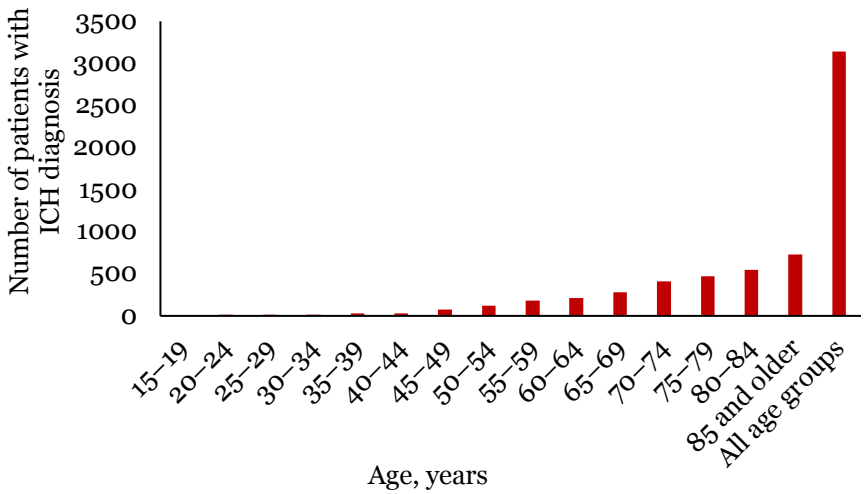

Figure 2. Number of Swedish in-patients with an ICH diagnosis in different age groups in 2019 according to the National Board of Health and Welfare, Sweden

Pathogenesis

ICHs can be further classified according to underlying conditions and pathogenetic processes. In some cases, the underlying cause is unknown or there is a combination of several underlying causes of the ICH event. Two classification systems that can be used to classify ICH by etiology are H-ATOMIC (hypertension, cerebral amyloid angiopathy, tumor, oral anticoagulants, vascular malformation, infrequent causes, cryptogenic) and SMASH-U (structural vascular lesion, medication, amyloid angiopathy, systemic diseases, hypertension, and undetermined) (14, 15). The distribution of different ICH etiologies in the SMASH-U study (including 1,013 ICH cases) and a recent Italian study (including 726 ICH cases) were as follows; structural vascular lesions 5–9%, medication 14–18%, amyloid angiopathy 4–20%, systemic diseases 5–10%, hypertension 34–35%, and undetermined 21–25% (15, 16).

Primary ICH and secondary ICH are terms that in some cases are used for ICH classification. Commonly, primary ICH denotes ICH events that are due to the spontaneous rupture of small arterial blood vessels affected either by hypertension or amyloid angiopathy or by a combination of the two, whereas secondary ICHs are due to other underlying causes (17). Examples can be ICH events associated with coagulopathies (including antithrombotic treatment),

arteriovenous malformations, mycotic aneurysms, cerebral venous thrombosis, vasculitis, brain tumors and hemorrhagic conversion of ischemic stroke (17). Hypertensive vasculopathy and cerebral amyloid angiopathy are discussed in more detail below.

Hypertensive vasculopathy

Hypertension can give rise to hypertensive vasculopathy. Hypertensive vasculopathy is classically associated with deep ICHs located in the basal ganglia, thalamus and brainstem and can give rise to lipohyalinosis, microatheromas, and microaneurysms of small vessels in the brain (18). The cerebral circulation contains small arterioles that branch directly from larger vessels in the proximity of the circle of Willis. These vessels can be subjected to high pressure due to their location. There are no branches prior to the arterioles that allow stepwise reduction of arterial pressure (19). In persons with hypertensive vasculopathy, this may result in a reactive hyperplasia of the smooth muscle cells of the vessel wall. Subsequently, the smooth muscle cells are replaced by collagen fibers, causing the vessel to become more prone to rupture (18, 19).

Cerebral amyloid angiopathy

Cerebral amyloid angiopathy is more often seen in patients with lobar ICHs compared to deep ICHs. Lobar ICHs are located in the cortical and subcortical regions of the cerebrum. Cerebral amyloid angiopathy is characterized by amyloid-β peptide deposits within the vessel walls (19). The affected vessels are typically small and medium-sized arteries, arterioles and capillaries located in the cortex and in the meningeal pia mater and subarachnoid mater (18). Depositions of amyloid-β peptides in the vessel wall replace smooth muscle cells which results in reduced vessel compliance (19). The reduced compliance increases the propensity to vessel rupture and can result in splitting of the vessel wall, microaneurysms, microhemorrhages and fibrinoid necrosis making the vessel more prone to rupture (18, 19). The Boston criteria can be used to estimate the likelihood of an ICH being caused by cerebral amyloid angiopathy. These criteria include factors such as the age of the individual, the number of ICHs and their localization (20).

Clinical presentation

ICH symptoms vary depending on the location and size of the hemorrhagic event. Symptoms of ICH can be sensory or motor impairments, aphasia, hemianopsia, gaze deviation, neglect and signs of brain stem dysfunction such as cranial nerve deficits (21). Patients with ICH can also experience headache, nausea and vomiting. Reduced consciousness in an ICH patient indicates a large ICH volume or an ICH located in the brainstem. Seizures can occur, most often within the first 24 hours of the ICH event (21).

In the Surgical Trial in Intracerebral Hemorrhage, which included 1,033 persons with acute ICH, 58% of the participants had arm paralysis, 59% had dysphasia or aphasia, and 48% had leg paralysis. One in five individuals had a Glasgow Coma Scale score ≤ 8 (22).

It is not possible to distinguish between ischemic stroke and ICH only using clinical examination or biomarkers. Brain imaging is necessary. A decreased level of consciousness, vomiting, severe headache, and systolic blood pressure above 220 mm Hg are examples of signs and symptoms that suggest a hemorrhagic stroke event rather than an ischemic event (23).

Treatment

The acute management of ICH has several important aspects. Care in a dedicated stroke unit improves survival in ICH patients (24, 25)

Some aspects of ICH management can vary depending on the cause of the event (17). In patients undergoing anticoagulant treatment it is important to rapidly reverse the anticoagulant effect, but it remains uncertain whether patients with ICH who are taking platelet inhibitors benefit from platelet transfusion (26, 27). Management of glucose levels, treatment of epileptic seizures, avoidance of hyperthermia and efforts to prevent secondary complications such as pneumonia and venous thromboembolism, are other cornerstones of ICH care (27).

Another aspect to consider is blood pressure control. In the acute setting, blood pressure reduction is considered safe and it may be beneficial to lower systolic pressure <140 mm Hg if no other contraindications exist (27-29). However, a post-hoc analysis showed that caution is advisable when considering intensive blood pressure lowering measures in patients with very high blood pressure (systolic blood pressure ≥ 220 mm Hg) (30).

On balance, there is no strong evidence supporting surgical treatment of ICH. However, surgery can be beneficial in some specific subgroups. Current guidelines (27) advocate that patients with an ICH located in the cerebellum with worsening neurological status, compression of the brain stem and/or ventricular obstruction causing hydrocephalus should be considered to undergo neurosurgery to remove the hematoma. For supratentorial hematomas, decompressive craniectomy and hematoma evacuation can be considered in selected patients (27).

One factor impeding progress in ICH management could be that more than one in three patients with ICH are ineligible for participation in clinical trials regarding ICH treatment due to restrictive inclusion and exclusion criteria (31).

Prognosis and functional outcome

In a study of consecutive ICH patients, that excluded ICH due to underlying causes such as tumors and arteriovenous malformations, the mortality of ICH at one month was 25% (32). According to a meta-analysis with similar exclusion criteria, the mortality of ICH at one year was 54 % and mortality at 5 years was 71% (33). Patients who experience an ICH event during oral anticoagulant

treatment have a higher in-hospital mortality compared to persons without anticoagulant treatment (34).

In Sweden, it has been shown that 23% of individuals with ICH have died after one month (2), 47% after one year, 61% after five years and 82% 13 years after the ICH event (35). In persons who had survived the first year after the ICH, the mortality was higher than that of the general population (35).

The ICH score is a widely used prognostic model for 30-day-mortality of ICH. Older age (≥ 80 years), greater hemorrhage volume, infratentorial location of ICH, lower Glasgow Coma Scale score at admission and presence of intraventricular hemorrhage are factors associated with worse prognosis (36, 37). Level of consciousness at admission is the strongest predictor of death at both one month and one year after the ICH event (38).

Caution has been suggested regarding early decisions of "Do not resuscitate" in the management of acute ICH as such a decision can limit optimal care and in itself worsen prognosis. Offering maximum care for 5 days resulted in better outcomes than the initial ICH score suggested (39) and according to current guidelines it is graded as probably recommended to offer early aggressive care and postpone new "do-not-resuscitate" decisions until at least 48 hours have passed from ICH onset (27).

The annual recurrence rate of ICH among ICH survivors varies between 1% and 7% (33). Lobar ICH has a higher risk of ICH recurrence than deep ICH. ICH survivors had more ischemic stroke events than ICH events during a three-year follow-up period (8). In patients with an indication for anticoagulants or antiplatelets, such treatments may be beneficial even in persons with a previous ICH. There are several studies investigating this issue (40-42). Results from one of the studies suggests that for ICH survivors who restart a previous antiplatelet therapy, the established benefits of the therapy probably outweigh the risks of a recurrent ICH (40).

Functional outcome in stroke patients can be measured using the modified Rankin Scale (mRS), a scale ranging from zero to five with a higher number indicating a higher disability (43). In a Norwegian cohort of patients with ICH with a mean age of 75 years at onset, the functional outcome at three months after the event were as follows: 39% had died, 12% had a severe disability (mRS 5) and 33% percent had a slight to moderately severe disability (mRS 2–4). Only a modest proportion of patients (16%) could live independently (mRS 0–1) (9).

Risk markers for ICH

Age

The incidence of ICH increases with age (12). In middle-aged to elderly persons the risk of ICH doubles for each decade (44). The risk of conditions associated

with ICH risk, such as hypertension and cerebral amyloid angiopathy, increases with age (18, 45).

Sex

Studies have shown that an equal number of men and women experience an ICH (12) or, alternatively, there can be a male predominance (46, 47). Women who experience an ICH event are older and more often have lobar ICH when compared to men (46). The cardiovascular risk factor burden differs between men and women who experience an ICH event, where men more often have a history of ischemic heart disease or peripheral arterial disease and are more likely to be current smokers or overconsume alcohol (46).

Hypertension

Hypertension can be defined as an in-office systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg (48). It is a contributor to death and disability (49) and is globally the leading predictor for stroke mortality (50). It is estimated that approximately one third of the world's adults have hypertension (51). In Västerbotten County, Sweden, 25% of women and 35% of men aged 50 years have hypertension (45). The prevalence of hypertension in the Nordic countries has decreased during the past decades (45, 52).

Hypertension is the most important modifiable risk factor for ICH (44, 53) and the population-attributable risk associated with hypertension was 56% for ICH in the INTERSTROKE study (53). It has been shown that persons with a genetic predisposition for high blood pressure have a higher risk of ICH (54). In a meta-analysis of individuals without known vascular disease, the risk of fatal ICH was lower in persons with a lower baseline blood pressure (55). The risk of ICH in an individual with systolic blood pressure ≥ 160 mmHg or a diastolic blood pressure ≥ 110 mm Hg is 5.6 times that of a normotensive individual (44). Non-treated hypertension is a stronger predictor of ICH than treated hypertension (56). Among persons experiencing an ICH event, 78% have a history of hypertension (56). It is estimated that one in four hemorrhagic strokes among hypertensive patients could be prevented if all hypertension was treated (57). The association between hypertension and risk of ICH is stronger for non-lobar ICH, but there is an association between hypertension and risk of lobar ICH as well (58). Treatment with angiotensin-converting enzyme inhibitors alone or in combination with thiazide-like diuretics in patients with previous strokes or transient ischemic attacks has been shown to reduce the risk of ICH irrespective of presence of hypertension at baseline (59).

Smoking

Smoking is a global contributor to disease risk. In 2015, there were more than one billion smokers worldwide (60). In the Nordic countries, the prevalence of smoking has decreased in recent years (61, 62). It is estimated that 11.5% of all deaths are due to smoking (63) and smoking is the fifth most important predictor of stroke mortality (50).

It is not certain whether smoking is a risk marker for ICH as results of studies have been inconclusive (44, 53, 64, 65). An older meta-analysis found an association between current smoking, but not ever smoking, and an increased risk of ICH (66). A recent meta-analysis, focusing on location-specific risk markers, found no association between smoking and risk of ICH (58). It is possible that only heavy smoking is associated with a substantial increase in risk of ICH (53, 67). Genetic markers for smoking initiation are not associated with an increased risk of ICH (68).

Diabetes mellitus

Globally, diabetes mellitus and glucose intolerance taken together was the third leading risk factor for stroke mortality in 2017 (50). There is no consistent association between diabetes mellitus and ICH. A meta-analysis of unadjusted data from case-control studies found an association between diabetes mellitus and risk of ICH, but no association in an analysis of the association between diabetes mellitus and ICH risk in cohort studies (69). In another meta-analysis, including three prospective studies, a positive association between diabetes mellitus and ICH was seen (64). A recent meta-analysis concluded that diabetes mellitus was only associated with risk of non-lobar ICH (58). In the INTERSTROKE study, a negative association between diabetes mellitus and ICH was found (53). The duration of diabetes mellitus seems to affect the association between diabetes mellitus and ICH risk (70). A fasting plasma glucose < 4.0 mmol/L or ≥ 6.1 mmol/L has been shown to be associated with increased risk of ICH. The same is true for very high and very low HbA1c in persons with diabetes mellitus (70, 71). Among persons experiencing an ICH event, 16% to 28% have a history of diabetes mellitus (32, 72).

Lipids

The population mean cholesterol has decreased in the past decades in the Nordic countries. It has been reasoned that this, at least in part, is due to changes in diet and an increased use of lipid-lowering drugs (52, 73).

Epidemiological studies have shown that low cholesterol levels (74) and statin use (75) are associated with an increased risk of ICH or deep ICH events respectively. A meta-analysis of randomized trials of statin treatment concluded that high-dose statin treatment significantly increases the risk of ICH but reduces the risk of all stroke, ischemic stroke and all-cause mortality. Low-dose statin therapy significantly reduced the incidence of all stroke and all-cause mortality but was not associated with an increased risk of ICH (76). Genetically elevated levels of low-density lipoprotein are associated with a lower risk of ICH. This finding indicates that there may be a causal link between low-density lipoprotein levels and ICH occurrence (77).

Overweight and obesity

Obesity is an increasing health concern in many countries. In 2016, more than 1.9 billion adults were overweight and among these, 650 million were obese (78). Both overweight and obesity are associated with increased all-cause mortality (79). In 2015, four million deaths globally were due to overweight and obesity (80). Obesity is the fourth most important modifiable risk factor for stroke mortality (50). Body mass index (BMI) categories can be used to define overweight and obesity (81). A high BMI is associated with an increased risk of stroke. Three quarters of this association can be explained by the fact that persons with high BMI are likely to have higher blood pressure, cholesterol and glucose levels (82).

Both underweight and obesity have been associated with an increased risk of deep ICH, but not lobar ICH (58, 83), although an association between BMI and risk of ICH was not seen in all studies (44, 84, 85). Obesity has also been reported to be associated with a decreased risk of hemorrhagic stroke (64). A high waist hip ratio is associated with an increased risk of ICH (53). A Mendelian randomization study showed that genetic predisposition to abdominal obesity, but not genetic predisposition to high BMI, was associated with risk of non-lobar ICH. The association was not mediated by hypertension or glucose levels (86).

Education and socioeconomic status

There are differences in risk marker patterns, such as BMI, between different socioeconomic groups (87). In a Finnish study, the incidence of ICH was higher in adults younger than 60 years with low income compared to adults in the same age group with high income (88). In contrast, a Swedish study showed no association between low socioeconomic status and risk of hemorrhagic stroke (89). Higher education level has also been associated with a lower risk of ICH (90, 91) although not in all (44) studies. Living in an area with a low neighborhood economic status is associated with a higher incidence of first ICH (92).

Alcohol

An increased consumption of alcohol is associated with risk of alcohol-related medical conditions, as well as an increased risk of accidents (93) and all-cause mortality (94). Alcohol consumption is the leading cause of death for those aged 15 to 49 years (93). Globally, alcohol consumption is expected to rise during the upcoming decade (95). According to sales statistics, alcohol consumption in Sweden has been stable during the past decade (96). However, among Swedish adolescents, alcohol consumption has decreased in recent years (97).

It is estimated that Swedes aged 15 years and older consume 8.8 L of pure alcohol each year (98). Of this amount, 82% is registered (sales of alcohol in the Swedish Retail Monopoly, in bars and restaurants and sales of beer with reduced alcohol content in other locations), and 18% is unregistered (for instance, alcohol that is purchased abroad, alcohol ordered on the internet or

homemade) (98). Surveys during the past two decades show that four in five Swedes have consumed alcohol during the past month (96).

There are many ways to measure self-reported alcohol consumption among which the Alcohol Use Disorders Identification Test (AUDIT) and timeline follow-back are examples of widely used tests (99, 100). There are direct and indirect biomarkers of alcohol consumption. Direct biomarkers measure ethanol or its metabolites while indirect biomarkers reflect consequences of ethanol on blood chemistry or organ function (101). Examples of direct biomarkers of alcohol consumption are ethanol in breath or blood, ethyl glucuronide in urine (reflecting alcohol consumed during the past five days), phosphatidylethanol (PEth) (reflecting alcohol consumed during the past weeks) and ethyl glucuronide in hair (reflecting alcohol consumed during the past three to six months). Examples of indirect markers of alcohol consumption are γ-glutamyl transferase and carbohydrate-deficient-transferrin (101).

