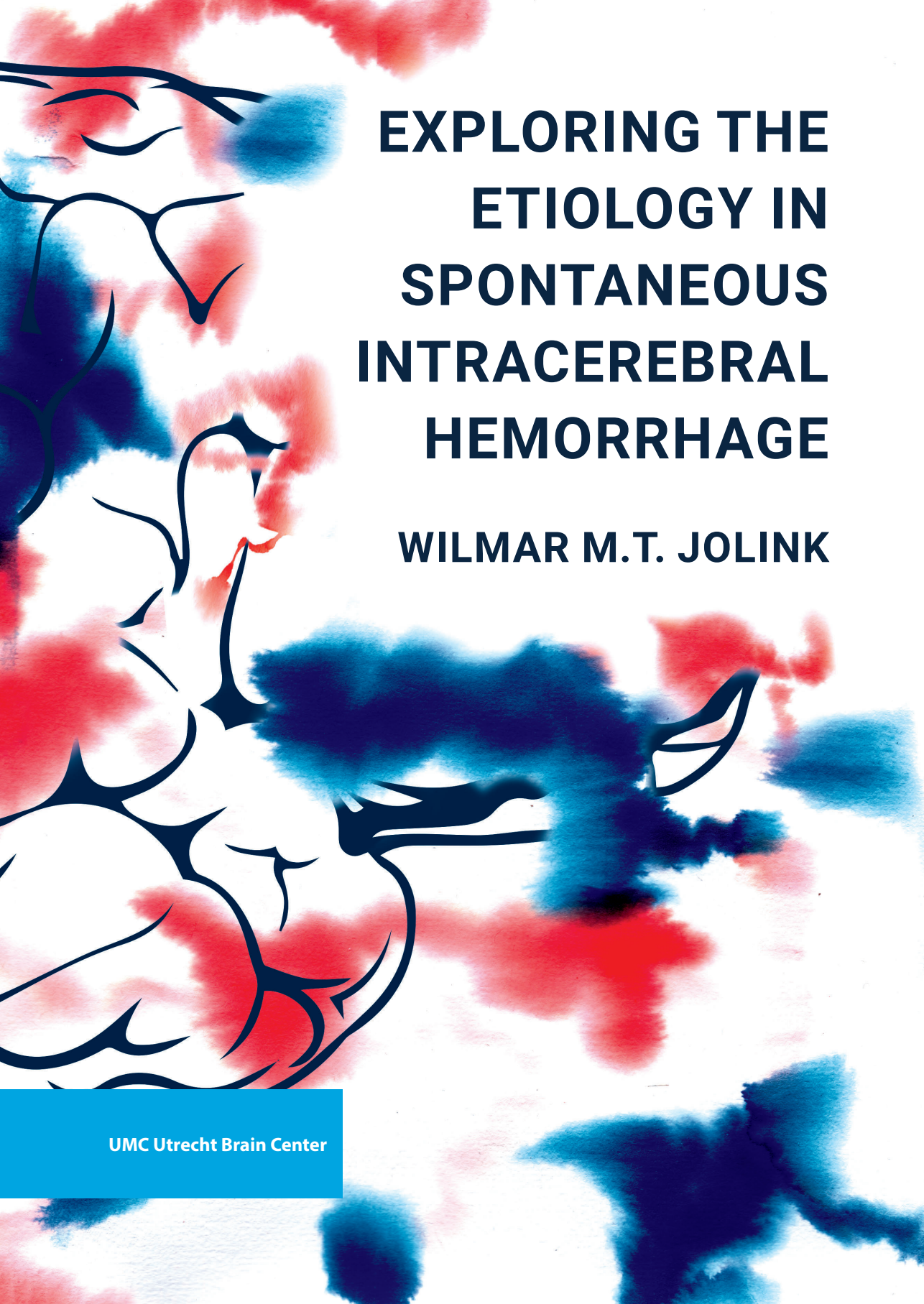

EXPLORING THE ETIOLOGY IN SPONTANEOUS INTRACEREBRAL HEMORRHAGE

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COLOFON

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Exploring the etiology in spontaneous intracerebral hemorrhage

Een zoektocht naar de oorzaken van spontane intracerebrale bloedingen

(met een samenvatting in het Nederlands)

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door

Wilhelmus Martinus Tim Jolink

geboren op 1 februari 1988
te Gouda

Promotoren:
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Prof. dr. C.J.M. Klijn

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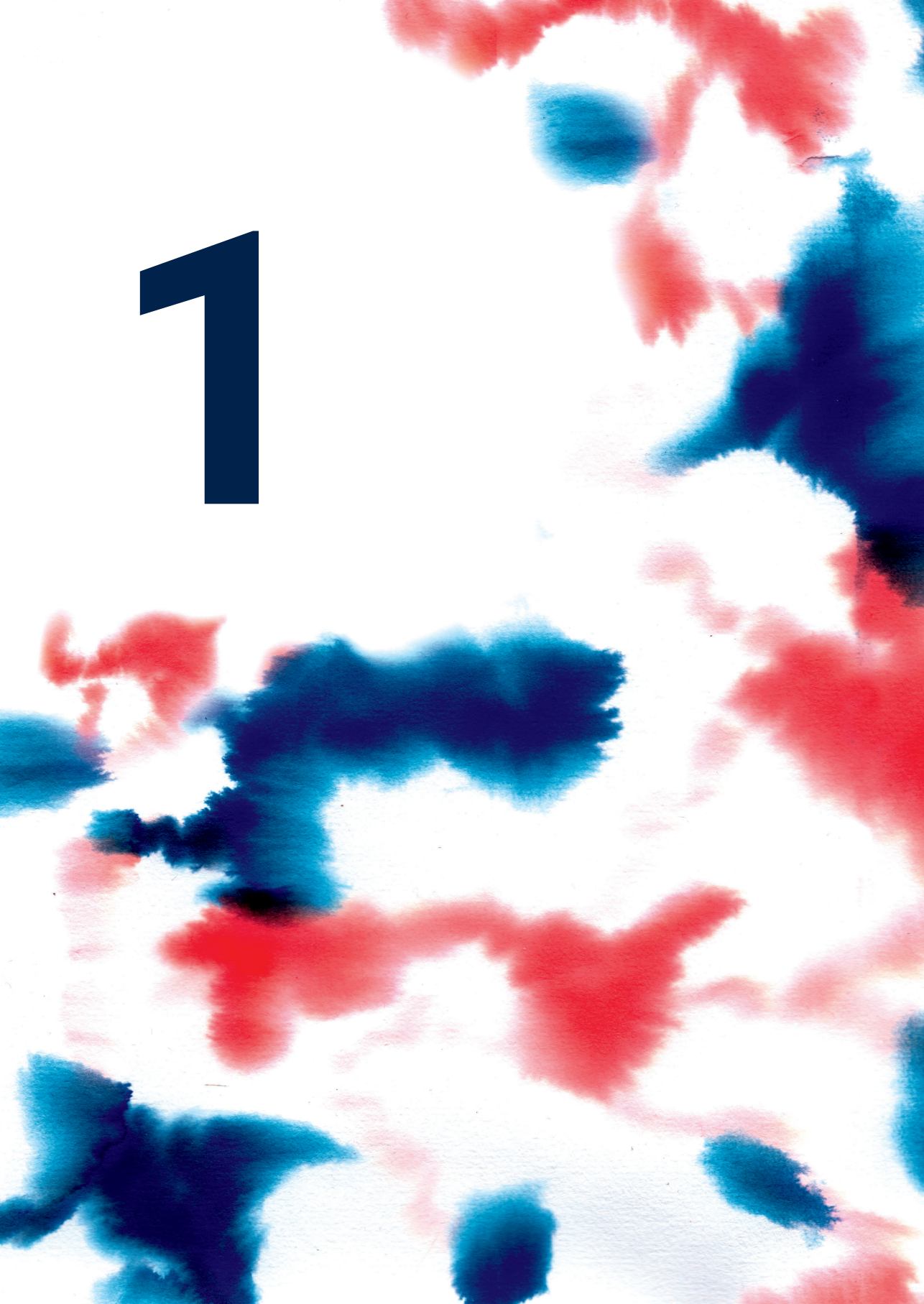