A high self-reported alcohol consumption is associated with an increased risk of ICH (94) A Mendelian randomization study showed that genotype predicted alcohol intake was associated with both ICH and increased blood pressure (102). Increased levels of γ-glutamyl transferase are associated with increased risk of hemorrhagic stroke (103). PEth is discussed in a separate section, see page 19.

Other risk markers of ICH

Several additional markers have been investigated as potential risk markers of ICH. Anticoagulant treatment is associated with risk of ICH (104). In the United States, the proportion of anticoagulant-related ICHs has increased from 4% 1987–1999 to 14% 2000–2014 (75). In 2019, 400,000 Swedes were treated with anticoagulants and among these, 654 (0.17%) had an ICH that year. One fourth of all ICH events in Sweden in 2019 occurred in persons treated with anticoagulants (105). Antiplatelet treatment, such as aspirin, is associated with increased risk of hemorrhagic stroke (106).

A healthy diet and a high level of physical activity has been associated with a decreased risk of ICH (53). East and Southeast Asian ethnicity is associated with increased risk of ICH (12). In one study, Hispanic and Black persons had an increased risk of non-lobar ICH in a meta-analysis (58).

Studies have been conducted regarding the association between genetic markers and risk of ICH. Apolipoprotein alleles are associated with deposition of β-amyloid in cerebral amyloid angiopathy (107). Apolipoprotein E allele ε2 has been shown to be associated with an increased risk of lobar ICH and apolipoprotein E allele ε4 has been shown to be associated with an increased risk of both lobar and non-lobar ICH (108). The heritability for ICH risk is estimated at 29% for non-apolipoprotein E loci and 15% for apolipoprotein E loci (109).

Potential novel biomarkers for risk of ICH

Biomarkers

The term biomarker can be defined as “a defined characteristic that is measured as an indicator of normal biological processes, pathogenetic processes, or biological responses to an exposure or intervention, including therapeutic interventions.” (110) . A biomarker can be used in different contexts, as shown in Figure 3. For example, it can be used to assess risk of disease, to screen for disease, for disease diagnosis, for monitoring disease activity and treatment effect, and for prognostic purposes (110).

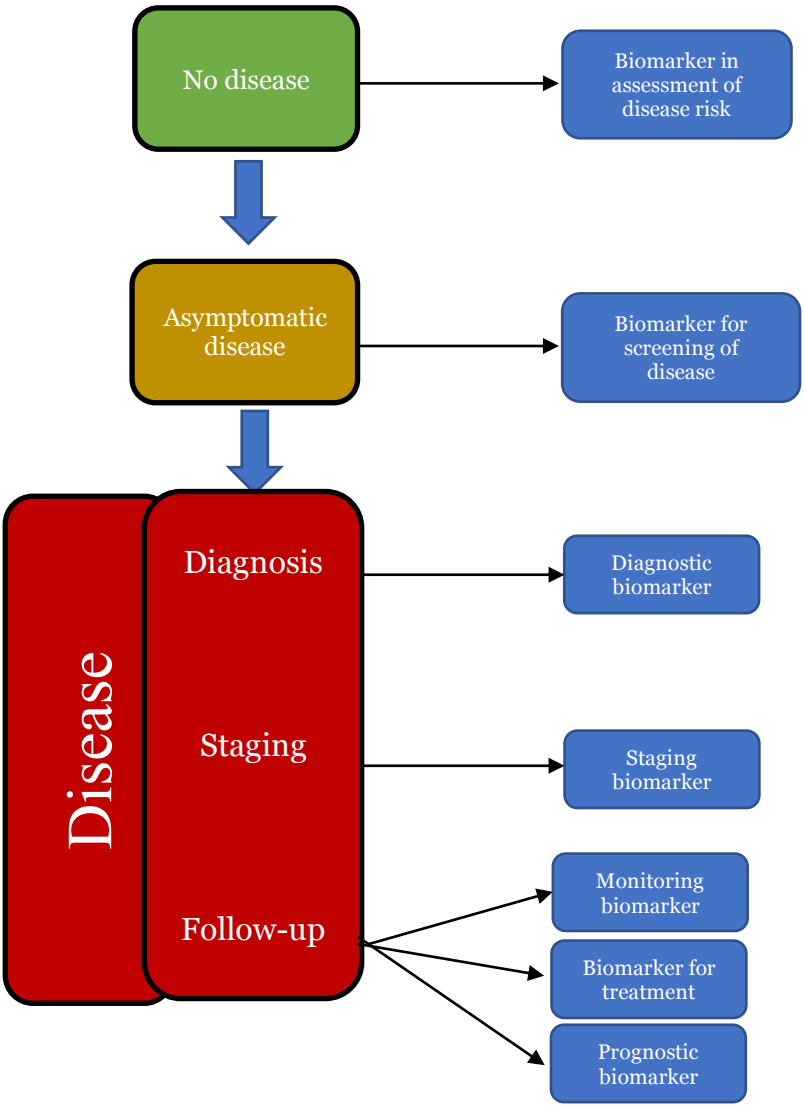

Figure 3. Different contexts of biomarker use.

When considering a novel biomarker, it is important to endeavor to comprehend the underlying pathophysiological mechanisms of the proposed biomarker. Furthermore, it is of essence to investigate the synthesis, distribution and elimination of the biomarker in both healthy and affected individuals (110).

Several factors should be considered when evaluating whether or not a biomarker could be of clinical interest.

Firstly, it must be shown that the biomarker levels differ between persons with and without the investigated outcome. Secondly, the predictive properties of the biomarker must be compared with those of already established tests. Thirdly, the properties of the biomarker must be shown to positively influence decision making such as leading to an earlier disease diagnosis. Additionally, the usefulness of the biomarker must be considered. This term encompasses the cost, analysis availability, patient discomfort and inherent risks of biomarker use. The prevalence and severity of the condition are also important to consider when determining biomarker usefulness. Lastly, an intervention study should be conducted. The aim of this is to demonstrate that the use of the biomarker improves clinically relevant outcomes (111). The different stages of the development of potential new biomarkers are illustrated in Figure 4.

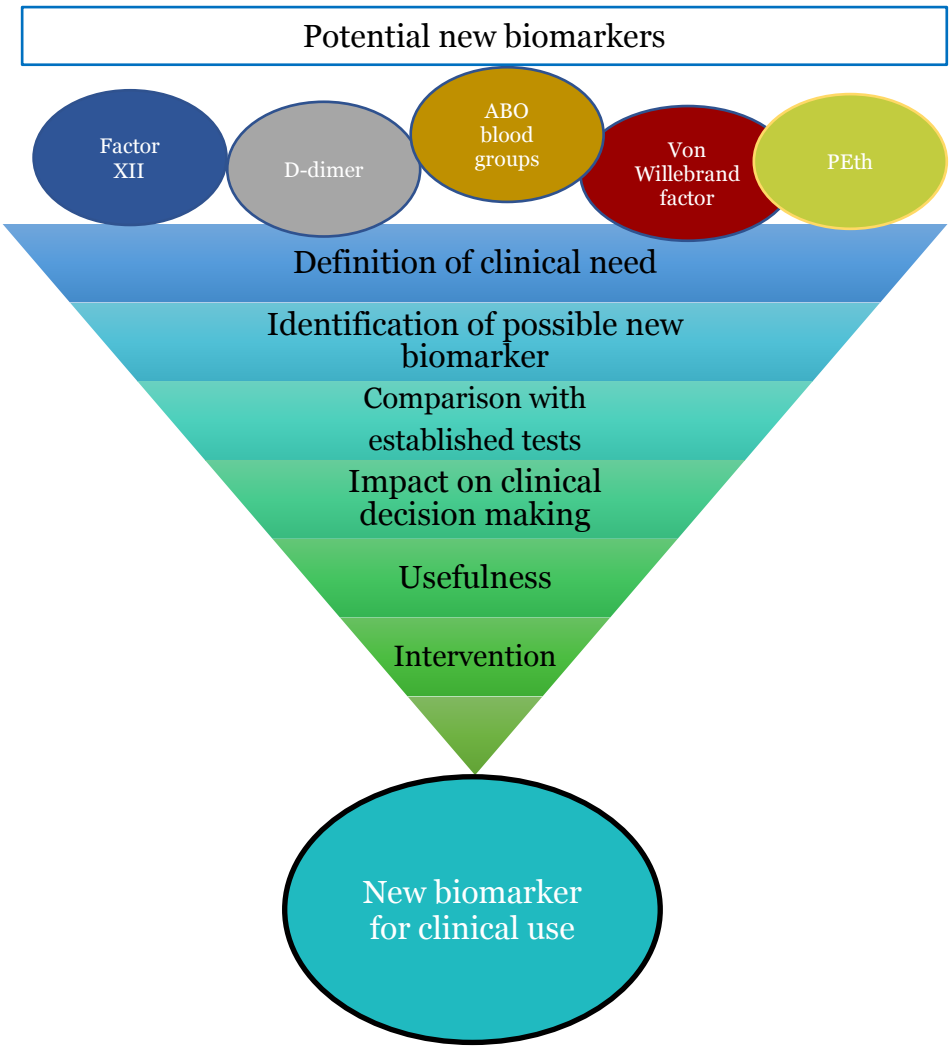

Figure 4. The different stages of development of potential new biomarkers.

Biomarkers in different contexts

Many different biomarkers are used in current clinical practice. For example, infection with certain human papillomavirus subtypes can be used as a biomarker in the assessment of risk for cervical cancer (112). Troponin-T may be used as a diagnostic biomarker for acute myocardial infarction (113). Prothrombin complex is a monitoring biomarker in the context of warfarin treatment (114) and the TP53 mutation can be used as a prognostic biomarker in chronic lymphocytic leukemia (115).

Biomarkers have also been studied in the context of hemorrhagic stroke. For example, levels of high-sensitivity troponin-I (116) and γ-glutamyl transferase (117) have been shown to be associated with incident hemorrhagic stroke. Several biomarkers have been studied with the aim of differentiating ICH and ischemic stroke in the acute setting (118). S100β concentrations has been shown to be significantly higher in persons with acute ICH compared to persons with acute ischemic stroke in some (119) but not in other (118) studies. There are also studies addressing the utility of β-amyloid in the classification of ICH and of D-dimer, which might be used to predict prognosis of ICH (120).

Biobanks

Biobanks can be valuable resources when attempting to explore and validate potential new blood biomarkers. A biobank is defined as a "collection of human biological material, retained for one or more purposes, and information about this material" (121). Stored blood samples can enable prospective studies of medical conditions which are yet to occur. For the blood samples to be usable, it is essential that the biologic materials are handled correctly during transportation and storage. It must be ensured that stored samples are appropriate for the analysis in question. It is important to have knowledge of the degradation of the biomarker and how it is affected by storage time (122).

Potential mechanisms of novel biomarkers

Even though an ICH event often comes as a surprise to the affected individual, many antecedent pathogenetic processes have likely occurred before the defining clinical event. Subclinical ischemic events and microhemorrhages in individuals with small vessel disease may precede the ICH event by years (18, 123). It is possible that these processes could be identified using biomarkers. Proteins and protein fragments involved in hemostasis and fibrinolysis could be potential biomarkers for ICH. It may be that these biomarkers are not part of the causal chain but could nevertheless be used as risk indicators to better define high-risk groups. If markers of hemostasis and/or fibrinolysis are found to be associated with ICH, that could lead to further studies that probe deeper into aspects of ICH pathogenesis related to these processes.

Another approach to novel biomarkers could be to measure already established risk markers for ICH such as alcohol consumption (53) in a more accurate way. That could increase measurement reliability and the accuracy of the risk estimates.

Factor XII (FXII)

FXII is a protein involved in the intrinsic pathway of blood coagulation and in inflammation (124). FXII deficiency results in a prolonged activated partial thromboplastin time and can be found through routine blood testing (125). FXII has been described as a "neglected player" in stroke pathophysiology, and even though FXII was discovered more than 60 years ago, the in vivo role of FXII is still not fully elucidated (126). In the past decades, more FXII functions have been discovered, for example related to angiogenesis (127), wound healing (128) and immune response (129).

FXII is synthesized in the liver (130) and FXII concentrations are not affected by age or sex (131). FXII is activated by negatively charged biological and artificial materials, such as molecules released from activated platelets and collagen exposed beneath damaged endothelium (124). FXII activates factor XI, which in turn triggers the coagulation cascade (132). FXII also initiates proinflammatory actions through the kallikrein-kinin system triggering the formation of the inflammatory peptide bradykinin (124).

FXII deficiency has not been shown to be a marker of hemorrhage risk in humans (133). On the contrary, for many years FXII concentrations and FXII genotype were investigated as potential risk markers for thromboembolic disease (134-136) without definite evidence for such associations. During the 21st century, inhibition of FXII has been shown to reduce brain inflammation and infarction size without increasing bleeding in experimental ischemic stroke models (126).

FXII has been proposed as a potential new target for anticoagulant medications since a FXII inhibitor would be expected to have an acceptable safety profile as FXII deficiency is not associated with an increased bleeding risk (137).

D-dimer

D-dimer is a product of fibrin degradation. D-dimer concentrations increases as a consequence of fibrin formation when blood clots are formed and in other situations with activation of the coagulation pathway (138). D-dimer has been investigated as a biomarker in different settings and found to be associated with both coronary heart disease (139) and all stroke (140). D-dimer can be used as a diagnostic biomarker in venous thromboembolism (141). Elevated D-dimer measured at the time of admission for an ICH event has also been shown to be associated with a worse prognosis (142).

High D-dimer concentrations are associated with bleeding events in patients treated with oral anticoagulants (143, 144). The potential role of D-dimer as a predictor of hemorrhagic stroke was investigated in a recent meta-analysis (140). This meta-analysis included three prospective studies, one cohort study with 91 cases (145) and two case-cohort studies (146, 147) with 66 hemorrhagic stroke cases and 59 ICH cases respectively. One study showed an association between high D-dimer concentrations and risk of hemorrhagic stroke (146). Another did not find an association between D-dimer as a continuous variable

or between tertiles of D-dimer and risk of ICH, although having a D-dimer concentration in the upper 5% was associated with an increased risk of ICH (147). In the third study, there was no association between D-dimer, either as a continuous variable or between quintiles of D-dimer and risk of hemorrhagic stroke (145). The conclusion of the meta-analysis was that D-dimer was not a risk marker for hemorrhagic stroke (140).

In addition to the studies included in the meta-analysis mentioned above, the French three-city study, which included 21 cases of hemorrhagic stroke, showed no association between D-dimer concentrations and risk of hemorrhagic stroke (148).

Von Willebrand factor (VWF) and ABO blood groups

VWF has an important role in primary hemostasis. In primary hemostasis, a platelet plug is formed at the site of vessel injury. In addition, VWF functions as a carrier protein for coagulation factor VIII. VWF is synthesized in the endothelium and in megakaryocytes (149). Upon vascular injury, VWF is released into the blood stream and forms multimers. Larger multimers are more thrombogenic than smaller ones. The VWF multimers in the blood stream can be cleaved by the protein A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) to smaller, less thrombogenic VWF monomers. ADAMTS13 influences the effect of VWF on hemostasis in this manner (150). The half-life of VWF multimers is approximately 12 hours (149).

ABO blood groups (A, B, AB, O) are defined by different carbohydrate structures that can be found on red blood cells and some other cell types, including the vascular endothelium. Persons can form antibodies against the ABO carbohydrate structures which are not recognized by their own immune system. This has implications for the transfusion of blood products (151).

ABO blood groups interact with VWF in several ways. Mean VWF concentration differs between different blood groups. Individuals with blood group O have a 25% lower mean VWF concentration compared to individuals with a non-O blood group (152). It has been proposed that the ABO blood groups influence the susceptibility of VWF to cleavage by ADAMTS13 (150). It is estimated that approximately 70% of the intraindividual variation in VWF concentrations is genetically determined and that almost one third of the genetic variation is related to the ABO blood group of the individual (153).

Decreased VWF concentrations and/or impaired VWF function is associated with an increased risk of bleeding, as is seen in individuals with von Willebrand disease. Persons with this condition have an increased bleeding risk, especially in situations with mucocutaneous bleedings and bleeding after trauma and surgery (154, 155).

Increased VWF concentrations have also been shown to predict major bleeding during oral anticoagulant treatment (156). The association between VWF and

risk of ICH has been investigated in prospective population-based studies with conflicting results. An association between low concentrations of VWF and increased risk of ICH (157), high concentrations of VWF and increased risk of ICH (158), and no association between VWF concentrations and risk of ICH at all (159, 160) have been noted.

ABO blood groups have also been investigated as possible risk markers for a wide range of diseases. Non-O blood group is associated with an increased risk of venous thrombosis and cancer compared to O blood group (161, 162). Non-O blood group has also been studied as a potential risk marker for arterial thromboembolic disease, but the results are more inconsistent (163-165). O blood group, on the other hand, was a risk marker for hemorrhage in a study where bleedings in different sites were included as outcomes (166). Studies of the relationship between ABO blood group and risk of intracranial bleeding have not shown any association (167-169). It is possible that the presence or absence of an association between ABO blood groups and risk of bleeding is dependent on bleeding location. Blood group O has also been associated with a higher grade of hematoma expansion in ICH patients (170) although this was not the case in all studies (171).

It has been suggested that the associations between ABO blood group, thromboembolic disease and bleeding risk are driven by the tight connection between ABO blood group and VWF concentrations or possibly by an influence of ABO blood groups on platelet function (150).

PEth

PEth is an accumulation of abnormal phospholipids formed only in the presence of ethanol. This means that PEth has a theoretical specificity of 100% for alcohol intake (172). PEth concentrations in erythrocytes can be used as a biomarker of alcohol consumption (101) and can be detected in blood after up to four weeks of sobriety (173). As the phosphatidylcholine molecules of the cell membrane of the erythrocytes are exposed to ethanol, the enzyme phospholipase D catalyzes a transphosphatidylation reaction and PEth is formed. A schematic illustration of the formation of PEth in erythrocytes is shown in Figure 5 (172).

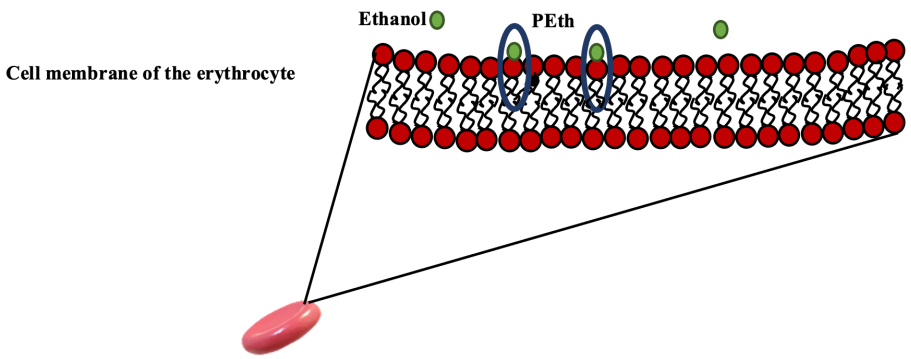

Figure 5. A schematic illustration of PEth in the cell membrane of the erythrocyte.

In recent years, the possibility of measuring exhaled PEth has been investigated, but the research in this field is still limited (174).

To date, almost 50 homologues of PEth have been found in humans. Each has a phosphoethanol head and a unique combination of fatty acid chains. One of the most abundant homologues is PEth 16:0/18:1 (175). PEth 16:0/18:1 has a half-life of four to ten days (176) and PEth levels in blood are correlated to the amount of alcohol consumed (177, 178). PEth 16:0/18:1 is currently used in clinical practice in Sweden as a marker of alcohol consumption. A PEth 16:0/18:1 concentration of > 0.30 µmol/L (equivalent to 210 ng/mL) is used to indicate heavy alcohol consumption (179, 180). A cutoff of 300 ng/mL (equivalent to 0.43 µmol/L) of PEth 16:0/18:1 had a 90% specificity and a 71% sensitivity for detecting alcohol consumption of 56 g ethanol (approximately four standard drinks) daily (178). Small amounts of alcohol, such as those in regular use of mouthwash, do not result in PEth concentrations above 0.05 µmol/L (181).

During a five-day drinking experiment, where participants had a daily alcohol intake calculated to give rise to a blood ethanol concentration of 1 g/kg, the peak concentration of PEth reached 237 ng/mL (equivalent to 0.34 µmol/L) (182). After a single ingestion of alcohol, leading to an estimated blood ethanol concentration of 1 g/kg, PEth 16:0/18:1 can be detected in blood during the ensuing three to twelve days (183). The elimination of PEth does not seem to be influenced by age (178), sex (173, 184) or degree of liver disease (178).

Epidemiological concepts

Risk

Epidemiologically, risk is defined as the “probability of an event during a specified period of time” (185). A risk marker is “a factor that is non-causally

associated with the risk of a disease or other specified outcome" (186). There is no universally accepted definition of the term risk factor. In one of the stricter definitions, a risk factor is described as "an environmental, behavioral, or biologic factor confirmed by temporal sequence, usually in longitudinal studies, which if present, directly increases the probability of a disease occurring, and if absent or removed, reduces the probability. Risk factors are a part of the causal chain or expose the host to the causal chain. Once disease occurs, removal of a risk factor may not result in a cure." (187). A determinant is "an attribute or exposure that increases the probability of occurrence of disease or other specified outcome" (187).

For some diseases, a single factor can be identified as the causal factor for that condition. This is especially common among infectious diseases and nutritional deficiency diseases. In absence of that factor, the disease cannot occur. In other conditions, such as cardiovascular diseases, there is not one essential causal factor. Instead, there are risk factors. A risk factor is associated with an increased probability of developing the disease, but it is not necessary for disease development. Neither do all persons with the risk factor develop the disease (188, 189).

In some way or another, risk factors reflect the underlying disease biology. A risk factor can sometimes be a proxy for another, more proximal risk factor, and can also reflect a combination of multiple biological processes. For example, age is a risk factor for multiple diseases, but little is known about the direct biological mechanisms involved. Probably, age is one way of measuring many different processes. Risk factor and risk marker research can further the understanding of the direct, biological causes of disease and can also be used to improve the prediction of disease risk (188).

Validity

The term validity encompasses internal and external validity.

Internal validity can be defined as "the degree to which a study is free from bias or systematic error" (186). The precision, or level of random error, is not a component of internal validity. As a norm, the level of external validity is unimportant if a study lacks sufficient internal validity (186).

External validity, which can be interpreted as to what degree the study results can be generalized to a broader population, is important when interpreting study results. Systematic differences between the source population, from which the study participants are drawn, and the target population, the population to which the results are supposed to be applied, can reduce the external validity (190).

Observational studies

An observational study is defined as "A study that does not involve any intervention (experimental or otherwise) on the part of the investigator" (186).

Examples of observational studies are cohort studies and case-control studies (189).

Observational studies are prone to bias and confounding and therefore considered less suitable to give proof of causality compared to randomized controlled trials (191). Associations found in observational studies can contribute to the knowledge within a field and give rise to ideas for exploration.

In prospective observational studies, where the exposure is measured before the outcome occurs, the temporality criterion, a necessary criterion for causality, is met. This criterion states that a cause must happen before an effect. An important limitation of observational studies is that they are more prone to bias when compared to randomized controlled studies where a successful randomization can limit the distortion of the results caused by residual confounding (185).

However, there are situations where randomized controlled trials are not appropriate. First, not all exposures can be randomized. For example, it would be unethical to randomize individuals to possibly harmful exposures (192) such as a high alcohol consumption. In such cases, observational studies can have an important role.

The external validity of randomized controlled trials can be lower than that of observational studies due to more restrictive inclusion and exclusion criteria and persons declining to participate in interventional studies (193). Furthermore, observational studies are usually less expensive and less time consuming to perform compared to randomized controlled trials (192, 194). At times, an observational study can be the best way to maximize the amount of knowledge gained for the available assets, especially if the outcome is rare and very large randomized controlled trials are necessary (195).

Confounding

Confounding can be defined as a distortion of the estimated effect of an exposure on an outcome caused by an association between the exposure and other factors (confounding variables or confounders) that influence the outcome. This results in an apparent association between the exposure and the outcome that is actually explained, at least in part, by the confounding variable (186).

In observational studies, care must be taken to identify and measure confounders. Likewise, the influence of the confounders must be averted. This may be accomplished by employing different techniques, such as using different inclusion and exclusion criteria, matching, stratification or adjustment in regression models (189).

If used properly, adjustment for confounding variables reduces confounding bias (186). Both pre-existing knowledge and observed statistical associations

can influence the choice of which variables that should be considered to be confounders (196).

Aims

The aim of this thesis was to evaluate the associations between biomarkers (reflecting hemostasis, fibrinolysis and lifestyle factors) and risk of ICH

The specific aims of this thesis were:

- To investigate the associations between FXII concentrations and risk of hemorrhagic stroke as well as between FXII concentrations and risk of ICH separately.

- To investigate the association between D-dimer concentrations and risk of ICH.

- To investigate the association between VWF concentrations, ABO blood group and risk of ICH.

- To investigate the association between alcohol consumption, measured as PEth concentrations, and risk of ICH.

Materials and Methods

Northern Sweden Health and Disease Study cohort (NSHDS)

The NSHDS cohort consists of three subcohorts: the Västerbotten Intervention Programme (VIP) cohort, the northern Sweden MONItoring trends and determinants in CArdiocascular disease (MONICA) cohort and the Mammography Screening Project cohort, each described in more detail below. The VIP is a health screening and intervention program targeting middle-aged persons in Västerbotten County aiming to reduce the burden of cardiovascular risk factors and diabetes mellitus. The MONICA is a population-based health survey. The Mammography Screening Project cohort consists of women who participated in breast cancer screening in Västerbotten. Between 1985 and March 2007, the VIP and the MONICA cohorts combined consisted of 98,625 unique individuals. The Mammography Screening Project cohort consists of 28,800 women, of which some were included in the VIP and/or MONICA cohorts. A schematic illustration of the inclusion of participants in the VIP, MONICA and Mammography Screening Project cohorts is shown in Figure 6.

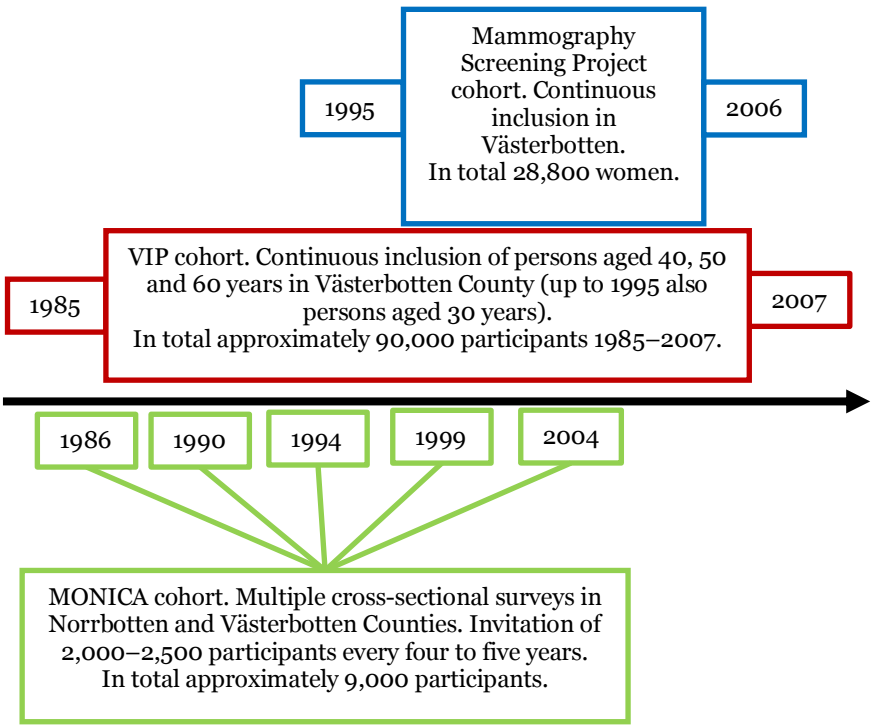

Figure 6. A schematic illustration of the inclusion of participants in the VIP, MONICA and Mammography Screening Project cohorts.

Study design and overview

Paper I, aiming to investigate the association between FXII and risk of hemorrhagic stroke, was designed as a prospective, population-based cohort study where participants of the MONICA health survey performed in 1994 are followed as a cohort until the occurrence of a hemorrhagic stroke event, death or study termination on December 31, 2011. The associations between FXII and risk of myocardial infarction and ischemic stroke were also published. Those associations are not the subject of this thesis and will not be discussed further. The study design of paper I is shown in Figure 7.

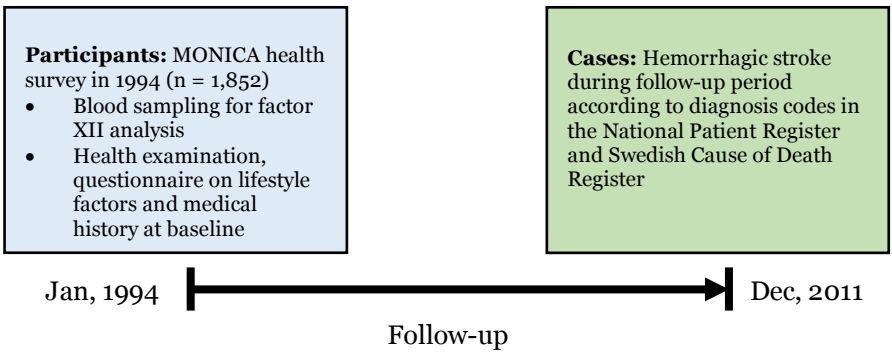

Figure 7. Schematic overview of the study design of paper I, a cohort study.

Papers II–V, aiming to investigate the association between FXII, D-dimer, VWF and ABO blood groups, PEth and risk of ICH, were designed as prospective, population-based case-referents studies. Cases were defined as participants of the NSHDS 1985–2007 who had donated blood samples for research purposes and developed an ICH event between the blood sampling at health examination and end of study in March 2007. Two referents are matched to each case. The study design of papers II–V is shown in Figure 8.

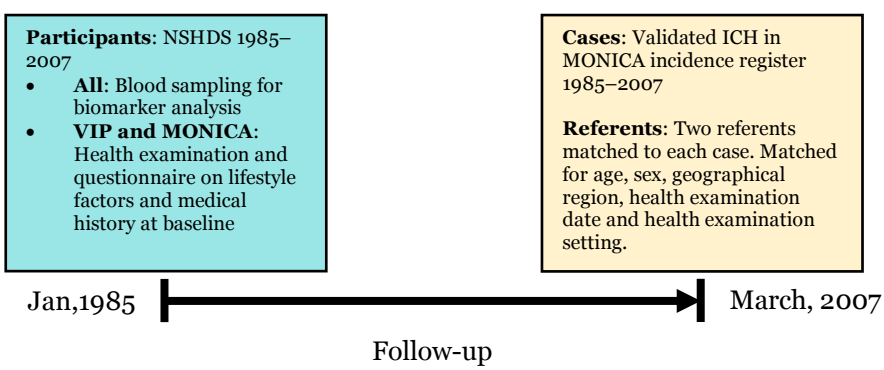

Figure 8. Schematic overview of method of paper II–V, case-referent studies performed between January, 1985 and March, 2007.

An overview of the studies included in this thesis is shown in Table 2.

Table 2. Overview of the papers of this thesis. All studies are prospective.

	Paper I	Paper II	Paper III	Paper IV	Paper IV	Paper V
Biomarker	FXII	FXII	D-dimer	VWF	Blood group*	PEth
Design	Cohort	Case-referent	Case-referent	Case-referent	Case-referent	Case-referent
Study population	MONICA	VIP	VIP, MONICA	VIP, MONICA	VIP, MONICA, MSP	VIP, MONICA
Year of inclusion	1994	1985–2000	1985–2007	1985–2007	1985–2007	1985–2007
Method	Prekallikrein/ DS-method	ELISA	ELISA	ELISA	Reverse blood grouping	LC/MS-MS
Blood sample	Plasma	Plasma	Plasma	Plasma	Plasma	Packed erythrocytes
Outcome	Hemorrhagic stroke**	First-ever ICH	First-ever ICH	First-ever ICH	First-ever ICH	First-ever ICH
Cases, n	30	70	141	139	162	97

**ABO blood groups*
***Hemorrhagic stroke includes ICH and subarachnoid hemorrhage. In an exploratory analysis, ICH was analyzed as a separate outcome.*
MSP, Mammography Screening Project, DS, dextran sulfate, ELISA, enzyme-linked immunosorbent assay, LC/MS-MS, liquid chromatography tandem mass spectrometry

NSHDS subcohorts and blood sample collection

MONICA

The Northern Sweden MONICA study was launched as a part of a multinational World Health Organization survey on risk factors for cardiovascular disease. In northern Sweden, the MONICA was performed in Norrbotten and Västerbotten Counties with a combined population of 511,878 in year 2000 (197). By 2007 a total of five population-based MONICA surveys had been performed including the years 1986, 1990, 1994, 1999 and 2004. Two thousand randomly selected inhabitants aged 25–64 were invited to participate in the surveys during the years 1986 and 1990. Corresponding participation totals in 1994, 1999 and 2004 included 2500 randomly selected inhabitants aged 25–74 years. The invited participants were stratified for age and sex; 250 women and 250 men were invited for each 10-year age category.

Participants were asked to attend a survey where specialized teams performed a health examination including blood sampling and a questionnaire investigating lifestyle factors and medical history.

VIP

The VIP is a population-based health examination and intervention in Västerbotten County. Västerbotten County encompasses an area of 55,000 km^2 (198) with a population of 256,000 in the year 2000 (197). In VIP, all county inhabitants aged 40, 50 and 60 years were invited to participate in the program. Until 1995, inhabitants aged 30 years were also invited to participate (199).

The VIP was initiated in 1985. The program started as a pilot project at one primary health care center and was then rapidly implemented in the entire county. Half of the primary health care centers in Västerbotten County offered VIP health examinations by 1990 and from 1992 onward, all primary health care centers in the area were involved. The VIP was integrated into routine practice within primary health care in 1995 (199).

The aim of the VIP is to improve health on the population-level and to identify individuals at high risk of cardiovascular disease and diabetes mellitus. The VIP includes a health examination, a questionnaire, and health counselling by a nurse. If needed, the nurse will direct participants to a primary health care provider. As part of the VIP health examination, participants are asked to consent to storage of health examination results and questionnaire data for use in future research. They are also asked if they are willing to donate blood samples to the Biobank Research Unit for research purposes (199).

Mammography Screening Project cohort

In conjunction with the population-based mammography screening in Västerbotten, women who attended the mammography screening were asked if they consented to participate in research. If willing, they answered a short questionnaire and donated blood samples for later research use. The

Mammography Screening Project cohort included participants between 1995 and 2006 and included a total of 28,800 women. Among the participants, 95% were aged between 48 and 70 years at inclusion. For participants of the Mammography Screening Project cohort only blood samples were used in the papers of this thesis.

Blood sample collection

Participants in the MONICA, VIP and Mammography Screening Project cohort were asked to donate blood samples for future research. Data management of these samples was provided by the Biobank Research Unit at Umeå University, Umeå, Sweden. The samples were stored in a biobank and were linked to questionnaire data and, if available, health examination data. The cumulative number of blood samples from participants of NSHDS between 1985 and 2007 is shown in Figure 9.