1

GENERAL INTRODUCTION

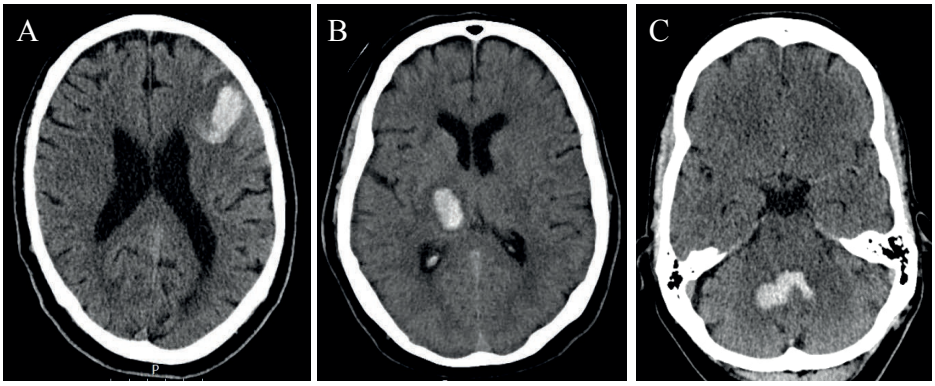
GENERAL INTRODUCTION

Intracerebral hemorrhage

Non-traumatic intracerebral hemorrhage (ICH) is caused by rupture of a small artery or arteriole into the brain parenchyma. This may result from a variety of causes.[1,2] ICH can be classified as either spontaneous (in the literature often named primary) or ICH from easily identifiable causes (often referred to as secondary). Such causes include a vascular malformation from larger vessels such as an arteriovenous malformation, a dural arteriovenous fistula or aneurysm, a cavernous malformation, an intracranial venous thrombosis, a hemorrhagic transformation of cerebral infarction, a severe clotting deficiency (e.g. hemophilia), a tumor, vasculitis, an infective endocarditis or a posterior reversible encephalopathy syndrome.[3] Secondary ICH will not be discussed in this thesis. Spontaneous ICH without causes as mentioned above, further referred to as ICH, compromises between 80 to 90% of cases with ICH.[2] ICH is often considered one entity that should be diagnosed and treated similarly, whereas in fact many different etiologies may underlie ICH,[1] which may require different treatments and different strategies for secondary prevention.

Classically, deep (i.e. in the basal ganglia or thalamus, figure 1.1) ICH is associated with hypertension, which causes damage to the small perforating arteries ultimately resulting in a hematoma. In lobar (i.e. cortical areas of the frontal, temporal, parietal or occipital lobe, figure 1.1) ICH in elderly patients cerebral amyloid angiopathy (CAA) plays an important role. CAA is caused by the accumulation of the protein amyloid β in the walls of cortical and leptomeningeal vessels, which disrupts the structure of the vessel walls, leading to cerebral microbleeds and cortical superficial siderosis, besides the lobar macrobleed.[4] The modified Boston criteria are used as diagnostic criteria to identify patients with probable CAA.[5] The diagnosis can be made in patients of 55 years and older with multiple (macro- or micro)bleeds restricted to lobar regions with or without cortical superficial siderosis (cSS). The diagnosis definite CAA can only be made after post-mortem examination.[5] The subdivision of ICH in deep and lobar ICH however, is an oversimplification, because not all patients with deep ICH have hypertension and in only one-third of elderly patients with lobar ICH probable CAA is diagnosed.[6] Moreover, infratentorial ICH (i.e. brainstem or cerebellum, figure 1.1) and lobar ICH in younger patients (<50 years) are not covered by this dichotomy.[7]

Figure 1.1 A. Left frontal ICH in a 77 years old man; B. Right deep ICH in a 63 years old man; C. Right cerebellar ICH in a 61 years old man



Worldwide ICH affects more than three million people each year.[8,9] In 2018, around 6.000 persons were admitted to a hospital in the Netherlands because of ICH.[10] ICH accounts for approximately 25% of all strokes, with proportions ranging from 19% in high income countries to 28% in low- to middle-income countries.[9] Around 40% of ICH patients die within the first month of onset.[11] Survivors have differing degrees of residual disability with a high risk of vascular events, including recurrent ICH, and other complications such as epilepsy and dementia.[3] In the last decades, incidence, case fatality and functional outcome of ICH did not change over time.[11] However, changes over time might vary in different subgroups, for example due to sex differences and in different age groups.

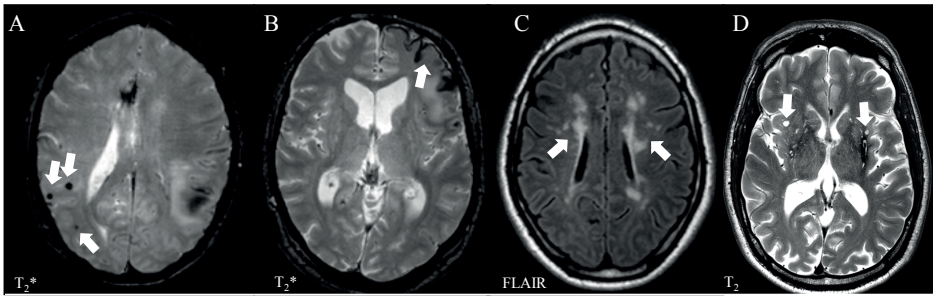
Identifying risk factors for ICH might help in understanding the etiology of subgroups of ICH. In a review published in 2003 the modifiable risk factors hypertension and high alcohol intake were identified, as well as the non-modifiable risk factors higher age and male sex.[12] As pathogenesis of ICH may vary according to location of the ICH, risk factor profiles may differ in patients with non-lobar (deep or infratentorial [i.e. brainstem or cerebellum]) and lobar ICH.[13,14]

Cerebral small vessel disease

Advances in imaging and histopathologic assessment of imaging biomarkers have shown that ICH is predominantly caused by pathological changes of arterioles, capillaries and venules, in other words: cerebral small vessel disease (SVD).[1] Neuro-imaging markers of SVD might provide insight in the disease processes underlying ICH. Differences in patterns of presence, severity and distribution of SVD markers might indicate distinct etiologies of ICH. CAA is associated with strictly lobar cerebral microbleeds (CMBs),[4] cSS,[15] multiple subcortical spots of white matter hyperintensities (WMH),[16] and enlarged perivascular spaces (EPVS) in the centrum semiovale,[17]

whereas predominantly deep CMBs,[4] peri-basal ganglia WMH[16] and EPVS more pronounced in the basal ganglia[17] are more often found in patients with ICH associated with vascular risk factors (figure 1.2). Definitions of the neuro-imaging SVD markers have been described in the STandards for Reporting Vascular changes on nEuroimaging (STRIVE) criteria.[18]

Figure 1.2. Examples of MRI markers of SVD in ICH on 3 tesla MRI. Cerebral microbleeds [A], cortical superficial siderosis [B], white matter hyperintensities [C] and enlarged perivascular spaces [D] indicated by the white arrows



Cerebral microinfarcts (CMIs) may be an additional marker of SVD and have been described in cerebrovascular disease and dementia in autopsy studies.[19] The arrival of ultra-high-field (7 tesla [T]) MRI allows detection of CMIs in vivo.[20] Up till now, the meaning of CMIs in spontaneous ICH and its underlying SVD remains unclear.