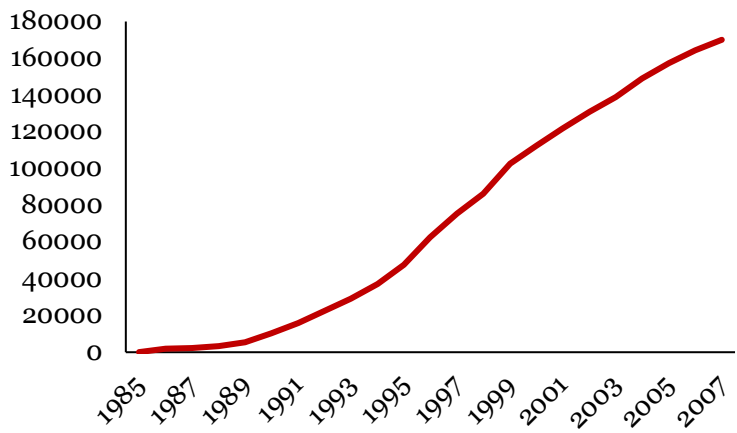

Figure 9. The cumulative number of blood samples from participants of NSHDS between 1985 and 2007.

In the MONICA and VIP, blood samples were drawn with minimal stasis after an overnight fast (80%) or at least a four-hour fast (20%). In the Mammography Screening Project cohort, fasting was not required. The blood samples (a total of 20 ml) were drawn into evacuated glass tubes (Venoject). One glass tube contained 1/100 volume of 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA) and the other one contained heparin. The blood samples were centrifuged at 1500 g for 15 minutes to separate plasma, erythrocytes and buffy coat. They were then frozen to at least -20 degrees C within one hour, and later aliquoted before being frozen to -80 degrees C.

Measurements and definitions

Health examination and questionnaire data

Health examination and questionnaire data was collected at the MONICA and VIP health examinations.

Hypertension

In the MONICA survey, participants were asked to rest for five minutes in a sitting position. Blood pressure was then measured two times with a Hawksley random zero sphygmomanometer. The mean of the two measurements was recorded. In the VIP, blood pressure was measured once using a mercury sphygmomanometer after the participant had rested for five minutes in a supine position. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive drugs during the two weeks preceding the health survey according to the MONICA and VIP questionnaires. In papers III, IV and V, where MONICA and VIP data are combined, blood pressure measurements taken in a supine position were recalculated to sitting blood pressure measurements. Recalculations were based on a comparison between blood pressure measurements taken in a sitting and supine position in a sample of VIP health examinations participants (45).

Smoking

Data on smoking habits was collected using the MONICA and VIP questionnaires. In paper I, smokers were defined as persons with self-reported current or former smoking of at least one cigarette per day. Previous smokers of less than one cigarette per day, occasional smokers and non-smokers were classified as non-smokers. In papers II–V, smokers were divided into daily smokers and non-smokers. Previous smokers, occasional smokers and never-smokers were classified as non-smokers.

Diabetes mellitus

Fasting plasma glucose measurements, an oral glucose tolerance test (OGTT) and questionnaire answers (self-reported diabetes mellitus) were used to define diabetes mellitus in MONICA and VIP participants. The OGTT was performed by dissolving 75 g of glucose in 300 mL of water. The solution was then ingested by the participant. A capillary (VIP) or venous (MONICA) blood sample was taken from each participant before and two hours after drinking the solution. In the MONICA, the OGTT was offered to 65% of participants without diabetes mellitus. In the VIP, all participants without known diabetes mellitus and with a fasting plasma glucose below 7.0 mmol/L were offered to undergo an OGTT.

A fasting plasma glucose ≥ 7.0 mmol/L and/or an OGTT venous plasma glucose ≥ 11.1 or an OGTT capillary plasma glucose ≥ 12.2 mmol/L was defined as diabetes mellitus. Diabetes mellitus defined by self-reporting comprised pharmacologically treated diabetes mellitus (papers I and III) or all self-reported diabetes mellitus regardless of treatment (papers II, IV and V).

Lipids

In MONICA, total cholesterol was measured in venous blood samples after at least four hours of fasting using an enzymatic method (Boehringer Mannheim GmbH, Mannheim, Germany). The analysis was performed at the Department of Clinical Chemistry, Umeå university hospital. The quality of the total cholesterol measurements in MONICA was regularly compared to WHO reference laboratory standards. In VIP, total cholesterol was measured at each health care center in venous samples after at least four hours of fasting. An enzymatic method with Reflotron analyzers (Boehringer Mannheim GmbH, Mannheim, Germany) was used. The Reflotron cholesterol measurements were recalculated to make them comparable to the MONICA cholesterol measurements (200).

BMI

In VIP and MONICA, BMI was calculated as weight (kg) divided by height (meters) squared. In VIP, height and weight was measured in light clothing. In MONICA, a participant's weight was measured to the nearest 0.2 kg while wearing light clothing and no shoes. Height was measured to the nearest centimeter.

Education

Data on education level were obtained from questionnaires used in MONICA and VIP respectively. Education was categorized into secondary school or less, or above secondary school education.

Self-reported alcohol consumption

In the MONICA and VIP, data on self-reported alcohol consumption was obtained from food frequency questionnaires. In the questionnaire, the participants reported their intake of light beer (1.8 weight percent of ethanol), medium strong beer (2.8 weight percent of ethanol), strong beer (4.5 weight percent of ethanol), wine (9.9 weight percent of ethanol), and liquor (32 weight percent of ethanol). For each of these beverages, the participant answered the question "How often do you consume the following products? Choose the category that matches your average consumption during the past year". The participant could then select one of nine different descriptors ranging from "never" to "four times per day or more". These frequencies of alcohol intake were converted to number of weekly intakes and subsequently multiplied by an age- and sex-specific intake amount to derive the weekly intake of each beverage in grams. The weekly intake in grams was multiplied by the weight percent of ethanol in each beverage to derive the amount of ethanol consumed per week from each source beverage. Lastly, these amounts were added to obtain total intake of ethanol in grams per week. This was divided by 12 to get a total intake of alcohol in standard drinks per week where one standard drink was defined as 12 g of ethanol.

Biomarker analyses

All biomarker analyses were performed in accordance with current health and safety guidelines. Laboratory staff had no knowledge of case/referent status of the participants. In the case-referents studies, cases and referents were analyzed in sets of three, with one case and two referents in each set. The positions of the cases and referents within each set varied randomly.

FXII

In paper I and paper II FXII concentrations were analyzed in plasma. In paper I, FXII was analyzed using a chromogenic substrate assay described by Tankersley et al (201). This method is based on the activation of prekallikrein to kallikrein by activated FXII. The rate of kallikrein formation is proportional to the original plasma FXII zymogen concentration in the sample of the participant. The rate of kallikrein generation was recorded by S-2302, a chromogenic substrate sensitive to kallikrein. A Hitachi 911 automatic analyzer was used to perform the analyses. Sensitivity for the method was high, samples had to be diluted > 1000-fold. Pooled plasma was used to obtain standard curves and WHO calibrator plasma was used for calibration to international units (IU). All samples were analyzed at the same location and at the same time (1997, Umeå, Sweden). The variation coefficient was 6.0%.

In paper II, FXII was analysed using a sandwich enzyme-linked immunosorbent assay (ELISA) method from Abcam (ab108835). Sensitivity of this ELISA method is high and plasma samples had to be diluted > 1000-fold in the wells. The intra-assay coefficient of variation was 4.6%.

D-dimer

In paper III, D-dimer concentrations were analyzed with a sandwich ELISA method from Abcam (ab196269). The analyses were performed in 2016 and 2017 in Umeå, Sweden. The intra-assay coefficient of variation was 3%. The inter-assay coefficient for the method is 5%.

VWF

In paper IV, VWF antigen concentrations were analyzed with a sandwich ELISA method from Dako, Denmark (reference A0082 for coating and P00226 for conjugate). The analyses were performed in 2017 in Skellefteå, Sweden. VWF antigen concentrations are given as kIU/L. The local standard plasma used in the analysis is regularly calibrated.

Blood group

In paper IV, blood group was analyzed. For blood group analysis, reverse blood grouping using gel cards was performed. With this method, the presence of ABO antibodies is assessed in plasma. The gel cards were analyzed using a Banjo ID-reader. The analyses were performed in 2018 in Växjö, Sweden.

PEth

In paper V, PEth 16:0/18:1 concentrations were analyzed in packed erythrocytes using liquid chromatography with tandem mass spectrometry. The method is

described in detail in the publication by Lakso et al. (202). The analyses were performed in 2018 in Umeå, Sweden. The limit of quantification for this method was 0.01 µmol/L. The between-day-precision for two samples with concentrations of 0.32 µmol/L and 1.94 µmol/L had coefficients of variation of 5.4% and 3.1%, respectively. This method is not the same that is currently used in usual practice in the area. The method used in clinical practice is robot-based, has a limit of quantification of 0.05 µmol/L and PEth 16:0/18:1 is analyzed in whole blood. The PEth concentrations in packed erythrocytes were recalculated to make them comparable to PEth concentrations in whole blood.

Event registration and case definition

ICD diagnosis codes

In paper I, the National Patient Register and the Swedish Cause of Death Register were searched for ICD-8, ICD-9 and ICD-10 diagnosis codes for hemorrhagic stroke (430, 431, I60, I61) for the years 1985 through 2011. Ten individuals with unspecified stroke (436, I64) were excluded from analyses. Only the first occurrence of the studied outcome (hemorrhagic stroke) after inclusion in the study at health examination was considered. In an additional analysis, only the first occurrence of ICH was considered.

MONICA incidence register

In papers II–V, the MONICA incidence register was used to define outcome ICH events. In the MONICA incidence register, all stroke events that occurred in the inhabitants of Norrbotten and Västerbotten Counties aged 25 to 74 years were registered. The register was active between January 1, 1985 and December 31, 2009. Registration of the stroke event in the MONICA incidence register was independent of whether or not an individual had participated in a health examination such as the VIP, the MONICA or the Mammography Screening Project.

Possible stroke cases were mainly detected in the following ways: screening of hospital and nursing home discharge registers, reports from hospitals and general practitioners, and screening of death certificates. The Swedish Cause of Death Register was also used to detect possible stroke cases. The stroke-related diagnoses that were submitted to the MONICA incidence registry and subsequently validated are listed in Table 3.

Table 3. ICD codes used to detect potential stroke cases for inclusion in the MONICA incidence register

	ICD 8, ICD 9	ICD 10
Discharge records	430-438 (cerebrovascular diseases)	I60–I69 (cerebrovascular diseases) G45 (transient ischemic attack) G46 (vascular syndromes of brain in cerebrovascular diseases)
Death certificates	430-438 (cerebrovascular diseases) 440 (atherosclerosis) 798–799 (sudden or ill-defined death)	I60–I69 (cerebrovascular diseases) R96-R99 (sudden death)

All stroke cases were validated by specially trained nurses using medical records, radiology reports and autopsy protocols. An experienced stroke physician could be consulted if needed. Stroke cases were defined as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin" according the MONICA criteria which are based on the World Health Organization stroke definition (203). Global clinical signs were only accepted as symptoms of stroke in persons with subarachnoid hemorrhage or in a deep, comatose state. Brain lesions that were radiologically detected but not accompanied with acute neurological signs, detailed above, were not accepted as stroke. Extradural and subdural hemorrhages were not defined as stroke. Potential stroke cases with brain tumor, trauma or a severe blood disease were excluded from the register.

Stroke cases were classified into stroke subtypes. A valid ICH event that was registered in the MONICA incidence register required a finding of an ICH on a computerized tomography (CT) scan or at autopsy. Information about the cases was collected from medical records (summarized in Table 4).

Table 4. Information collected at the time of the stroke event for patients registered in the MONICA incidence register.

	Examples of information collected
Demographic information	Age, sex
Medical history and tobacco use	Hypertension, diabetes mellitus, previous myocardial infarction or stroke, tobacco use
Medications used at the stroke event	Antihypertensive and antithrombotic drugs
Clinical signs at onset	Level of consciousness, neurological deficits
Radiological examinations	CT scan, ultrasound, magnetic resonance imaging, angiography
Dates regarding the stroke diagnosis	Date of symptom onset, admission, discharge and death
Diagnoses	Type of stroke

Referents

In papers II–V, two referents were matched to each person with ICH. Matching was performed for age ± 2 years, sex, health examination setting, health examination date ± 1 year and geographical region. A referent was excluded if he or she experienced a stroke, according to the northern Sweden MONICA incidence register, before the date of the ICH of their matched case. Potential referents were also excluded if they had moved out of the study region or died before the date of the ICH of their matched case.

Statistical methods

Number of observations, percentages, medians, interquartile ranges, means and standard deviations (SDs) were used to describe baseline characteristics and to describe characteristics at the ICH event. Between-group differences were tested with the Mann-Whitney U-test, the Student's t-test and the $\chi 2$ test. If the expected frequency for any one cell was < 5, Fisher's exact test was used instead of the $\chi 2$ test when testing for between-group differences in the distribution of categorical variables. For correlation assessments, Spearman's rank-order correlation was used. Two-sided P-values < 0.05 were deemed to be significant.

The association between biomarker concentrations and risk of hemorrhagic stroke or ICH, except for PEth concentrations, was assessed with the studied biomarker expressed as a continuous variable per SD and as a categorical variable in tertiles. As D-dimer and VWF were not completely normally distributed, sensitivity analyses were made where the association between the natural logarithm of these variables and the risk of ICH was investigated. For PEth concentrations, no analysis with PEth as a continuous variable was made as more than half of the participants had PEth concentrations below the lower limit of quantification. PEth category boundaries were based on clinically

relevant cut-off points. ABO blood group was analyzed in the four established ABO blood group categories and dichotomized as O/non-O blood group.

In the cohort study, uni- and multivariable Cox proportional hazards regression was used to study the association between FXII concentrations and risk of hemorrhagic stroke. The proportional hazards assumption was tested using Kaplan-Meier and log-minus-log plots for categorical variables. For continuous variables, the partial residuals were plotted versus survival time. In the case-referent studies, univariable and multivariable conditional logistic regression analysis was used to study the association between biomarker concentrations and ICH risk. Potential confounders were included in the multivariable models based on their known relationship with cardiovascular disease. In paper II and paper V, only variables with a P-value < 0.10 in the univariable analysis were entered into the multivariable model. The adjustment variables included in papers I–V are listed in Table 5.

Table 5. Adjustment variables included in papers I–V

	Paper I	Paper II	Paper III	Paper IV	Paper IV	Paper V
Investigated biomarker	FXII	FXII	D-dimer	VWF	Blood group	PEth
Age	X	Matched	Matched	Matched	Matched	Matched
Sex	X	Matched	Matched	Matched	Matched	Matched
Hypertension	X	X	X	X		X
Smoking	X	X	X	X		X
Diabetes	X	X	X	X		X
BMI	X	X	X	X		X
Cholesterol	X		X	X		
Education		X				X

In a sensitivity analysis for paper I, where the outcome was an ICH event, a backward elimination model was used to minimize the number of adjustment variables. In this model, the variables with the highest p-values were removed stepwise until only variables with a p-value < 0.20 remained in the model. Age and sex were kept in the model regardless of p-value.

In a sensitivity analysis for paper III, the multivariable model was adjusted for systolic blood pressure at health examination as a continuous variable and blood pressure lowering medication instead of for the compound variable hypertension.

Stratified analyses were used to study the association between biomarker concentrations and risk of ICH in different groups separately by stratifying analyses based on, for example, the time between blood sampling and ICH event.

Additive and multiplicative interaction analyses were performed.

Sample size calculations for papers I and II showed that an OR of ≥ 4.5 and ≥ 2.6 respectively could be detected with a power of 80% and a significance level of 0.05 for an increased risk of hemorrhagic stroke and ICH respectively in individuals with FXII concentrations in the highest tertile. For papers III and IV, an OR of ≥ 2.0 could be detected with a power of 80% and a significance level of 0.05 for an increased risk of ICH in individuals with D-dimer and VWF concentrations in the highest tertile. Sample size calculations for paper V showed that an OR of ≥ 3.0 could be detected with a power of 80% and a significance level of 0.05 for an increased risk of ICH in individuals with PEth > 0.30 μmol/L if 10% of the referents had PEth > 0.30 μmol/L.

In papers I and III, complete cases analyses were performed, i.e. participants with missing data on a variable were not included in the multivariable model. In papers II, IV and V, participants with missing data on a categorical variable were placed in a separate category (data not shown). In paper IV, participants with missing data on a continuous variable were not included in the multivariable model. No continuous adjustment variables were included in papers II and V.

A sensitivity analysis was performed for paper V in which multiple imputation was used to handle missing data for adjustment variables. Multiple imputation was performed using chained equations. Ten imputed datasets were generated.

Statistical analyses were performed using IBM SPSS Statistics for Macintosh (IBM Corp, Armonk, NY). The multiple imputation analysis was performed in Stata Statistical Software: Release 14 (StataCorp. College Station, TX).

Ethical considerations

All studies in this thesis have been approved by the Regional Ethics Review Board, Umeå, and were conducted in accordance with the Declaration of Helsinki. Ethics applications were approved 1985-05-07/§1 and 1985-11-05/§19. Further ethics applications have approval numbers 94-289 with amendment, approval number 349/96-277 with amendments, approval number 00-142 and approval number 2017-239-31 with amendment.

All individuals participating in the studies of this thesis have given written informed consent to participate in future research when attending the VIP, MONICA or Mammography Screening Project. If a health situation requiring medical attention was discovered during a health examination, for example an elevated blood pressure, participants were referred to a provider in the health care system. At the health examinations approximately 20 mL of blood was drawn. This is considered to pose a minimal risk to the participants. The participants were not subjected to any study specific medical interventions other than blood sampling.

Regarding case registration, all surviving persons with ICH received a letter with information about the MONICA incidence register and the purpose of the register before the case data was recorded. The persons were asked to contact

the registry if they did not consent to the recording of personal data. In that case, their personal information was omitted.

All data analyses were performed with datasets where each individual had been given a code number and the researchers performing the analyses of the data set did not have access to the encryption key. The encryption key was kept at the Biobank Research Unit, Umeå.

All results are presented on group level and no individuals can be identified. This is especially important since ICH is a relatively uncommon disease and reports of specific cases carry a risk of violating personal integrity. The participants do not directly benefit from being a part of the studies of this thesis, but hopefully persons with the same or similar medical conditions can benefit in the future. Participation in a health examination, such as MONICA and VIP, can potentially benefit the participant as previously unknown health issues can be discovered, although this could also be a potential cause of distress for the participant. The results of the laboratory analyses performed in our studies have not been communicated to the participants and we have not taken any action when encountering laboratory values outside the normal range. We consider our studies to be of an exploratory nature. For the majority of the participants, a long period of time had passed between their blood sampling and analysis of our biomarkers (up to three decades).

Results

Study population, paper I

Paper I included 1,852 participants, 51% of which were women. Characteristics of the participants at the time of the health examination are shown in Table 6.

Table 6. Data collected at MONICA health examination in 1994. Values presented as mean (standard deviation) or n (%).

	Men (n = 914)	Women (n = 938)
Age	50.4 (14.1)	49.6 (14.1)
Present or previous daily smoking	468 (51.4)	455 (48.6)
Body mass index, kg/m^2	26.2 (3.7)	25.8 (4.7)
Cholesterol, mmol/L	6.2 (1.3)	6.2 (1.4)
Hypertension	377 (41.3)	333 (35.5)
Diabetes mellitus	42 (4.6)	42 (4.5)
Above secondary school education	229 (25.4)	275 (29.5)
Factor XII, IU/mL	1.11 (0.29)	1.11 (0.31)

The mean age at the MONICA health examination was 50 years (SD 14) and the mean age at hemorrhagic stroke event was 68 years (SD 13). Hypertension was present in 796 participants at the health examination and of these, 197 (28%) had been taking blood pressure lowering medication during the two weeks preceding the health examination. A total of 30 participants experienced a hemorrhagic stroke event during the follow-up period. Among these, 21 had an ICH event and 10 had a subarachnoid hemorrhage (one of the individuals with a subarachnoid hemorrhage later experienced an ICH event). The median time between blood sampling at health examination and ICH event was 9.5 years and the median time between blood sampling and subarachnoid hemorrhage was 9.4 years.

Study population, papers II–V

Characteristics at health examination for VIP and MONICA participants

The mean age at health examination was 55 years (SD 8). Of identified cases, 88% had participated in the VIP and 12% had participated in the MONICA. Data collected at the health examination for the ICH cases and their referents is shown in Table 7.

Table 7. Data collected at VIP and MONICA health examinations for cases and referents. Values presented as mean (standard deviation) or n (%).

	Cases (n = 164)	Referents (n = 327)
Age	55.4 (7.7)	55.4 (7.6)
Female sex	58 (35.4)	117 (35.8)
Daily smoking	36 (23.4)	67 (21.3)
Body mass index, kg/m^2	28.1 (5.2)	26.1 (3.5)
Cholesterol, mmol/L	6.2 (1.1)	6.2 (1.1)
Hypertension	122 (76.3)	149 (47.5)
Diabetes mellitus	18 (11.8)	12 (4.0)
Above secondary school education	4 (4.7)	25 (15.3)

Among the cases, 22% stated intake of blood pressure lowering medication two weeks preceding the health examination. Only one in six of the cases treated with antihypertensive medications at the time of the health examination had a systolic blood pressure < 140 mmHg and a diastolic blood pressure < 90 mmHg. Among the referents, 12% stated intake of antihypertensive medication and among those, approximately one in five had a systolic blood pressure < 140 mmHg and a diastolic blood pressure < 90 mmHg at the time of the health examination.

Characteristics of cases at the ICH event

In the MONICA incidence register, data collected at a first-ever ICH event between 1985 and March 2007 was available for 200 NSHDS participants. Blood samples for biomarker analysis were not available for all of the 200 ICH cases.

Among the 200 cases, 94 were women and 106 were men. The mean age at the ICH event was 64 years for women (SD 7 years) and 61 years for men (SD 8 years). The age distribution at the ICH event is shown in Figure 10.

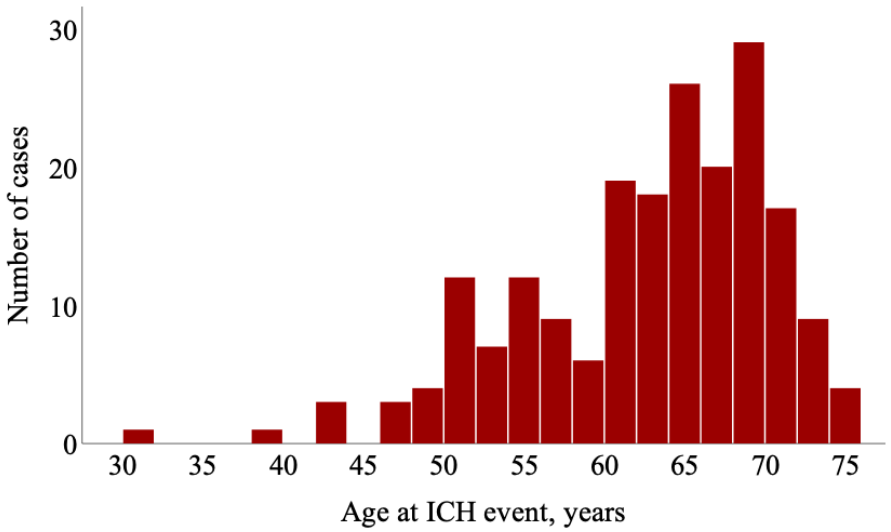

Figure 10. The age distribution of the cases (n = 200) at the time of ICH event.

The time that elapsed between the blood sampling at baseline and the ICH event is shown in Figure 11.

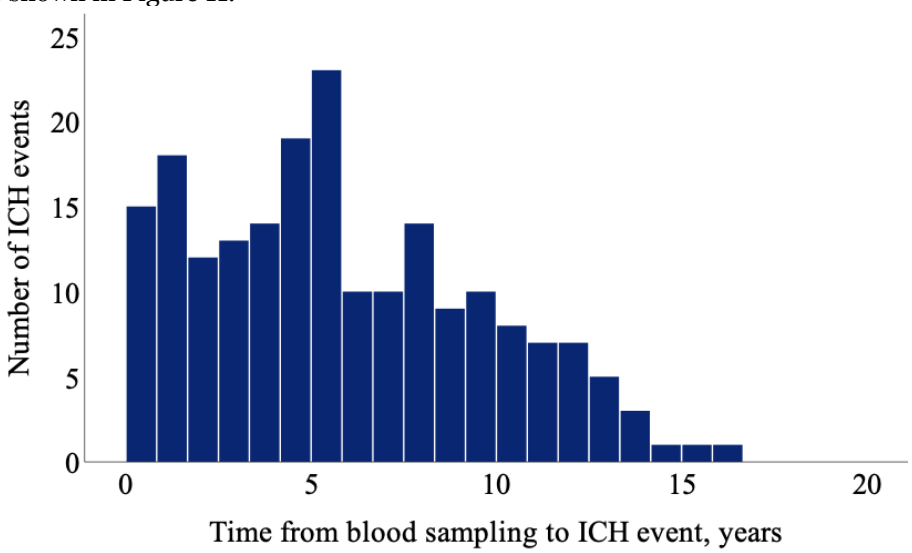

Figure 11. Time in years for the cases (n = 200) between baseline blood sampling and ICH event.

The calendar years when the ICH events occurred are shown in Figure 12.

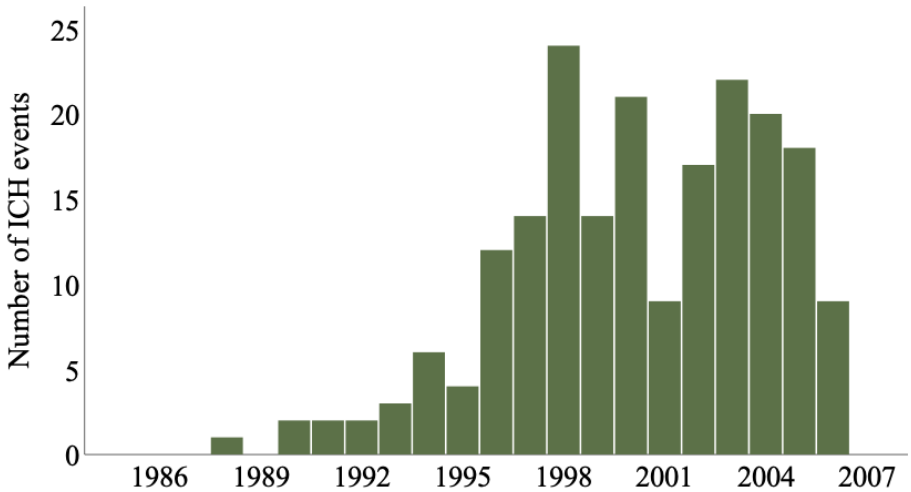

Figure 12. Calendar year of the ICH event for the cases (n = 200).

Before admission for the ICH event 175 participants (88%) were independent in their activities of daily living. Upon seeking medical attention 30% of the ICH cases had a reduced level of consciousness and 67% had a motor deficit.

Data on anticoagulant and antiplatelet treatment was available for 189 cases among which seven (4%) had antecedent anticoagulant treatment while 37 (20%) had ongoing antiplatelet treatment at ICH onset. One participant was treated with a combination of the two. According to medical records, 54% of the cases had a diagnosis of hypertension and 10% had a diagnosis of diabetes mellitus established prior to the ICH event.

At discharge, 41% of the ICH cases were independent in their activities of daily living, 3% were partly dependent, 32% were fully dependent and 24% were deceased. Among the persons with ICH, one in five died within the first 48 hours after the ICH event. The 28-day mortality was 26%. Among persons who were comatose at admission (n = 25) the in-hospital mortality was 80% while the remaining five individuals were discharged highly disabled.

FXII and risk of hemorrhagic stroke and ICH respectively (papers I–II)

MONICA 1994 cohort (paper I)

The distribution of FXII concentrations in the study population is shown in Figure 13.

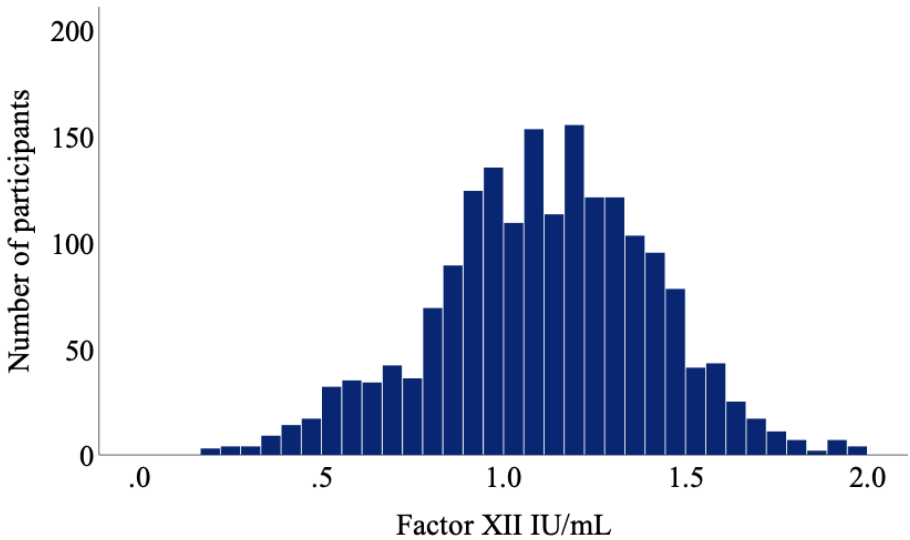

Figure 13. The distribution of FXII concentrations (n = 1,852).

The hazard ratio in univariable analysis for the association between FXII concentrations and hemorrhagic stroke was 1.42 (95% CI 0.99–2.05) per SD of FXII. In a multivariable model adjusted for age, sex, diabetes mellitus, hypertension, smoking, BMI and cholesterol levels, the corresponding hazard ratio was 1.51 (95% CI 1.03–2.21) per SD of FXII.

A similar hazard ratio was obtained in an exploratory analysis where the association between FXII and risk of ICH was investigated in a multivariable model with the same adjustment variables (hazard ratio 1.83; 95% CI 1.15–2.90 per SD of FXII). Due to few cases with ICH, sensitivity analyses with fewer adjustments variables were performed. A univariable analysis resulted in a hazard ratio for the association between FXII per SD and ICH of 1.64 (95% CI 1.06–2.55). In a backward elimination model, the variables with the highest p-values were removed stepwise until only variables with a p-value < 0.20 remained in the model. Age and sex were kept in the model regardless of p-value. This resulted in a model where the association between FXII and risk of ICH was adjusted for age, sex and smoking (hazard ratio 1.81; 95% CI 1.15–2.86 per SD of FXII).

VIP case-referent study (paper II)

We also investigated the association between FXII and risk of ICH in a case-referent study where we included 70 cases and 137 matched referents. The mean age of the study participants at health examination was 54 years and 33% were women.

In this study, we found no association between FXII concentrations and risk of ICH. In a univariable conditional logistic regression model, the OR for the association between FXII per SD and risk of ICH was 1.20 (95% CI 0.74–1.94).

In a model adjusted for hypertension, the OR was 1.15 (95% CI 0.66–1.98) and in a model adjusted for hypertension, diabetes mellitus, smoking and BMI, the OR was 1.06 (95% CI 0.57–1.97).

Interaction analyses for the interaction between FXII and hypertension in the context of ICH risk were performed. There was no interaction between FXII and hypertension, neither on the additive nor on the multiplicative scale.

D-dimer and risk of ICH (paper III)

We investigated the association between D-dimer and risk of ICH in a study including 141 cases (39% women) and 255 matched referents. The mean age for men at ICH event was 60 years (SD 8) and for women 62 years (SD 7.5). The mean D-dimer concentration in the study population was 269 μg/L (SD 178). The cases had higher D-dimer concentrations compared to the referents, p-value 0.002.

In a univariable analysis, the OR for the association between D-dimer concentrations and risk of ICH was 1.41 (95% CI 1.12–1.77) per SD of D-dimer. In a model adjusted for hypertension, the OR for the association between D-dimer and risk of ICH was 1.38 (95% CI 1.08–1.77) per SD of D-dimer. The association was similar in a model adjusted for diabetes mellitus, hypertension, daily smoking, BMI and cholesterol levels (OR 1.36; 95% CI 1.05–1.77). There was no evidence of multicollinearity for the adjustment variables (Spearman's correlation coefficient ≤ 0.6 for all variables).

The univariable and multivariable associations between D-dimer as a continuous and categorical variable and risk of ICH are presented in Figure 14.

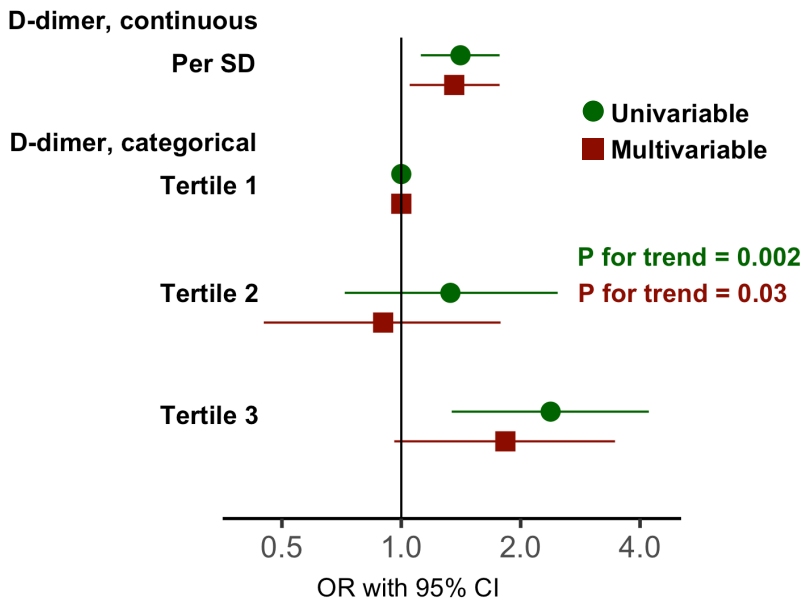

Figure 14. The association between D-dimer concentrations and risk of ICH, presented as ORs with 95% CIs. D-dimer concentrations are treated as a continuous variable per SD of 178 µg/L and as a categorical variable in tertiles. Tertile 1 = < 153 µg/L, tertile 2 = 153–257 µg/L and tertile 3 ≥ 258 µg/L. The multivariable models are adjusted for hypertension, diabetes mellitus, daily smoking, BMI and cholesterol.

A sensitivity analysis was performed were the multivariable model described above was adjusted for systolic blood pressure at health examination (continuous) and blood pressure lowering medication (categorical) instead of for the compound variable hypertension. In this model, the OR for the association between D-dimer per SD and risk of ICH was similar to the main analysis (OR 1.30; 95% CI 0.99–1.69).

The association between the natural logarithm of D-dimer and risk of ICH was also investigated. In a univariable conditional logistic regression the OR was 1.92 (95% CI 1.30–2.83), p-value 0.001 for the association between the natural logarithm of D-dimer and risk of ICH. In a multivariable model adjusted for diabetes mellitus, hypertension, daily smoking, BMI and cholesterol levels the OR for the natural logarithm of D-dimer was 1.67 (95% CI 1.07–2.61), p-value 0.02.

In addition, we explored the associations between D-dimer concentrations and risk of ICH in participants with different times from health examination, when blood samples were drawn, to ICH event. We divided the cases and their

matched referents into three groups according to the time from blood sampling to ICH event and analyzed the association between D-dimer and of risk of ICH in each group separately. Using the same multivariable model as in the main analysis, the association seemed to be most pronounced in the participants with the shortest time from blood sampling to ICH event, < 3.5 years (OR 1.78; 95% CI 1.05–3.05 per SD of D-dimer). The separate univariable and multivariable associations for the three groups are presented in Figure 15.