Finally, disruption of the blood-brain barrier (BBB) might play a role in SVD-related ICH.[21–23] The BBB is the unique boundary of microvasculature between the blood and the central nervous system, consisting of endothelial cells connected by tight-junctions. The BBB restricts the transport of toxins, maintains ion homeostasis, and controls the immune system to protect the neuronal environment.[21,24] Derangement of the BBB results in damage to the wall of small vessels.[24] Leakage of gadolinium contrast on MRI can be used as a marker of BBB disruption. It has been described in the acute phase after ICH, but it is unclear if this is a cause or consequence of the ICH.[25]

Aim and outline of this thesis

The key aim of this thesis is to explore the etiology in spontaneous ICH by identifying time trends for and risk factors in different subgroups of ICH and assessing presence, severity and distribution of CMIs and BBB disruption on MRI, as novel markers of SVD in patients with ICH.

In **part I**, we assess the epidemiology and risk factors of ICH. Chapter 2 shows the time trends of ICH in the Netherlands. We describe changes in incidence, 30-day case fatality and 1-year

case fatality between 1998 and 2010 and mortality between 1980 and 2010, stratified by age-groups and sex. In chapter 3 we describe the results of a systematic review and meta-analysis of studies reporting on risk factors according to lobar or non-lobar location of the ICH.

In **part II**, we investigate the importance of novel markers of small vessel disease in understanding the etiology of ICH. In chapter 4 we describe the presence of CMIs on 7 T MRI in a small case series of patients with ICH. Chapter 5 addresses the role of CMIs and CMBs in ICH with the combination of post-mortem 7 T MRI and histopathological examination. In chapter 6 we study CMIs on 7 T MRI in a cohort study of lobar and non-lobar ICH patients and assess associations with SVD severity. Chapter 7 describes contrast leakage on 7 T MRI as a marker of BBB disruption.

In chapter 8 we summarize the most important results of the research presented in this thesis and we discuss implications for clinical practice, remaining challenges and future directions.

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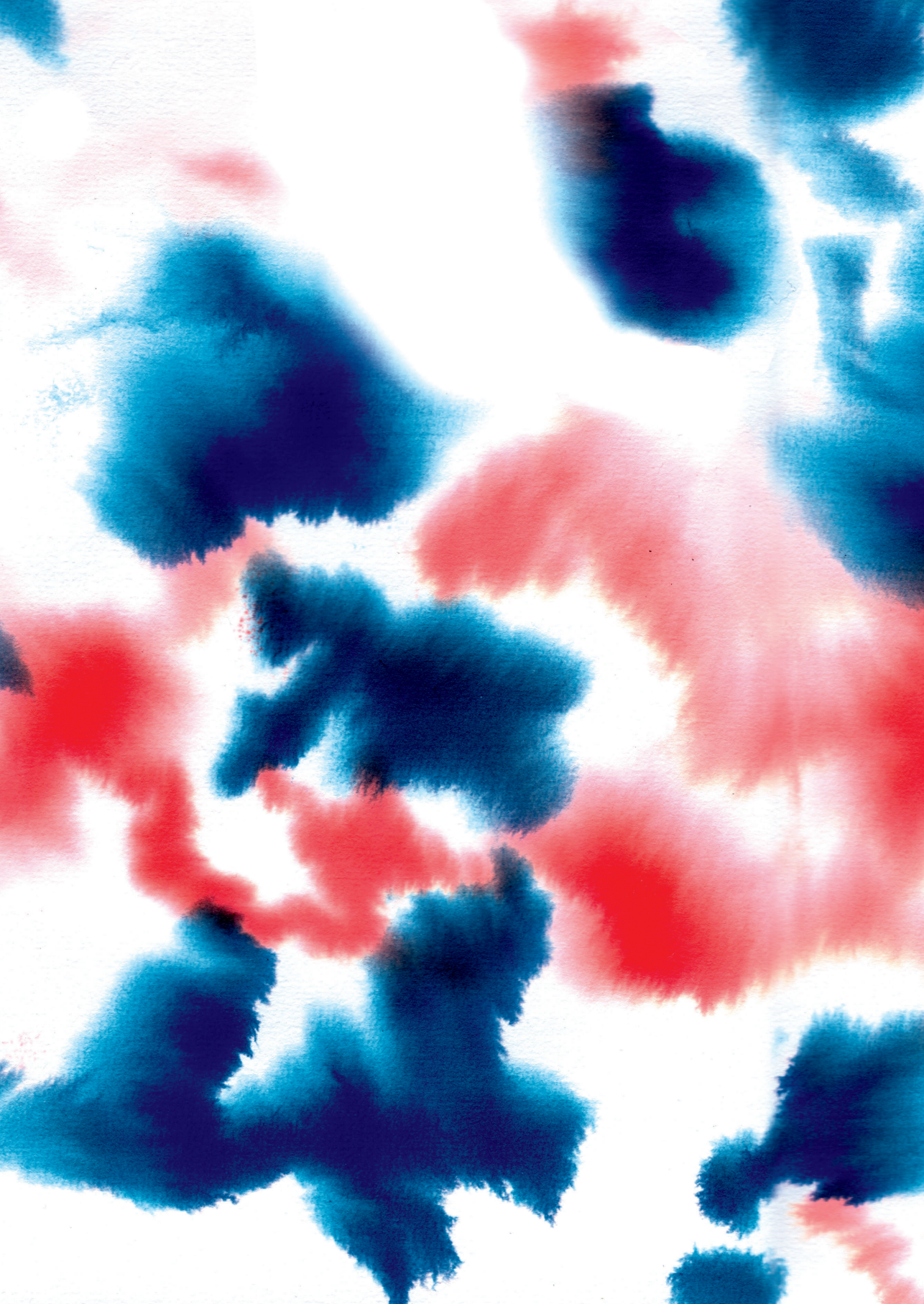
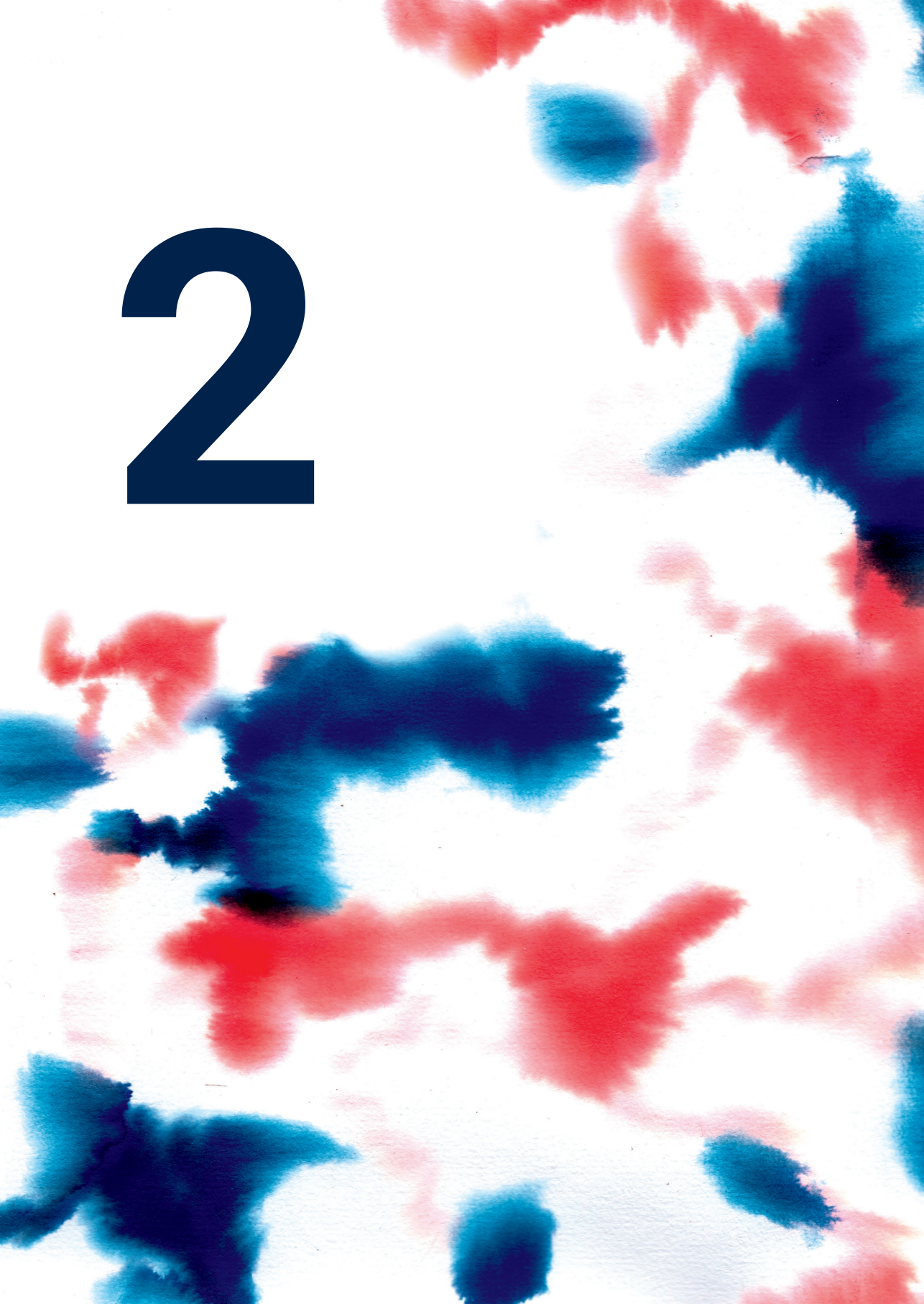