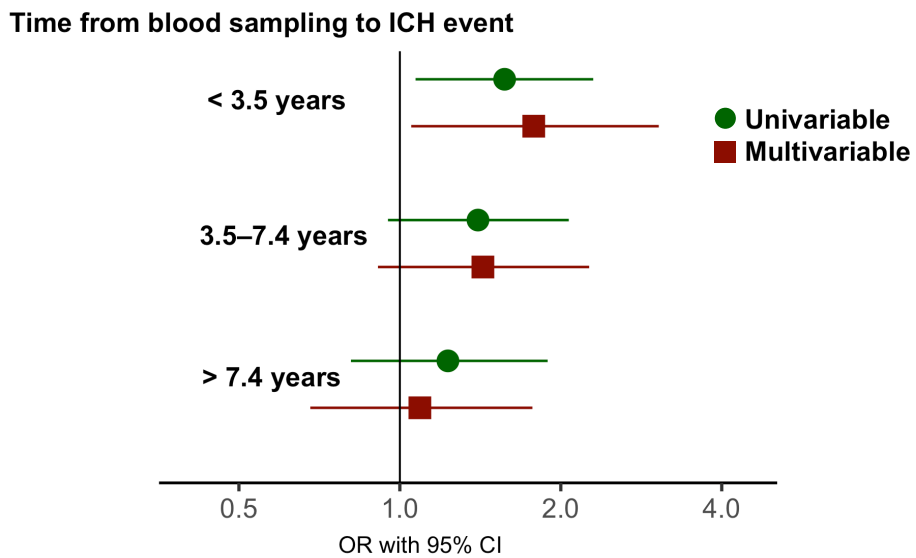

Figure 15. The association between D-dimer and risk of ICH stratified by time between blood sampling and ICH event. The multivariable model is adjusted for diabetes mellitus, hypertension, daily smoking, BMI and cholesterol. Associations are shown as OR with 95% CI per standard deviation of D-dimer.

VWF, ABO blood groups and risk of ICH (paper IV)

We investigated the association between VWF, blood groups with focus on blood group O, and risk of ICH in a nested case-referent study with 176 cases and 349 referents. The median age at the time of health examination was 60 years (1st quartile 50 years, 3rd quartile 60 years) and 50% were women. The mean age for the cases was 63 years at the ICH event.

In a univariable model, the OR for the association between VWF per SD and risk of ICH was 0.99 (95% CI 0.77–1.28). In a multivariable model adjusted for hypertension, smoking, BMI, cholesterol and diabetes mellitus, the corresponding OR was 0.85 (95% CI 0.54–1.34) per SD of VWF. Neither was there an association between VWF and risk of ICH when using the natural logarithm of VWF. There was no additive or multiplicative interaction between VWF and hypertension in the context of ICH risk.

We also explored the association between VWF and risk of ICH stratified by the time between health examination and ICH event in tertiles. In this analysis, cases (n = 103) and referents (n = 204) who had participated in the VIP and MONICA health examinations were included. In the strata with the shortest time between health examination and ICH event (≤ 5.4 years), the OR for the association between VWF per SD and risk of ICH was 1.06 (95% CI 0.51–2.18) in a multivariable model. The model was adjusted for hypertension, smoking, BMI, cholesterol and diabetes mellitus. The corresponding ORs for the strata with 5.4–9.6 years and ≥ 9.6 years between health examination and ICH event were 0.50 (95% CI 0.20–1.25) and 0.86 (95% CI 0.25–2.96) respectively.

Blood group O was also evaluated as a potential risk marker for ICH. The median VWF concentration was lower in individuals with blood group O (1.17 kIU/L) compared to individuals with non-O blood group (1.41 kIU/L). The cardiovascular risk factors evaluated at the baseline health examination did not differ between participants with O and non-O blood groups, as presented in Table 8.

Table 8. Characteristics evaluated at the health examination for participants (n = 374) with O blood group and non-O blood group. Values presented as median (1^{st}–3^{rd} quartile) or n (%).

	O blood group (n = 155)	Non-O blood group (n = 219)	p-value
VWF, kIU/L	1.17 (0.85–1.53)	1.41 (1.12–1.87)	<0.001
Hypertension	85 (57.0)	123 (58.9)	0.73
Diabetes mellitus	7 (4.9)	14 (6.9)	0.44
Daily smoking	30 (19.9)	50 (24.0)	0.35
Education*	14 (10.0)	28 (14.7)	0.20
SBP, mm Hg	140 (128–149)	140 (125–150)	0.53
DBP, mm Hg	87 (82–92)	86 (79–93)	0.44
BMI, kg/m²	26.1 (23.9–28.2)	25.9 (23.7–28.7)	0.69
Cholesterol, mmol/L	6.1 (5.5–6.9)	6.1 (5.6–6.8)	0.69

**above secondary school education*
SBP, systolic blood pressure, DBP, diastolic blood pressure

There was no association between blood group O and risk of ICH compared to non-O blood groups in a univariable conditional logistic regression analysis (OR 0.96; 95% CI 0.65–1.42). Neither was there an association between any of the three blood groups A, B and AB compared to blood group O and the risk of ICH as presented in paper IV. In an analysis where only participants of the VIP and MONICA health examinations were included, a multivariable model adjusted for hypertension, smoking, BMI, cholesterol and diabetes mellitus was used. The OR in the multivariable model was 1.09 (95% CI 0.64–1.88) for the association between blood group O and risk of ICH compared to non-O blood group.

In three explorative analyses, the blood groups A, B and AB were used as reference levels in conditional logistic regression models investigating the association between each of the other blood groups and risk of ICH. There was an association between blood group B and lower risk of ICH compared to blood group A (OR 0.47; 95% CI 0.23–0.95). There were no significant associations when using AB as reference category.

PEth and risk of ICH (paper V)

The study regarding the association between PEth concentrations and risk of ICH included 97 cases (40% female) and 180 referents. Baseline characteristics stratified by PEth concentration are shown in Table 9.

Table 9. Baseline characteristics for participants (n = 277) stratified by PEth concentration. Values presented as mean (SD) or n (%).

	PEth, µmol/L			
	< 0.01 (n = 150)	0.01–0.049 (n = 57)	0.05–0.30 (n = 49)	> 0.30 (n = 21)
Hypertension	79 (54.4)	26 (48.1)	28 (58.3)	12 (60.0)
Diabetes mellitus	7 (5.1)	6 (11.7)	2 (4.6)	1 (5.6)
Daily smoking	19 (12.9)	10 (18.5)	15 (31.9)	12 (60.0)
BMI, kg/m^2	26.6 (4.1)	26.1 (3.5)	27.6 (3.9)	27.7 (5.2)
Education*	10 (8.4)	9 (19.1)	5 (12.5)	2 (11.8)

*above secondary school education

The distribution of PEth concentrations among cases and referents are shown in Figure 16. Among the cases, 13% had PEth concentrations > 0.30 µmol/L, compared to 4% among the referents.

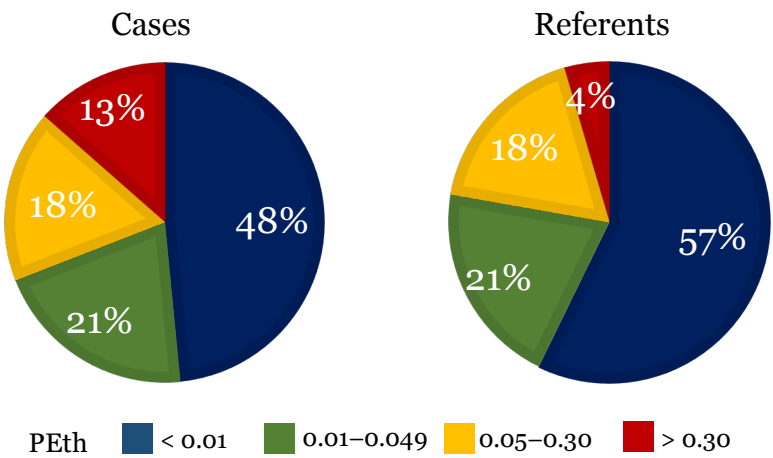

Figure 16. PEth concentrations, measured in µmol/L, among cases (n = 97) and referents (n = 180).

We found an association between PEth concentrations > 0.30 µmol/L compared to < 0.01 µmol/L and risk of ICH with an OR of 4.01 (95% CI 1.53–10.56) in a univariable conditional logistic regression analysis. In a multivariable model adjusted for hypertension, diabetes mellitus, education level and BMI, the OR for the association between PEth and risk of ICH was 4.64 (95% CI 1.49–14.40).

In a sensitivity analysis, a conditional logistic regression was performed in which multiple imputation was used to handle missing data on adjustment variables. The result of this analysis, when adjusted for the same variables as the main analysis, was similar to those of the main analysis (OR 4.67; 95% CI, 1.48–14.75 for risk of ICH in persons with PEth concentrations > 0.30 µmol/L compared to < 0.01 µmol/L).

More men than women had PEth concentrations in the higher categories, p < 0.001. A PEth concentration > 0.30 µmol/L was noted in 14 men and 7 women. The distributions of different PEth concentrations for men and women are shown in Figure 17.

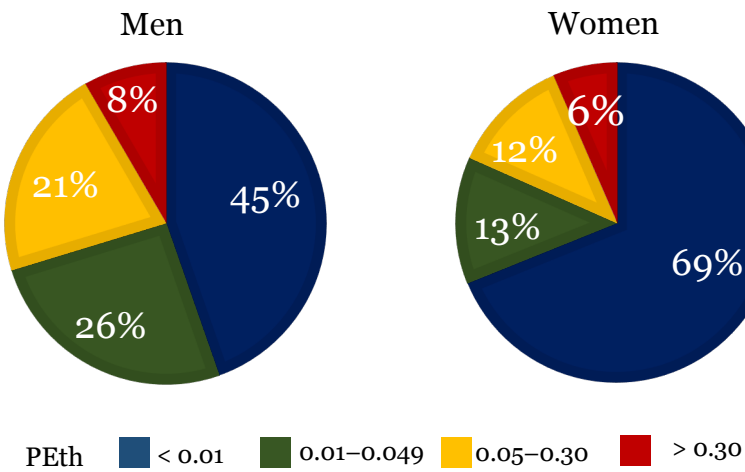

Figure 17. PEth concentrations, measured in µmol/L, among men (n = 168) and women (n = 109).

A conditional logistic regression model stratified for sex and adjusted for hypertension was used to investigate the association between PEth and risk of ICH compared to the reference level PEth < 0.01 µmol/L. The OR for the association between PEth > 0.30 µmol/L and risk of ICH in men was 3.97 (95% CI 0.99–16.01) and in women OR was 7.71 (95% CI 1.07–55.80). It should be noted that only 7 women (4 cases and 3 referents) had a PEth concentration > 0.30 µmol/L.

An additive interaction analysis for the interaction between PEth concentrations and hypertension in the context of ICH risk was performed. Participants with valid PEth measurements and data on hypertension status were included in the analysis (n = 267). The analysis was adjusted for age, sex, diabetes mellitus, education level and BMI. The additive interaction analysis is shown in Figure 18.

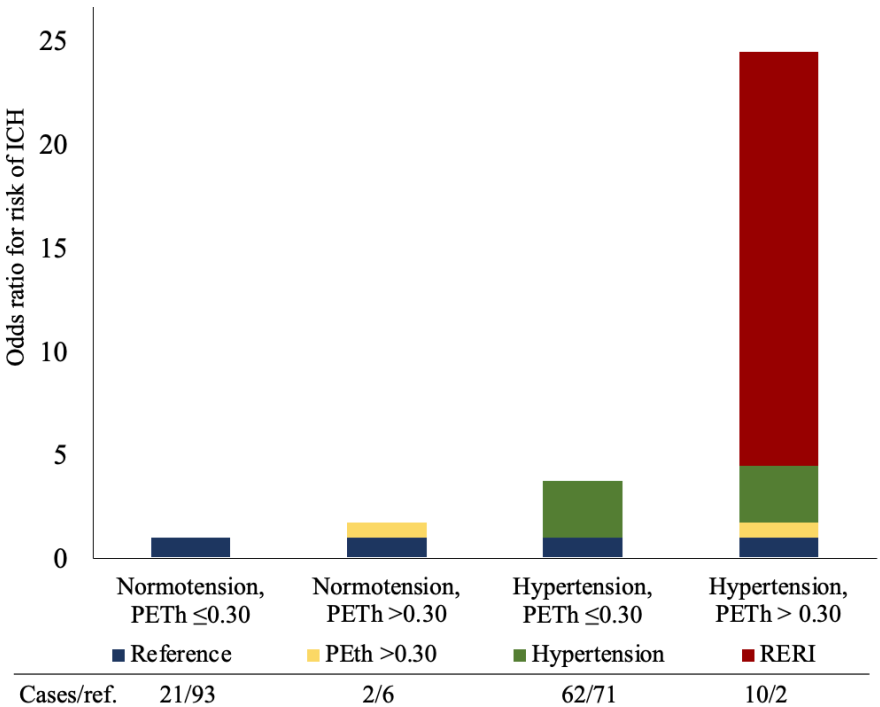

Figure 18. Additive interaction analysis for the interaction between PEth concentrations measured in µmol/L and hypertension in the context of ICH risk. The analysis is adjusted for age, sex, diabetes mellitus, education level and BMI.. RERI, relative excess risk due to interaction, Ref, referents

Among the participants with data on PEth concentrations, 81 cases and 148 referents had data on self-reported alcohol consumption. The percentage of participants with different levels of self-reported alcohol intake, stratified by PEth concentration, is shown in Figure 19. More than one third of the participants with a PEth concentration > 0.30 µmol/L had a self-reported alcohol intake < 3.0 standard drinks per week.

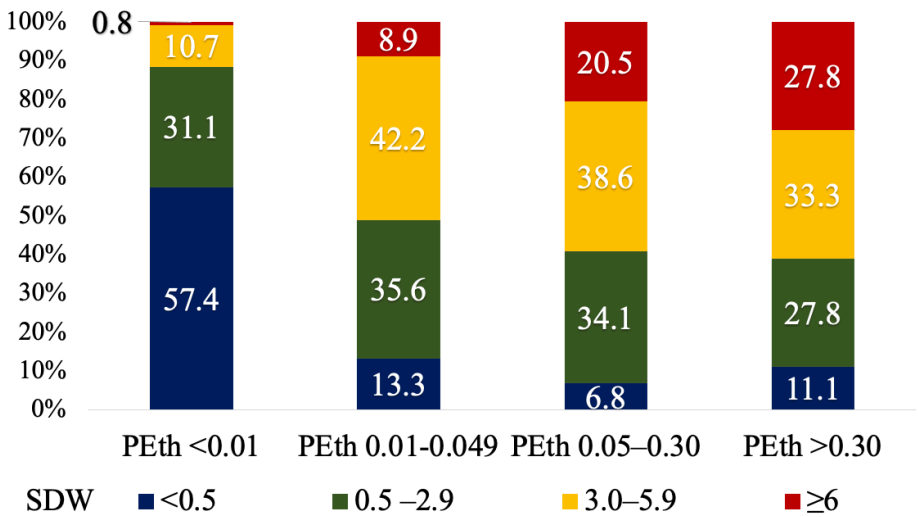

Figure 19. Self-reported alcohol intake in standard drinks weekly (SDW) stratified by PEth concentration in µmol/L, PEth < 0.01 (n = 122), 0.01–0.049 (n = 45), 0.05–0.30 (n = 44) and > 0.30 (n = 18).

There was a significant correlation between PEth concentrations and self-reported alcohol consumption, shown in Figure 20. The correlation coefficient was 0.56.

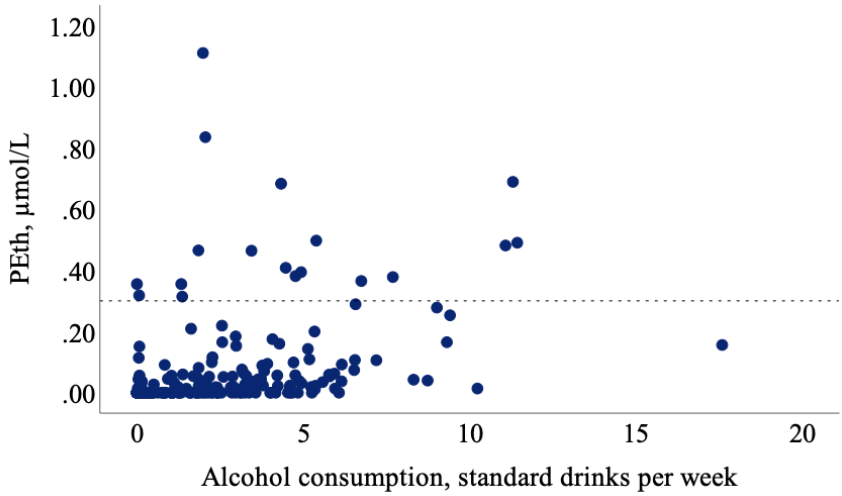

Figure 20. Correlation between PEth concentrations and self-reported alcohol consumption. Dashed line indicates PEth 0.30 µmol/L (n = 229).

Self-reported alcohol consumption consisted of intake of beer, wine and liquor. The percentages of the self-reported alcohol intake consumed as these three types of alcoholic beverages are shown in Figure 21. Cases consumed more liquor and less wine than the referents.