Part I

EPIDEMIOLOGY AND RISK FACTORS OF INTRACEREBRAL HEMORRHAGE


2

TIME TRENDS IN INCIDENCE, CASE FATALITY AND MORTALITY OF INTRACEREBRAL HEMORRHAGE

Wilmar M.T. Jolink, Catharina J.M. Klijn, Paul J.A.M. Brouwers, L. Jaap Kappelle, Ilonca Vaartjes

Neurology 2015; 85(15):1318-1324

ABSTRACT

Objective

To assess age- and sex-specific trends in incidence, 30-day and 1-year case fatality of intracerebral hemorrhage (ICH) in the Netherlands.

Methods

Patients hospitalized for first ICH were identified through linkage of the national hospital discharge register and population register using ICD-9 code 431. We identified out-of-hospital deaths in the national cause of death register. Incidence, 30-day and 1-year case fatality and total mortality rate were calculated by age and sex. We identified time trends by joinpoint regression analysis and Mann-Kendall tests.

Results

We identified 41,068 ICH cases (51% men) between 1998 and 2010, of which 6% out-of-hospital deaths. ICH incidence declined in men and women younger than 75 years ($p \leq 0.01$, not significantly in men 35-54), but remained stable in patients 75 years and older. Thirty-day and 1-year case fatality declined in patients younger than 75 years (not significantly in women 35-54). In patients 35 to 54 years ICH mortality remained stable until 2003 and then declined slightly (annual percentage change [APC] men -7.09%; 95% confidence interval [CI] -11.39 to -2.59; women -8.67%; 95% CI -15.18 to -1.66). In patients 55 to 74 years mortality declined in men between 1995 and 2010 (APC -4.55%; 95% CI -5.49 to -3.59) and in women between 1992 and 2010 (APC -3.51%; 95% CI -4.16 to -2.85). Mortality did not decline in patients 75 years and older.

Conclusion

In The Netherlands, ICH incidence, case fatality and mortality rates have declined significantly in men and women younger than 75 but remained stable in patients 75 years and older. The observed time trends may be explained by better prevention and treatment during the previous two decades of which the elderly do not seem to benefit.

INTRODUCTION

Intracerebral hemorrhage (ICH) is the most lethal subtype of stroke affecting more than two million people worldwide each year.[1] According to our meta-analysis of 36 population-based studies ICH incidence has not decreased between 1980 and 2008 with an overall incidence of 24.6 per 100,000 person-years.[2] Overall stable incidence of ICH may mask variation in time trends in incidence according to sex, age, deep or lobar ICH location and use of antithrombotic drugs. Time trends in case fatality may also vary according to different subgroups.[3-6] In the meta-analysis, 1-month case fatality was 40.4% and case fatality did not decrease over time,[2] but time trends were not studied separately in men and women, or in different age groups.

The aim of the current study was to assess age- and sex-specific trends in ICH incidence, 30-day and 1-year case fatality from 1998 to 2010, and total ICH mortality rates from 1980 to 2010 in The Netherlands.

METHODS

Cohort

ICH incidence, 30-day and 1-year case fatality. We defined ICH incidence as all patients with first hospitalization for ICH and patients who died from first ICH before hospitalization. We constructed a nationwide cohort of all patients admitted with first-ever ICH between 1998 and 2010 through linkage of the national hospital discharge register (HDR) and the Dutch Population register (PR) using International Classification of Diseases (ICD) version 9 code 431. The ICD-9 definition is as follows: hemorrhage (of): basilar, bulbar, cerebellar, cerebral, cerebromeningeal, cortical, internal capsule, intrapontine, pontine, subcortical, ventricular, rupture of blood vessel in brain including those caused by an arteriovenous or cavernous malformation and tumor- and aneurysm associated intraparenchymal hemorrhage, excluding traumatic hemorrhage. We linked this cohort with the cause of death register (CDR) of Statistics Netherlands to identify out-of-hospital deaths using ICD-10 code I61. Registries and linkage procedures have been described in detail previously.[7-9] Briefly, we selected all hospital admissions with a principal diagnosis of ICH (ICD-9 code 431) between January 1998 and December 2010 from the HDR. We merged this with the PR, using a combination of partially identifying variables (date of birth, sex, numeric part of the postal code) to identify different admissions from the same person. Approximately 85% of the Dutch population has a unique combination of these variables.[10] Between 1998 and 2010 we selected first admissions for individuals from all subsequent admissions of a person in the year of admission. Next we excluded persons with admission

for ICH three years prior to the admission. This resulted in a cohort of 41,068 patients aged 35 to 94 years with first-ever ICH between 1998 and 2010.

We assessed the accuracy of coding of ICH diagnosis, selecting a sample of all patients with a main diagnosis of ICH (ICD-9 code 431) from the hospital discharge registry of a university medical center and a large teaching nonuniversity hospital from January 1995 to December 2010. Medical records of 1,052 patients were checked against HDR files for the correct diagnosis. In 91% the diagnosis ICH had been correct (table 2.e-1).

From 2005 the number of participating hospitals in the HDR declined, resulting in an increasing number of missing records. From all hospital admissions in 2004, 1.1% of the records were missing. Between 2005 and 2010 the percentage of missing records varied between 3.3% and 14%.

Total mortality rates. Statistics Netherlands provided data on all deaths from first and recurrent ICH using ICD-9 code 431 for the period 1980 to 1995 and ICD-10 code I61 for the period 1996 to 2010 by year, sex, and age at time of death as well as accompanying population estimates.

Standard Protocol Approvals, Registrations, and Patient Consents

We performed all analyses according to Dutch privacy legislation and no patients were directly involved. The UMC Utrecht Medical Ethics Review Committee approved the study.

Data analysis

ICH incidence. Incidence per 100,000 persons was calculated by age and sex. We performed Mann-Kendall trend tests by age-sex group to investigate whether incidence changed between 1998 and 2010. In addition, we calculated the change in incidence rate over the period as (incidence rate 2010 – incidence rate 1998)/incidence rate 1998 for every age-sex group. We used the age groups 35-54, 55-74 and 75-94 years based on the sample sizes in order to have large enough groups for analysis.

The total number of patients with first ICH (hospital admission and out-of-hospital deaths) in this study is provided for those with a unique combination of date of birth, sex and the numeric part of the postal code (ranging from 88% [persons younger than 35 years] to 98% [persons older than 75 years]). We assumed that the risk of ICH does not depend on whether a unique combination of linkage variables is present or not. We estimated the absolute number of first ICH events in the total Dutch population using age- and sex-specific uniquity percentages as the adjusting factor.

Thirty-day and 1-year case fatality. We calculated survival time as time from initial admission date for ICH to the date of death from any cause. Thirty-day case fatality and 1-year case fatality (31-365 days) were calculated by age and sex according to the actuarial life-table method and expressed as percentage. We used the Mann-Kendall test to investigate whether 30-day case fatality and 1-year case fatality changed significantly between 1998 and 2010. In addition we computed changes in observed 30-day and 1-year case fatality for the periods between 1998 and 2010.

Total ICH mortality rate. We assessed age- and sex-specific trends in mortality between 1980 and 2010 by joinpoint regression analysis with software developed by the Surveillance Research Program for the US National Cancer Institute (joinpoint version 4.0.4).

This analysis allows identification of best-fitting points (joinpoints) when a significant change in the linear slope of the trend (on a log scale) is detected over the study period.[11] We used a Bayesian information criterion (BIC) approach to select the most parsimonious model that best fitted the data. The value of BIC is the log-likelihood value with penalizing the cost of extra parameters. The model with the minimum value of BIC is selected as the optimal model. In the model the dependent is rate and the independent year. We used a maximum of 3 joinpoints. The linear slope of the trend and probability value of the final model of the joinpoint regression analysis was calculated for every period, as well as annual percentages change (APC) with 95% confidence interval (CI) and minimum and maximum observed number of deaths per 100,000. The minimum observed number per period is determined by the year with the lowest mortality and the maximum by the year with highest mortality.

In addition, we calculated the change in observed ICH mortality rates per 100,000 per time period ([ICH mortality rate last year of period – ICH mortality rate first year of period]/ICH mortality rate first year of period). We graphically depicted mortality rates by age and sex using five-year averages for smoothing.

To interpret time trends in mortality in relation to incidence, and 30-day and 1-year case fatality, we calculated changes in mortality between 1998 and 2010.

RESULTS

Trends in ICH incidence: 1998-2010.

Between 1998 and 2010, we identified 41,068 new ICH cases (51% men). Six percent were out-of-hospital deaths. The estimated absolute number of patients with a first ICH in the Dutch

population was 3,180 in 1998 and 3,424 in 2010. The overall crude incidence (men and women, all age groups) was 20.3/100,000 persons in 1998 and 20.7/100,000 in 2010. Age distributions of the Dutch population in men and women can be found in figure 2.e-1.

Incidence remained stable in men 35-54 years from 6.1/100,000 to 5.9/100,000 (p=0.22), and declined in men 55-74 years from 53.3/100,000 to 37.2/100,000 (p<0.01), in women 35-54 years from 6.3/100,000 to 5.1/100,000 (p<0.01), and in women 55-74 years from 36.1/100,000 to 26.4/100,000 (p<0.01) (tables 2.1 and 2.2). In the age group 75-94 years incidence of ICH did not change in men and women. In patients 35-54 years, ICH incidence was similar in men and women, whereas ICH incidence was significantly higher in men 55-74 years than in women.

Trends in ICH 30-day and 1-year case fatality, and mortality: 1998-2010.

Thirty-day case fatality declined over time in men 35-54 years from 28.0% to 12.0% (p<0.01) and in the age group 55-74 years from 32.7% to 21.0% (p<0.01). In women 35-54 years it declined from 18.8% to 14.0% (p=0.39) and in women 55-74 years from 29.3% to 23.6% (p<0.01). In the age groups 75-94 years 30-day case fatality remained stable around 40% for men and women.

One-year case fatality declined in men 35-54 years from 33.6% to 15.0% (p<0.01) and in men 55-74 years from 40.2% to 28.2% (p=0.02). In women 35-54 years, 1-year case fatality declined from 24.6% to 18.2% (p=0.20) and in women 55-74 years from 36.0% to 27.7% (p<0.01). In patients 75-94 years 1-year case fatality remained stable and varied between 55.4% and 46.4% in men and between 54.4% and 45.5% in women (tables 2.1 and 2.2).

Between 1998 and 2010 the mortality rate per 100,000 persons declined in the age groups 35-54 years by 42% in men (table 2.1) and by 63% in women (table 2.2). In the age group 55-74 years, mortality rate decreased by 40% in men and women. No changes in mortality rates were observed in men and women 75-94 years of age (tables 2.1 and 2.2).

Trends in age- and sex-specific total ICH mortality between 1980 and 2010.

ICH mortality rate per 100,000 persons declined from 3.2 to 1.8 (43%) in men and from 2.5 to 1.1 (55%) in women 35-54 years. In this age group ICH mortality remained stable until 2003 and then declined slightly (APC men -7.09%, 95% CI -11.39 to -2.59; women -8.67%, 95% CI -15.18 to -1.66),figure 2.1, supplementary tables 2.e-2 and 2.e-3). In the age group 55-74 years mortality rate declined from 28.4 to 14.8 (48%) in men, and from 20.5 to 11.1 (46%) in women, with the largest decline in men between 1995 and 2010 (APC=-4.55%, 95% CI -5.49 to -3.59; supplementary table 2.e-2) and in women between 1992 and 2010 (APC=-3.51, 95% CI -4.16 to -2.85; supplementary table 2.e-3). We observed no trend in mortality rate over time in patients 75-94 years (figure 2.1, supplementary tables 2.e-2 and 2.e-3).

Table 2.1 Intracerebral hemorrhage incidence, case fatality and mortality for men between 1998 and 2010 in the Netherlands

Age group		1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Change[a] 1998-2010	Trend[b]
35-54	Incidence per 100,000	6.1	6.9	7.5	7.0	7.7	7.3	8.3	7.5	6.6	6.4	6.1	6.6	5.9	-0.03	0.22
	30-day case fatality (%)	28.0	25.3	22.9	26.1	26.3	23.6	20.4	20.1	20.1	15.0	19.0	18.4	12.1	-0.57	<0.01
	1-year case fatality (%)	33.6	29.7	27.9	28.5	32.8	28.2	23.5	25.7	22.0	21.8	23.8	21.5	15.0	-0.55	<0.01
	Mortality rate per 100,000	3.1	2.8	2.6	2.9	3.3	3.2	3.3	2.5	2.2	2.3	2.0	2.4	1.8	-0.42	0.02
55-74	Incidence per 100,000	53.3	52.8	55.9	52.5	58.6	50.8	53.6	46.5	41.4	41.3	39.2	40.2	37.2	-0.30	<0.01
	30-day case fatality (%)	32.7	30.4	26.9	32.4	31.5	28.4	24.0	24.8	23.9	24.8	24.8	22.6	21.0	-0.36	<0.01
	1-year case fatality (%)	40.2	39.8	35.3	39.4	38.9	36.8	31.1	32.4	32.2	32.2	33.5	32.3	28.2	-0.30	0.02
	Mortality rate per 100,000	24.6	24.7	23.3	26.1	25.6	24.0	22.4	19.4	17.6	18.1	19.3	14.5	14.8	-0.40	<0.01
75-94	Incidence per 100,000	179.5	193.2	206.3	198.8	212.2	193.9	220.3	219.6	197.3	185.3	169.4	173.1	176.3	-0.02	0.30
	30-day case fatality (%)	39.8	40.3	42.2	44.7	41.2	38.1	42.6	42.6	37.9	39.6	38.6	42.8	38.9	-0.02	0.71
	1-year case fatality (%)	55.4	53.9	55.1	59.6	55.9	53.9	54.8	56.0	52.5	54.8	51.4	58.1	46.4	-0.16	0.25
	Mortality rate per 100,000	105.2	106.7	113.5	100.2	112.4	115.6	133.5	121.5	118.8	110.0	94.2	111.1	108.5	0.03	0.86