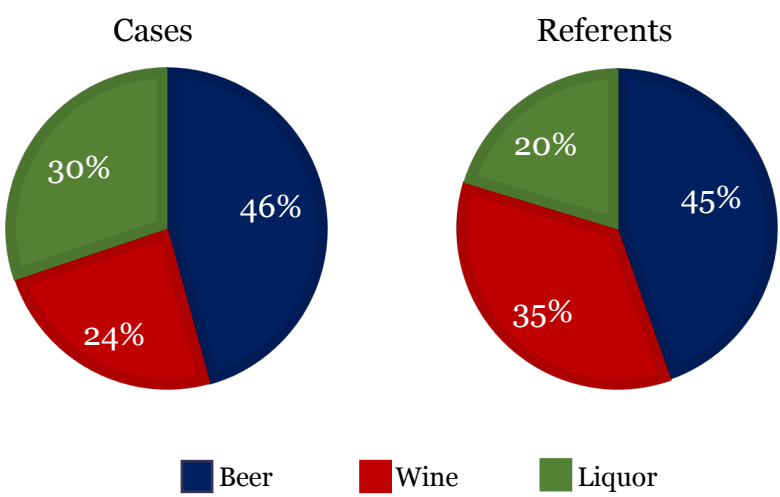

Figure 21. The percentage of self-reported alcohol intake consumed as beer, wine and liquor for cases (n = 81) and referents (n = 148).

Discussion

Main findings

FXII and risk of ICH

We found an association between FXII concentrations and risk of hemorrhagic stroke in a hypothesis generating cohort study. This association could not be confirmed in our larger case-referent study where the outcome was a validated first-ever ICH event.

An important limitation of the initial study is the small study size with only 30 hemorrhagic stroke cases, among which 21 had ICH. It is possible that our finding of an association between FXII and risk of hemorrhagic stroke in the cohort study is due to a type I error, i.e. a false positive result that can be due to chance. It is well-known that much of biomedical research, even highly cited articles, cannot be reproduced (204). Thus, it is important that efforts are made to replicate studies, especially if the results are unexpected. We therefore aimed to replicate our findings in a nested case-referent study. Although a larger number of cases were included, no association between FXII and ICH could be found.

There could be several reasons for the discrepancy in results between the studies. In the present studies, we used two different methods for FXII determination. The analysis method used in the replication study is more specific for FXII but the use of a different method means that the case-referent study does not exactly replicate the analytic procedures of the cohort study. In the case-referent study, we chose a FXII analysis method that was not dependent on the rate of kallikrein generation, thus eliminating the risk that other factors contributing to this process could influence FXII measurements.

We also noted that there was a difference in time from blood sampling to outcome event between the two studies with a longer time between blood sampling and outcome event in the cohort study. However, it is unclear if this had any bearing on the differences in results between our studies.

Furthermore, in the cohort study, the main outcome studied was hemorrhagic stroke, including both ICH and subarachnoid hemorrhage. In the case-referent study, it is an advantage that ICH was studied as a separate outcome. It is preferable to study ICH and subarachnoid hemorrhage as different entities as the pathogenesis differs between the two conditions (205).

In conclusion, the overall interpretation of the two studies indicates that there is no association between FXII and risk of future ICH. To further distinguish the impact of the different FXII analysis methods on the association between FXII and ICH risk, it could have been of value to analyze the same blood samples with the two different methods.

D-dimer and risk of ICH

In this thesis, we found an association between D-dimer and risk of ICH. The association was only significant for the cases with shortest time between blood sampling and ICH event.

The underlying mechanism explaining an association between high D-dimer concentrations and risk of ICH is not clear. One mechanism could be that increased D-dimer concentrations reflect changes in cerebral small vessels preceding the ICH event such as reactive hyperplasia of the smooth muscle cells or amyloid deposits. Increased D-dimer concentrations are associated with other types of vascular disease such as ischemic stroke (140) and D-dimer has been investigated in relation to cerebral small vessel disease. Although there is no established association between D-dimer and small vessel disease (206), high D-dimer concentrations have been associated with silent lacunar infarctions (207) and reduced brain volume (208).

There is a published meta-analysis on the relationship between D-dimer and risk of hemorrhagic stroke (140) and another on the subject of D-dimer and risk of ICH (209). The meta-analysis by Zhang et al. (140) did not show any association between D-dimer and risk of hemorrhagic stroke whereas Zhou et al. (209) demonstrated that the mean concentration of D-dimer was higher in ICH cases than in controls.

The meta-analysis by Zhang et al. includes prospective studies with D-dimer concentrations measured before the hemorrhagic stroke event while the meta-analysis by Zhou et al. measured the D-dimer concentrations at the time of hospital admission for the ICH event in the majority of the studies. A reason for the discrepancy in results between the two meta-analyses could be that Zhou et al. included non-prospective studies. It is known that D-dimer concentrations increase during acute medical conditions such as ischemic stroke (210). It is possible that the increase in D-dimer concentrations seen in the cases was due to an acute phase reaction triggered by the ICH event, rather than reflecting changes in D-dimer concentrations occurring in the time period before the acute event. As two of three studies included in the meta-analysis by Zhang et al. adjusted their analyses for systolic blood pressure and antihypertensive medication instead of adjusting for hypertension, we made an additional analysis with this adjustment strategy which yielded results similar to those of our main analyses. It is therefore unlikely that our finding of an association between high D-dimer concentrations and risk of ICH was due to higher D-dimer concentrations in individuals with the highest blood pressures at baseline. This is supported by the fact that there was no strong correlation between D-dimer and systolic blood pressure.

Due to its prospective nature, our study resembles the component studies included in the meta-analysis by Zhang et al. One reason for our finding of an association between D-dimer and risk of ICH could be that the time between blood sampling and ICH event in our study was shorter. In our study, the median time between blood sampling and ICH event was 5.2 years whereas in the study by Folsom et al. (145) included in the aforementioned meta-analysis

by Zhang et al., the median time between blood sampling and hemorrhagic stroke event was 8.5 years (Folsom, personal communication). This hypothesis is supported by the fact that, in addition to being significant for the whole study population, the association between D-dimer and risk of ICH remained significant in a separate analysis including only the cases with the shortest time between blood sampling and ICH event.

Our blood samples had been stored frozen, with a similar storage time for cases and their matched referents, before D-dimer analysis. It has been shown that D-dimer concentrations are only marginally affected by storage time when samples are stored at -70 degrees C (211). As there is an intraindividual variation in D-dimer concentrations (212), one limitation is that D-dimer was measured only once during the study period. For example, increased disease activity in Crohn's disease and rheumatoid arthritis can affect D-dimer concentrations (213, 214). Thus, a single D-dimer measurement may not be representative for an individual's usual D-dimer concentration. It would have been preferable to measure D-dimer at least at two time-points.

VWF, ABO blood groups and risk of ICH

We found no association between VWF concentrations, blood group O and risk of ICH. In an exploratory analysis, there was an association between blood group B, as compared to blood group A and risk of ICH. Our finding of no association between VWF and risk of ICH is in line with two previous, prospective studies (158, 160). In contrast, a smaller study performed in northern Sweden did find an association between low VWF concentrations and risk of ICH (157).

Persons with von Willebrand disease have low concentrations of VWF or a defect in VWF function leading to an increased risk of bleeding (215). We have no data on the presence of VWF disease diagnosis in our study participants, but we do know that only 1% of the study participants had VWF below 0.50 kIU/L. VWF function was not assessed. It is possible that too few individuals in the population have a sufficiently low VWF concentration for an association between low VWF concentrations and risk of ICH to be detectable.

Although the hypothesis for our studies was that low concentrations of VWF would be associated with risk of ICH, it can also be argued that high VWF concentrations could be associated with ICH risk. High VWF is considered a marker of endothelial dysfunction (216). Furthermore, increased VWF concentrations have been shown to be associated with signs of cerebral small vessel disease (217-219), one of the underlying conditions preceding an ICH (220). An increase in VWF concentrations could be associated with ICH because of its association with small vessel disease. Another supportive example is the association seen between high VWF and bleeding risk in persons with oral anticoagulant treatment (156, 221).

We found no association between blood group O and risk of ICH. A meta-analysis showed an increased risk of bleeding associated with blood group O

compared to non-O blood groups (166). In the meta-analysis, the two studies that included cerebral hemorrhages were published in the 1970s (167, 168). A possible cause of the discrepancy in results between our study and this meta-analysis is that only a minority of the bleeding events included in the meta-analysis were ICH events. A genotype study showed no association between ABO genotype and risk of hemorrhagic stroke, which is in accordance with our results (222).

PEth and risk of ICH

We found an association between PEth concentrations, reflecting alcohol consumption, and risk of ICH.

To our knowledge, the association between PEth concentrations and ICH risk has not been previously studied, but our results are consistent with studies investigating the relationship between high self-reported alcohol consumption and risk of ICH (94, 223). A meta-analysis including 11 prospective studies found that an alcohol intake of more than four drinks per day was associated with an increased risk of ICH (224).

A possible mechanism for the association between alcohol consumption and ICH risk is the known effect of alcohol on coagulation factor levels and thrombocyte count (225, 226). Another explanation could be an increased risk of small vessel disease associated with alcohol intake. Some support for this theory is given by a study comparing heavy drinkers and non-heavy drinkers with ICH (226). In this study, brain atrophy score was used as a marker of small vessel disease. Heavy drinkers had the same brain atrophy score as the non-heavy drinkers although they were, on average, 14 years younger at the time of the ICH event.

Furthermore, there is a well-known association between alcohol consumption and risk of hypertension. Heavy alcohol intake in normotensive individuals predisposes for development of hypertension (227, 228). A large meta-analysis showed that persons who consumed six or more drinks daily reduced their blood pressure by, on average, 5.5/4.0 mmHg if their alcohol consumption was halved (229). Several mechanisms have been suggested for the association between alcohol consumption and increased blood pressure, for example alcohol-related effects on the autonomic nervous system, cortisol secretion, endogenous vasoconstrictors and the baroreflex (230).

In our study, the mean systolic and diastolic blood pressure at baseline did not differ between persons with PEth > 0.30 μmol/L and persons with PEth ≤ 0.30 μmol/L but we found an interaction between PEth > 0.30 μmol/L and hypertension in the context of ICH risk. As there were few individuals with PEth > 0.30 μmol/L, the confidence interval for this interaction was wide. If the study was to be replicated in a larger population, we would expect the OR for the interaction to be lower. There are several possible explanations for the interaction. In the study population, 54% had hypertension at baseline. It is reasonable to believe that a substantial proportion of these persons were

prescribed antihypertensive medication as a result of the health examination or during the study period. It has been shown that alcohol drinkers have a lower compliance with antihypertensive medication (231). Thus, it is possible that the persons in our study with a high alcohol consumption had a lower adherence to medications and a poorer blood pressure control. This could result in a higher risk of ICH compared to individuals with hypertension at baseline and a lower alcohol consumption. This phenomenon would not be captured by our dichotomized hypertension variable which was evaluated only at baseline.

Using PEth as a marker of alcohol consumption has the advantage that PEth has a theoretical specificity of 100% (172) and is not subjected to recall bias and social desirability bias which could potentially be a problem with self-reports on alcohol consumption. In the VIP, the health examinations are performed at the participants' primary health care centers by nurses who are typically permanently employed at the health care center and who often resides in the local community. The questionnaire answers are not anonymous and are discussed with the participants as part of the health counselling component of the VIP. This can cause participants to be reluctant to share their true alcohol consumption. Social desirability bias (232) could be introduced.

There is substantial under-reporting when self-reported alcohol consumption is used to estimate true alcohol consumption (233). It has been shown that the degree of under-reporting of alcohol consumption is not equal between sexes, age groups and between persons with different levels of alcohol consumption (234). When there is a risk of biased questionnaire data on alcohol consumption, a blood biomarker reflecting alcohol intake can be valuable, both for research purposes and for individually tailored health counselling. The exact quantitative relationship between PEth and alcohol consumption is not known. PEth concentrations > 0.30 μmol/L correspond to approximately four standard drinks per day (178). In our study 8% of participants with data on both PEth and self-reported alcohol consumption had a PEth > 0.30 μmol/L. Among these participants, the highest self-reported alcohol consumption corresponded to 1.6 standard drinks per day. We believe that the use of a biomarker such as PEth yields valuable additional information on individuals with high alcohol consumption. The questions regarding self-reported alcohol consumption were not designed to capture binge drinking. It is possible that a questionnaire with more detailed questions in this area would have yielded a higher self-reported alcohol consumption for some of the participants.

PEth concentrations are largely unaffected by storage in -80 degrees C (202). This means that this biomarker is well-suited for use in studies where frozen blood samples stored in a biobank are thawed and analyzed. One disadvantage is that if ethanol is present in the blood when the sample is drawn, in vitro PEth formation can occur before the samples are frozen to -80 degrees C (235). As the samples in our study could be stored at -20 degrees C for a short time before being frozen to -80 degrees C, this could theoretically have influenced the measured PEth concentrations. However, it is improbable that this is an important source of preanalytical error as it is unlikely that a substantial

proportion of study participants had ethanol in their blood when attending the health examination.

There are some drawbacks to using PEth as a biomarker for alcohol consumption. There are interindividual variations in PEth formation which can result in persons exhibiting different PEth concentrations after a similar amount of alcohol consumption (183). This can make it difficult to translate a given PEth concentration to an exact amount alcohol consumed. Also, PEth only reflects the alcohol consumed during the past weeks (172, 173) and has a half-life of four to ten days (176). This can be a limitation when using PEth as a biomarker for assessment of disease risk. If PEth alone is used to gauge alcohol consumption, more remote periods of alcohol consumption, and even long episodes of heavy alcohol consumption, can remain undetected. In individuals with an alcohol consumption that varies over time, a single PEth measurement may not adequately represent their alcohol consumption. For example, in persons who practice binge drinking, the time elapsed since the last day with heavy alcohol consumption can affect the measured PEth value. Combining PEth with interviews or questionnaires can resolve some of these issues.

Methodological considerations

Study design

Paper I is a cohort study. As FXII concentrations were analyzed in all cohort members, it was possible to study the association between FXII concentrations and a range of different outcomes (myocardial infarction, ischemic stroke and hemorrhagic stroke). Papers II–V are prospective, nested case-referent studies within large cohorts. By using a study design where two referents were matched to each case represent the whole cohort, only a few hundred blood samples per biomarker needed to be analyzed. If the studies had been performed as cohort studies, approximately 100,000 samples would have been needed. The majority of the study participants were recruited from the VIP cohort in which data from a community intervention program is made available for research use. This is cost-effective as public health is improved (62) and research data is collected simultaneously.

Our studies are observational studies. Observational studies are prone to bias and confounding and therefore considered less suitable to give proof of causality when compared to randomized controlled trials (191). An isolated observational study is practically never sufficient to give definite proofs of causality (236). The prospective study design of all our studies ensures that the biomarker analyses are performed in blood drawn before the ICH event. Thus, the temporality criterion, the requirement that the studied exposure must occur before the outcome, for an association to be potentially causal, is fulfilled (237).

Matching

Cases and referents were matched for age, sex, geographical region, health examination date and source cohort in papers II–V.

Matching for source cohort ensures that the procedures and questionnaires used at the health examinations were the same for cases and referents. The VIP and MONICA health examinations and questionnaires are very similar, but there can be slight differences in the equipment used and in the questionnaires. Furthermore, participants of the VIP receive health counseling in conjunction with their health examination. Matching referents from the VIP to cases originating from the VIP cohort ensured that both cases and referents had received health counseling, which was not offered in the MONICA survey. Additionally, the biomarker concentrations and potential confounders were evaluated in the same way for cases and their matched referents, minimizing the risk of systematical errors causing between-group differences. Lastly, the inclusion criteria at baseline were the same for the cases and the referents. Thus, the referents represent the cohort from which the cases were drawn. An advantage of using this nested design in our studies is that no additional elements of selection bias are introduced when selecting the referents.

In a region such as Västerbotten, which consists of rural areas with small, sparsely populated municipalities and three cities, two of which are located in the coastal area, living conditions can differ depending on place of residence within the study area. Alcohol consumption, which is investigated in this thesis using the biomarker PEth, has been shown to differ between urban and rural areas (238), as do other risk factors associated with ICH risk such as diet (239). Matching cases and referents for geographical region will decrease the impact of between-community differences in lifestyle factors, environmental factors, dietary factors and socioeconomic status on study results. Access to recreational facilities, for example athletic facilities and parks, as well as the typical distance between home and workplace, differ greatly between urban and rural areas in northern Sweden. This may confer between-community differences in exercise habits which can introduce bias, as regular physical activity is associated with a reduced risk of ICH (53).

Sample handling and sample transportation times can differ between different geographical regions of the study area, often as a function of the distance to the nearest hospital. Pre-analytical factors can affect biomarker concentrations (240). Thus, systematical differences in pre-analytical factors between cases and referents can introduce bias. Matching for geographical region results in similar sample handling and transportation times in cases and their matched referents and thus reduces the risk of systematical differences in pre-analytical factors and thus the risk of bias.

Matching for health examination date reduces the risk that changes over time in the distribution and impact of cardiovascular risk factors on the population level introduce differences between cases and referents. Since alcohol consumption per capita in Sweden increased between 1985 and 2007, PEth concentrations in the population could be expected to have increased during the study period (241). Other factors, such as lifestyle factors and disease treatment and management have also changed since the 1980s. For example, physical activity increased in Västerbotten County during the study period (242). As physical

activity is associated with decreased risk of ICH, bias can be introduced if cases and referents are recruited at different time periods (53). To summarize, as there are simultaneous trends over time with an increase in PEth, a factor that we hypothesize is associated with an increased risk of ICH, and physical activity, a factor associated with a decreased risk of ICH, a true association between PEth and risk of ICH could remain undetected if cases and referents are not matched for health examination year. The same reasoning is applicable for treated hypertension and diabetes mellitus. The treatment options and management of these diseases have changed during the study period (243, 244). Consequently, the impact of these conditions on ICH risk has conceivably changed as well.