Incidence per 100,000, 30-day case fatality, 1- year case fatality (31-365 days) after admission for intracerebral hemorrhage and mortality rate per 100,000 by age in the Netherlands, 1998-2010 for men aged >35 years

[a] Change = (last year-first year)/first year

[b] Mann-Kendall test, p-values

Table 2.2 Intracerebral hemorrhage incidence, case fatality and mortality for women between 1998 and 2010 in the Netherlands

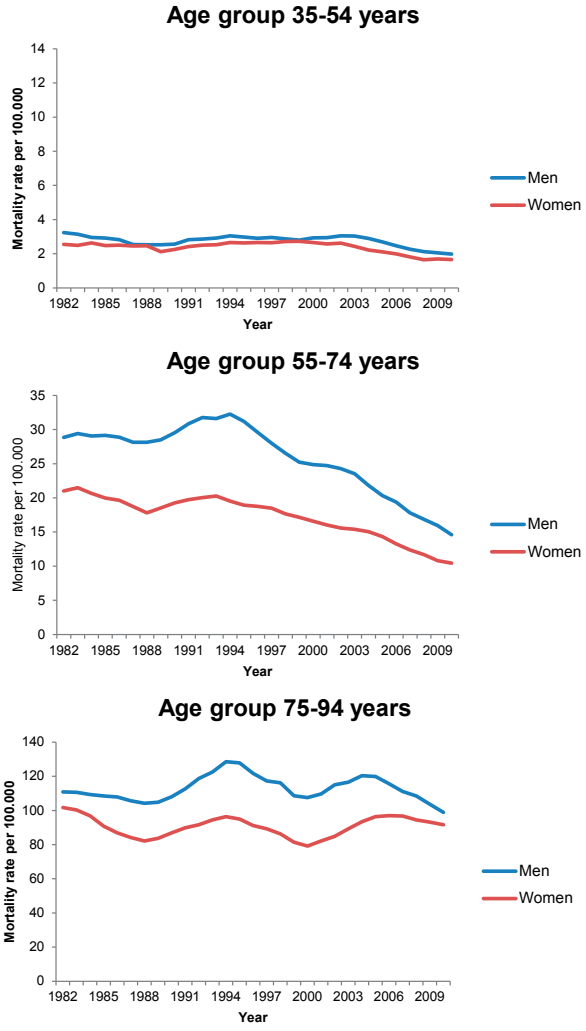
Age group		1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Change[a] 1998-2010	Trend[b]
35-54	Incidence per 100,000	6.3	6.2	6.6	6.6	6.0	6.4	6.4	6.4	5.6	5.2	5.3	5.1	5.1	-0.20	<0.01
	30-day case fatality (%)	18.8	22.8	26.2	21.0	18.7	21.1	22.0	23.7	18.8	19.2	22.7	19.0	14.0	-0.26	0.39
	1-year case fatality (%)	24.6	23.5	31.0	24.2	20.9	24.5	27.3	27.6	21.8	25.8	23.4	23.1	18.2	-0.26	0.20
	Mortality rate per 100,000	3.0	2.3	2.8	2.7	2.5	2.6	2.5	1.9	1.6	1.9	2.0	1.6	1.1	-0.63	<0.01
55-74	Incidence per 100,000	36.1	35.4	35.1	35.0	32.8	32.6	33.3	30.6	25.9	27.1	28.4	27.0	26.4	-0.27	<0.01
	30-day case fatality (%)	29.3	31.1	27.9	27.5	33.5	29.2	26.8	28.9	27.7	22.1	23.8	24.8	23.6	-0.19	<0.01
	1-year case fatality (%)	36.0	38.9	34.4	33.8	40.1	35.9	33.5	38.2	34.4	29.3	31.9	31.7	27.7	-0.23	<0.01
	Mortality rate per 100,000	18.5	18.1	15.3	15.4	15.5	15.8	15.8	14.5	13.5	12.0	10.3	11.5	11.1	-0.40	<0.01
75-94	Incidence per 100,000	136.3	134.8	130.3	136.3	149.6	151.2	152.2	159.0	147.9	150.3	141.6	145.0	140.1	0.03	0.33
	30-day case fatality (%)	38.4	42.8	39.7	39.4	38.4	41.3	38.8	41.5	37.3	38.5	39.4	38.6	39.1	0.02	0.58
	1-year case fatality (%)	54.4	57.1	53.3	53.4	52.4	55.4	53.3	53.6	49.8	51.1	52.6	52.3	45.5	-0.16	0.01
	Mortality rate per 100,000	79.7	81.6	80.4	75.8	78.3	94.6	95.3	102.1	96.5	93.4	97.8	93.9	90.6	0.14	0.10

Incidence per 100,000, 30-day case fatality, 1-year case fatality (31-365 days) after admission for intracerebral hemorrhage and mortality rate per 100,000 by age in the Netherlands, 1998-2010 for women aged >35 years

[a] Change = (last year-first year)/first year

[b] Mann-Kendall test, p-values

Figure 2.1 Five-year smoothed intracerebral hemorrhage mortality rate per 100,000 persons in men and women according to age group from 1982 to 2010



DISCUSSION

Between 1998 and 2010 ICH incidence in The Netherlands declined in men and women younger than 75 years, but not in patients 75 years and older. ICH mortality, 30-day case fatality and 1-year case fatality also declined in men and in women younger than 75 years. The most prominent decline in mortality was observed in patients 55-74 years, with a steeper decline in men than in women.

Possible explanations for the observed decline in total ICH mortality rates may be better primary prevention as reflected in a decreased incidence,[12, 13] better treatment in the acute phase as reflected in decreased 30-day case fatality,[14] or better secondary prevention as reflected in decreased 1-year case fatality.[15, 16] The benefit from organized stroke unit care for patients with ICH has been shown to be similar to that in patients with ischemic stroke with a decline in death and dependency (risk ratio 0.79, 95%CI 0.61-1.00).[14]

The decrease in 1-year case fatality may have been, at least in part, achieved by the introduction of updated guidelines concerning prevention in 2000.[15] This may have led to increased secondary preventive actions after stroke, such as control of blood pressure, as also observed in the PRoFESS (Prevention Regimen for Effectively Avoiding Second Strokes) trial.[16] In a previous study we observed that incidence of some risk factors declined (e.g. hypercholesterolemia and smoking) and incidence of other remained stable or increased (e.g. high blood pressure, BMI, diabetes) over time.[17] In patients 75 years and older, we observed no decrease in incidence and mortality, which may be the result of increasing use of antithrombotic treatments in this age group.[18] Another explanation could be that the impact of management of vascular risk factors mainly benefits patients in their 40s or 50s. Finally, the effect of vascular risk factor management may become less apparent in the elderly as a larger proportion of ICH is caused by cerebral amyloid angiopathy.[19]

The observed decline in total ICH mortality rates in patients younger than 75 years is in line with data from The Health Improvement Network in the United Kingdom.[20] In contrast to our results, the UK study found a decrease in mortality also in the older age group.[20] The UK study was based on data recording by general practitioners whereas our study was based on hospital admissions.

The overall crude incidence of 20.7/100,000 persons that we observed is within the range of the incidence of 24.5 (95% CI 19.7-30.7) reported in our recent meta-analysis of 36 population-based cohorts.[2] Also the higher incidence in men aged 55 years and older in comparison with women is in line with earlier studies.[19, 21, 22] In the meta-analysis no change in incidence was observed between 1983 and 2006, but time trends were not specified for different age groups.[2] Because the incidence is highest in the higher age group and incidence in this age group does not appear to change over time, time trends in patients younger than 75 years may have been missed in the meta-analysis. Indeed, age-specific data from the UK and France show a decrease in incidence of ICH in patients younger than 75 years for the period 1981 to 2006 in France and 1985 to 2008 in the UK.[19, 23] Similar to our results, a stable incidence over time in patients 75 years and older was observed in the United Kingdom,[23] whereas an increased incidence was found in this age group in Dijon.[19] In a recent study from South Texas the incidence of ICH declined in patients

60 to 74 years and in those 75 years and older, but not in the age group 45 to 59 years.[24] The authors suggested that the absence of a decline in incidence in patients younger than 60 may be attributable to limited awareness of the importance of controlling vascular risk factors such as hypertension. The South Texas population comprises 60% Mexican Americans and in this ethnic group ICH incidence is known to be higher than in non-Hispanic white populations.[25]

In contrast with the stable overall 30-day case fatality of around 40% between 1983 and 2006 reported in our meta-analysis,[2] we now observed an age- and sex-specific decline in 30-day and 1-year case fatality in patients younger than 75 years over the last 15 years, both in men and women. This was also observed in the UK study with an overall decline in 30-day case fatality from 53.1% in the time-period 2000-2001 to 35.8% in 2006-2008.[20] In the South Texas study case fatality did not decline but was not assessed for specific age groups.[24] The introduction of stroke units in the late 1990s may well have contributed to this decline.

The higher total mortality rate in men older than 54 years can partly be explained by both a higher incidence and slightly higher 30-day and 1-year case fatality. Other factors (e.g. risk factors, comorbidity, medication use) that could potentially explain this sex differences are not available in our own data. The current literature also lacks explanations regarding sex differences.[26, 27]

Strengths of our study are that we were able to obtain data from reliable registries with demonstrated high quality linkage.[7, 28, 29] Furthermore, our validation study showed correct coding of ICH in >91% of patients. Another strength of this registry study with nationwide coverage is that it has a population-based nature, large sample size, and broad range of age groups for both sexes. This is in contrast with hospital-based cohort studies that often include patients that are younger and healthier than average patients seen in daily practice and therefore report lower mortality rates.[30] Finally, we performed mortality trend analysis over a large time period (30 years) with joinpoint regression analysis, which identifies time periods objectively, avoiding potentially biased time trend analyses with prespecified periods.

Our study also has limitations. First, we could not correlate the change in incidence with a possible change in ICH according to location. Second, data on antithrombotic medication was not available. Third, nursing home data was not available for patients surviving their first ICH without being admitted to the hospital. This may have led to some underestimation of ICH incidence especially among the eldest. However, these numbers are small and it is unlikely that this has influenced the observed trends over time. Fourth, we had no access to information of previous admissions before 1995 and could only identify recurrent events in the previous three years. This might have resulted in labelling recurrent events as first-time events but affects only the absolute numbers, not the trends over time. Fifth, because incidence and mortality

estimates differ across ethnic groups our findings may not apply to other ethnic groups. On January 1st 2010, 80% of the Dutch population was ethnic Dutch with the remaining 20% mainly consisting of people from Turkey, Indonesia, Morocco, Suriname and the Netherlands Antilles.[31] Sixth, between 2005 and 2010 the percentage of missing records because of a decline in the hospitals participating in the HDR varied between 3.3% and 14%. Incidence estimates after 2005 could have been underestimated because some patients with first ICH could have been admitted to a hospital not participating in the HDR. To what extent our incidence estimates are influenced, is difficult to say because depending on the patient population of a hospital, more or less incident ICH patients could have been expected and thus have been missed if a hospital is not participating. The decline in participating hospitals is not related to the cause of death statistics and all patients could be followed over time for mortality. Therefore our estimates for total mortality, 30-day and 1-year case fatality have not been influenced by the decline in participating hospitals. Finally, for out-of-hospital deaths we used ICD-10 code I61. Most of these codes are determined without post-mortem examination. Over time, this has remained unchanged and therefore potential misclassification has not influenced time trends.

Our nationwide study shows that ICH incidence, 30-day and 1-year case fatality, and total ICH mortality in patients younger than 75 years have declined in The Netherlands. This is probably the result of increasing control of vascular risk factors, improved treatment in the acute phase, and better secondary prevention over the last 15 years. Because patients 75 years and older do not appear to benefit and because incidence, mortality, and case fatality are highest in this age group, specific efforts targeting the causes and consequences of ICH in the elderly may lead to lessening of the burden of ICH.

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SUPPLEMENTAL DATA

Table 2.e-1 Diagnosis of patients with discharge diagnosis ICH (ICD-9 code 431) in the hospital discharge registry of one university medical center and one non-university hospital

Diagnosis	No. of patients	%	Diagnosis	No. of patients	%
University medical center	523		*Non-university hospital*	529	
Correct diagnosis	463	89	Correct diagnosis	497	94
Lobar ICH	167	36	Lobar ICH	203	41
Deep ICH	141	36	Deep ICH	145	29
Infratentorial ICH	59	15	Infratentorial ICH	39	8
ICH, location unknown	30	7	ICH, location unknown	110	22
ICH, duplicate cases	66	14			
Incorrect diagnosis	60	11	Incorrect diagnosis	32	6
Perimesencephalic hemorrhage	25	42	Perimesencephalic hemorrhage	8	25
Ischemic stroke	20	3	Ischemic stroke	2	6
Cerebral infarction with hemorrhagic transformation	4	7	Cerebral infarction with hemorrhagic transformation	8	25
Subarachnoid hemorrhage	3	5	Subarachnoid hemorrhage	4	13
Other	4	7	Other	5	16
No ICH, duplicate cases	4	7	Unknown	5	16

Table 2.e-2 Intracerebral hemorrhage mortality trends by age in The Netherlands, 1980-2010 for men aged >35 years

Age group	Identified periods	APC[a]	95% confidence interval	Slope	Standard error	Number of deaths (min-max)	ICH mortality rates (min-max)	Change in ICH mortality rate[b]
35-54	1980-1988	-3.22	-7.14 to 0.86	-0.03	0.020	43-69	2.2-3.8	-0.31
	1988-2003	1.20	-0.44 to 2.87	0.01	0.008	43-81	2.2-3.5	0.46
	2003-2010	-7.09	-11.39 to -2.59	-0.07	0.022	45-83	1.8-3.3	-0.43
55-74	1980-1995	0.90	-0.06 to 1.86	0.01	0.005	289-400	26-33	0.08
	1995-2010	-4.55	-5.49 to -3.59	-0.05	0.005	249-409	14-33	-0.51
75-94	1980-1990	-0.95	-3.05 to 1.20	-0.01	0.010	256-298	99-115	-0.08
	1990-1993	7.22	-16.79 to 38.16	0.07	0.122	274-387	100-136	0.36
	1993-2010	-0.95	-1.73 to -0.16	-0.01	0.004	330-480	94-138	-0.20

Periods were identified by joinpoint regression analysis

[a] Annual percentage change

[b] (rate last year of period segment-rate first year of period segment) / rate first year of period segment

Table 2.e-3 Intracerebral hemorrhage mortality trends by age in The Netherlands, 1980-2010 for women aged >35 years