Small changes in the health examination questionnaires and procedures have been made over the years. To reduce the risk of systematical differences between cases and referents, matching for health examination date was performed. For example, from 1996 onwards some non-alcohol related items were removed from the food frequency questionnaire. This led to a more concise questionnaire, which hopefully improved the data accuracy of the remaining items, including self-reported alcohol intake.

Exposure variables

The biomarkers investigated as risk markers for ICH in this thesis were analyzed in frozen blood samples collected before the initial ICH event. This means that the acute phase reaction, triggered by the ICH event itself (245), could not alter biomarker concentrations as is a potential risk if the blood samples are collected at the time of or after an acute medical event. In paper I, specialized teams were responsible for blood sampling and sample handling. This approach ensures a uniform sample handling and reduces the risk of pre-analytical errors. In papers II–V, the majority of the participants were recruited from the VIP cohort. In the VIP, blood sampling and sample handling at the primary health care centers were integrated with usual primary health care practices and performed by primary health care staff. This may have resulted in a higher risk of pre-analytical differences. Matching cases and referents for geographical area is one way of reducing differences between cases and referents that are due to pre-analytical factors.

Regarding freezing time, previous studies has shown that analyses for D-dimer and PEth are largely unaffected by storage time (202, 211). However, as a precaution to minimize the risk of any impact of differences in storage time on blood biomarker results, we aimed for a similar freezing time irrespective of case/non-case status in paper I and in cases and their matched referents in papers II–V.

Blood samples were stored in the biobank as aliquots to minimize the number of freezing and thawing cycles that each sample was subjected to. Inter-assay variability can be a cause of systematic differences between different rounds of analysis, for example between different ELISA plates. To ensure that this could not be a cause of differences between cases and their matched referents, triplets

of one case sample and its matched referent samples were analyzed together in the same round of analysis.

Laboratory staff knowledge of case/referent-status can result in differential assessment of biomarker concentrations and thus introduce systematic differences between cases and referents (246). In papers II–V, we blinded laboratory staff to case/referent-status by randomly varying the order of the case and the matched referents within a triplet to avoid this risk.

Confounding variables

In this thesis, the inclusion of participants who had undergone a health examination at baseline enabled an extensive adjustment for possible confounders.

Some diseases such as hypertension and diabetes mellitus can remain undiagnosed, especially early in the disease process (247, 248). This means that the diagnosed cases do not necessarily reflect the true prevalence of the condition. To be able to better adjust for confounding due to these conditions it is important that persons who are hitherto undiagnosed are classified correctly. We combined health examination and questionnaire data in our studies in order to give an accurate representation of the risk factor status of each participant. For example, among the participants we defined as having diabetes mellitus, 37% would have been missed if only self-reported data of known diabetes mellitus had been used. It should be noted that our definition of diabetes mellitus required only one pathological glucose measurement whereas in the clinic, repeated measurements are usually required for a diagnosis of diabetes mellitus (249).

In this thesis, all studies are prospective and data on confounder variables were collected at the baseline health examinations. If data on confounder variables would only have been collected at ICH onset there would be a risk of bias since it would be hard to collect reliable data on seriously ill patients for example regarding smoking. This would be a substantial issue in our studies since 13% of the ICH cases were comatose at admission to hospital and one in five had died within 48 hours. A prospective design means that data accuracy and the proportion of missing data should not differ between cases and referents or between cases with better and worse prognosis. A prospective design also eliminates the risk of recall bias (250) which can occur, for example, when enquiring about smoking and self-reported alcohol consumption.

A limitation regarding our measurements of confounding factors is that they were only assessed at the start of the study. Later changes in cardiovascular risk factor status were not accounted for. The median time between health examination and ICH event varied between four and nine years in the studies of this thesis. It is reasonable to assume that changes in risk factor status occurred more often in paper I, where the time between health examination and ICH event was longer. Using data from repeated health examinations or complementing health examination data with data from diagnosis code registers

and medical records for the entire study period could have been of value to improve the measurement of confounder variables.

Another issue with the assessment of confounder variables at baseline only is the health intervention component of the VIP, from which the majority of the participants of papers II–V were recruited. It has been shown that cardiovascular risk factors improved at a faster rate in Västerbotten compared to a neighboring county without a similar intervention (62). This implies that cardiovascular risk factors measured at the health examination just before the intervention may not be representative for cardiovascular risk factor status after the health intervention. This can be problematic in studies of biomarkers correlated to cardiovascular risk factors, for example VWF (251), where it is important to adjust for cardiovascular risk factors.

Despite our measures to reduce confounding both through matching and adjustment in multivariable analyses, some residual confounding likely remains in the analyses of this thesis.

Outcome variables

In paper I, the National Patient Register and the Swedish Cause of Death Register were used for case identification. This is a reliable procedure as all inhabitants of Sweden have a personal identification number (252) which can be used to link different registers to the health examination data. The National Patient Register and the Swedish Cause of Death Register have a relatively high sensitivity to detect hemorrhagic stroke. In a validation study, 42 of 44 hemorrhagic stroke cases were found to be registered in at least one of the two registers (253). Likewise, a Danish study showed a high validity for ICH diagnoses in national registers (254). An advantage of using the National Patient Register and the Swedish Cause of Death Register data for case finding is the relatively low cost and time requirement associated with register-based studies (255). It also allowed us to register cases for a longer follow-up period compared to the MONICA incidence register in which data was not available for the whole study period.

The MONICA incidence register was used to define outcome events in papers II–V. The MONICA register has several strengths regarding the identification and validation of stroke cases. It has been estimated that the register includes approximately 96% of all stroke events that came to medical attention in the study area. As few as 0.6% of patients declined the recording of personal information in the MONICA incidence register (203). All diagnoses were validated by specially trained staff who had access to a physician to discuss ambiguities. The same diagnostic criteria were used throughout the study period. A limitation of the register is that only stroke events in persons aged 25 to 74 years were included. However, this does not largely affect the age distribution of ICH cases included in papers II–V as only a minority of the study participants reached an age of 75 years or more during the study period. We had no data on ICH location. This is a limitation since the pathophysiological mechanisms may differ between ICH locations.

ICH is a disease with a high early mortality. In the studies of this thesis, one in five died within 48 hours of ICH onset. To avoid excluding the most serious cases, it is important that the case finding procedure is not dependent on whether or not the individual survives the initial phase. Utilizing the MONICA incidence register and National Patient Register diagnostic codes meant that case finding was independent of ICH severity.

Representativity of participants

The NSHDS is a population-based cohort with an inclusion aiming at a low risk of selection bias. In the VIP, all Västerbotten County inhabitants of certain ages are invited to participate. Between 1990 and 2006, the participation rate was 56–65% (256). There are only minimal differences in age and education level between participants and those who declined to participate. More women than men participated in the VIP, but the difference in participation rate between sexes decreased over time (256).

Participation rate is higher for persons born in Sweden than for persons born outside Sweden, but the participation rates for persons born outside Sweden increased markedly with time. Persons with low income or who were single had a lower participation rate. A lower participation rate was also seen among persons who had previously been hospitalized (256). In the latter case, it is possible that they are already attending regular follow-up for chronic diseases and thus do not feel the need for a health examination and intervention such as the VIP. This could cause a selection bias where the persons who do participate in the VIP are healthier than the general population. A potential disadvantage of the integration of the VIP into standard health care practice could arise when more urgent medical needs are prioritized over performing health examinations. This could lead to a situation where all inhabitants are not invited to the VIP due to staff shortages or other difficulties with access to providers.

Attempts have been made to investigate the reason for VIP non-participation. A questionnaire was mailed to all persons eligible for VIP participation in 2001 investigating causes of non-participation. In total 8,151 persons answered the questionnaire (89%). Among those answering the questionnaire, 27% had not participated in the VIP. Among these non-participants, 21% declared that the reason that they had not participated was that they were already under medical care, 33% stated that they had not received an invitation, 21% had forgotten the appointment, 21% would have wished to have received more information about the VIP and 4% stated that they did not want to participate. (256)

In the MONICA database the participation rate was 80% or more for the age group 35–64 years in the surveys from 1986, 1990 and 1994. The study staff tried to contact all persons who declined to participate and conduct telephone interviews. Education level was similar for participants and non-participants. Among the non-participants, fewer had known hypertension, they more often smoked regularly, were more likely to be single, and had slightly lower BMI (in the case of non-participants, BMI was self-reported) (257).

There is limited information on the risk of selection bias in the Mammography Screening Project. Among the women who participated in breast cancer screening (85% of the women initially invited), one third donated blood samples to the NSHDS. The inclusion of participants of the Mammography Screening Project in the analysis of the association between blood group and risk of ICH caused an overrepresentation of women in that analysis.

Regarding the studies included in this thesis, NSHDS participants in whom an insufficient amount of the original blood samples remained were excluded as biomarker analyses could not be performed. It can be hypothesized that a higher proportion of persons for whom much of the stored samples had already been used for analyses in other studies had developed other medical conditions of scientific interest. This could also introduce an element of selection bias.

On the whole, the study population of this thesis is regarded as a representative sample of the population in the studied age-group, but it is important to be aware of the risk of selection bias when interpreting study results.

External validity

The external validity of this thesis depends on several factors.

In papers II-V, there was an upper age limit of 74 years. In 2019, the mean age of individuals suffering an ICH was 74 years in Sweden (105). Consequently, a substantial proportion of persons with ICH in Sweden are older than 74 years and would not be eligible for inclusion in papers II–V. This negatively affects the generalizability of the results to ICH in older persons. This applies especially to women as women in general acquire ICH later in life (258). ICH is very uncommon in persons younger than 25 years (13), so the limited generalizability to this age group should not have large clinical implications.

Previous studies have demonstrated that incidence of ICH and distribution of ICH locations differ between ethnic groups (12, 259). In Västerbotten County, approximately 94% of the population is native to Sweden (256) and the majority of the inhabitants are of European ancestry. The generalizability to persons of other ethnicities is therefore limited.

According to sales statistics from the Swedish Retail Monopoly, the alcohol consumption in Västerbotten County is similar to that in Sweden as a whole. According to the World Health Organization, the average annual alcohol consumption in 2017 in Swedes aged 15 years and older was 7.0 L which is similar to other Nordic countries where alcohol consumption varies between 6.0 and 9.3 L per annum (260). The estimated percentage of the alcohol intake from different alcoholic beverages in 2016 was, according to the World Health Organization, 36% from beer, 48% from wine, 14% from liquor and 2% from other alcoholic beverages (260). This can be compared with that of the referents in our study group in whom alcohol intake consisted of 45% beer, 35% wine and

20% liquor. Our conclusion is that the intake of different alcoholic beverages in our study population was approximately similar to that of Sweden as a whole.

To conclude, our study results can be generalized to persons in the studied age groups in countries with populations similar to that of Västerbotten County.

Gender perspective

In the papers of this thesis, we did not study men and women separately due to insufficient study size. This could be a focus of future studies. The fact that ICH demography and subtypes vary between men and women could be one reason for future sex-stratified studies. Women who experience an ICH are on average older and more often have lobar ICHs (46). Because of these differences, the relative importance of some ICH risk factors may vary between men and women.

Studies stratified by sex would be relevant with respect to PEth and risk of ICH as it is well established that men and women have different drinking habits (261). This was also seen in our study where more men than women had PEth concentrations in the higher PEth categories. In current Swedish clinical practice, a PEth concentration > 0.3 μmol/L is used as a cut-off for heavy drinking (180). This cut-off is the same for men and women. In the future, sex-specific cut-offs for PEth concentrations could be introduced if it is shown that disease risk, for example risk of ICH, increases at different PEth concentrations for men and women.

Clinical implications

The biomarkers studied in this thesis are biomarkers that could be of use in the assessment of disease risk (Figure 3). As ICH is a severe disease with limited treatment options (262), it is reasonable to conclude that there is a clinical need for better ICH prevention. A biomarker that could help to define risk groups for ICH could be one component of a strategy for ICH prevention. As described in Figure 4, there are different stages of developing a biomarker for clinical use. Our studies fall into the second stage: “Identification of possible new biomarkers”. There are several additional stages that need to be completed before a biomarker can come to clinical use.

As we found no association between FXII, VWF, blood group O and risk of ICH, we do not think that these study results will lead to any changes in clinical practice. Our unexpected finding of an association between blood group B and lower risk of ICH compared to blood group A could be due to chance as multiple comparisons were made. If this finding was to be confirmed in future studies, it could have implications for risk stratification for ICH in a clinical setting.

We found an association between D-dimer and risk of ICH. D-dimer could possibly be a component of an ICH risk score in the future. The first step towards this would be to perform a reclassification study where D-dimer would be added to established ICH risk factors to see if this would result in an

improved prediction of ICH risk. If so, a risk score including D-dimer could be used to assess an individual's ICH risk in future prospective studies. In the long run, such a score could be introduced in clinical practice for individually tailored risk factor management and surveillance.

We found an association between PEth and risk of ICH. PEth concentration measurements could contribute to assessment of ICH risk in the future. Hypertension is an important risk factor for ICH (53) and has a high prevalence in the population (45). Our results indicate that high PEth concentrations and hypertension interact in the context of ICH risk. Therefore, it is likely that the clinical benefit of PEth measurement for ICH risk stratification would be most pronounced in individuals with concomitant hypertension. Current guidelines advocate that persons with hypertension should moderate their alcohol intake (263). Only a small proportion of persons with hypertension reach blood pressure targets (264). The knowledge that a person has an additional marker of increased ICH risk, such as elevated PEth concentrations could help to stress the importance of proper management of the patient's blood pressure for both the patient and the physician.

Future perspectives

When a person experiences an ICH event there are great costs to the individual and his or her family in terms of suffering, disability and mortality. Furthermore, there are great costs to the society in the form of hospital- and community-based care. Treatment options are limited. Consequently, it is important to continue research on risk markers for ICH.

I now plan to proceed with a study on 265 individuals with ICH. The study, investigating ICH events that occurred in 2010–2017, is a more contemporary analysis which better reflects current medical practices such as an increasing proportion of anticoagulant-associated ICH (4% of individuals with ICH had anticoagulant treatment during the study period of the papers of this thesis compared to 25% of all individuals with an ICH registered in Sweden in 2019) (105).

In constructing this thesis, we did not have access to information about ICH location and limited information on the probable cause of the ICH. We had access to data on anticoagulant treatment at ICH onset and we excluded all persons with brain tumors or severe blood disease at the ICH event. However, we did not have information on whether the ICH was deemed to be due to specific processes such as hypertension, amyloid angiopathy or systemic diseases. This means that we were unable to investigate differences in risk marker profiles between ICH subtypes. In the planned follow-up study, detailed information on the ICH event, radiological exams and treatment has been collected at the time of case validation by using data from the medical records and autopsy reports. This will mean that ICH events can be classified both by location and by probable cause. This will allow me to evaluate the association between risk markers and different ICH subtypes which could be valuable as

different ICH subtypes may be associated with different causal processes and thus have different risk markers.

Since the incidence of ICH increases with age and the impact of ICH on morbidity and mortality is high in the elderly, it is important to study ICH in persons aged 75 and up in addition to younger persons. The upper age limit in papers II–V may have resulted in an under-representation of persons with lobar ICH who are generally older than persons with other types of ICH (265). In the planned study, there will be no upper age limit regarding the included ICH cases.

The study population in the planned investigation will consist of individuals from VIP and MONICA cohorts as in papers II–V of this thesis. In recent years the VIP and MONICA questionnaires have been supplemented with questions about, for example, sleep apnea. It would also be interesting to study lifestyle factors, such as diet or physical activity, in the context of ICH risk and different ICH subtypes.

For the association between PEth and risk of ICH, the next step could be to validate the association between PEth concentrations and risk of ICH in a new study population. Another future study could be focused on changes in PEth values over time and risk of ICH. Such a study could investigate if there are differences in risk of ICH between persons with multiple elevated PEth measurements and persons with one or no elevated PEth measurements. As Västerbotten inhabitants are invited to the VIP at 40, 50 and 60 years of age more than 40% of VIP participants have participated at least twice.

High alcohol consumption seems to be associated with non-lobar ICHs but not with lobar ICHs (58) and it would therefore be relevant to study PEth in a population with data on ICH location. There are several different methods of measuring self-reported alcohol consumption and problem drinking. In the planned study, I will have access to data on alcohol use at the health examination measured both with the AUDIT and the CAGE questionnaires. It would be interesting to investigate the association between the results of these questionnaires and risk of ICH and compare them with the association between PEth concentrations and risk of ICH in the same population.

Conclusions

- In an exploratory study we found an association between high FXII concentrations and risk of hemorrhagic stroke, this finding could not be confirmed in a larger study where the outcome was a validated first-ever ICH event.

- High plasma concentrations of D-dimer are associated with increased risk of first-ever ICH. The association is most pronounced in the cases with the shortest time from blood sampling to ICH event.

- We found no association between VWF or blood group O and risk of first-ever ICH.

- High PEth concentrations are associated with an increased risk of first-ever ICH.

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Appendix

Corrections to paper I

	Published text	Corrected text
Results, page 90	Of these individuals, 20 had intracerebral bleeding and 10 had subarachnoid bleeding.	Of these individuals, **21** had intracerebral bleeding and 10 had subarachnoid bleeding. **One of the individuals with a subarachnoid hemorrhage later experienced an intracerebral bleeding during follow-up.**
Results, page 90	When analyzing intracerebral bleeding and subarachnoid bleeding separately, the adjusted HR for the association between FXII and intracerebral bleeding was 1.50 per SD of FXII (95% CI: 0.95–2.34), and for subarachnoid bleeding, the HR was 1.30 per SD of FXII (95% CI: 0.69–2.44).	When analyzing intracerebral bleeding and subarachnoid bleeding separately, the adjusted HR for the association between FXII and intracerebral bleeding was **1.83 per SD of FXII (95% CI: 1.15–2.90)** and for subarachnoid bleeding, the HR was 1.30 per SD of FXII (95% CI: 0.69–2.44).