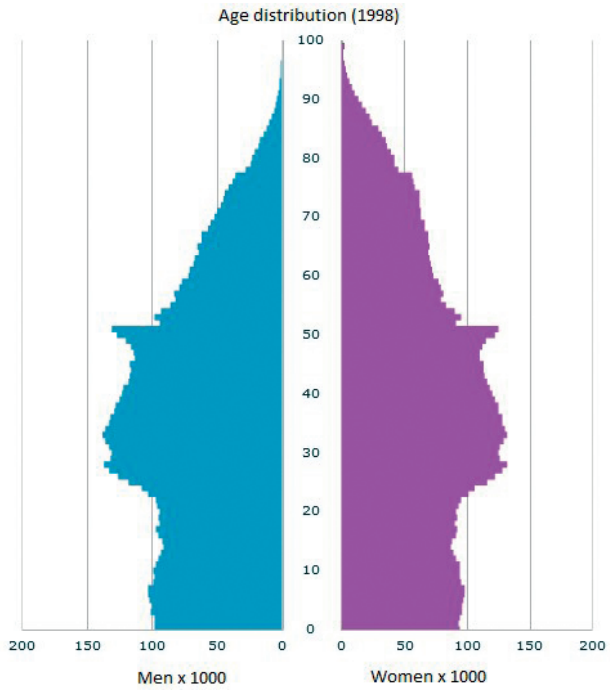
Age group	Identified periods	APC[a]	95% confidence interval	Slope	Standard error	Number of deaths (min-max)	ICH mortality rates (min-max)	Change in ICH mortality rate[b]
35-54	1980-2003	0.05	-1.06 to 1.17	0.0005	0.005	37-69	1.9-3.8	0.04
	2003-2010	-8.67	-15.18 to -1.66	-0.09	0.036	27-62	1.1-2.6	-0.57
55-74	1980-1985	0.69	-3.60 to 5.16	0.007	0.021	239-296	18.8-23	0.12
	1985-1988	-8.42	-25.66 to 12.83	-0.09	0.100	226-296	17.1-23	-0.25
	1988-1992	7.02	-3.02 to 18.10	0.07	0.047	226-289	17.1-22	0.25
	1992-2010	-3.51	-4.16 to -2.85	-0.04	0.003	178-289	10.4-21	-0.48
75-94	1980-1989	-3.24	-5.39 to -1.05	-0.03	0.011	382-464	80-110	-0.16
	1989-1993	5.96	-5.46 to 18.75	0.06	0.055	422-5434	81-99	0.20
	1993-1999	-3.32	-8.00 to 1.60	-0.03	0.024	474-573	80-100	-0.17
	1999-2010	1.71	0.30 to 3.15	0.02	0.007	472-664	76-102	0.11

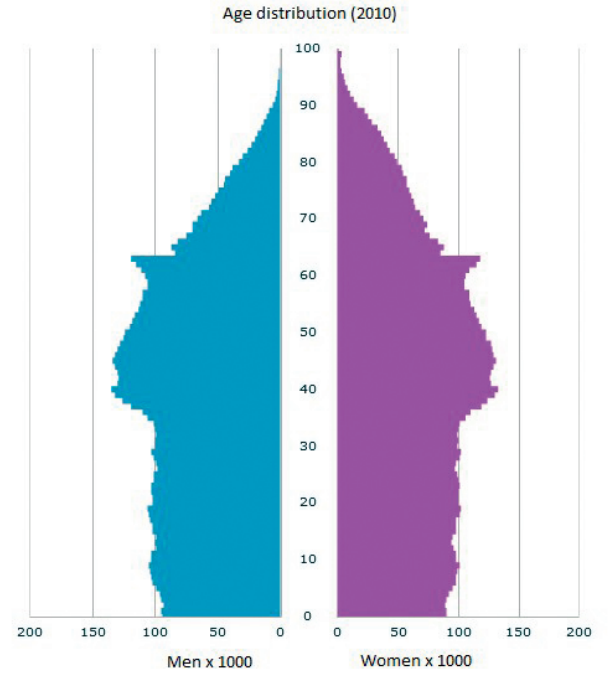
Periods were identified by joinpoint regression analysis

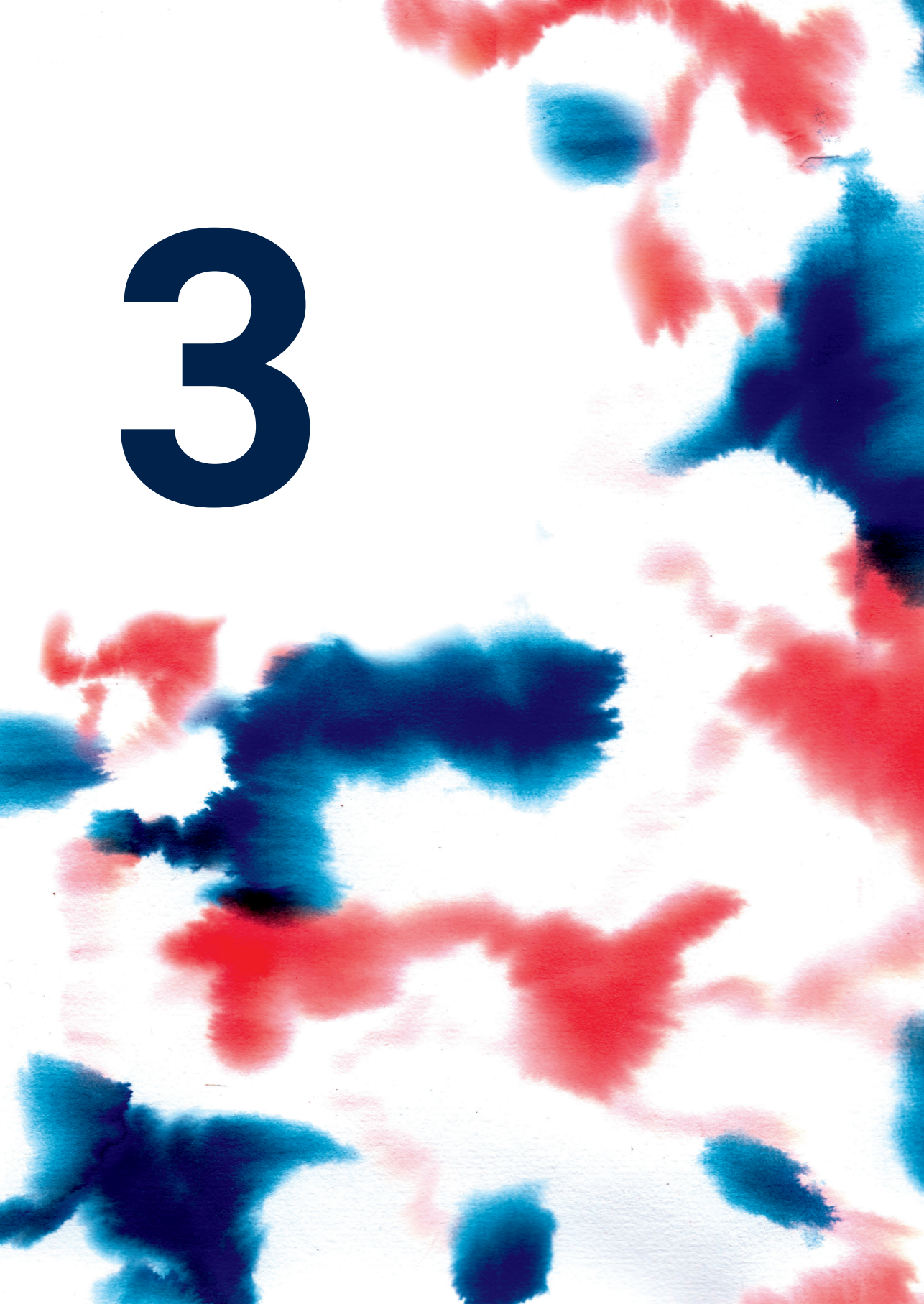
[a] Annual percentage change

[b] (rate last year of period segment-rate first year of period segment)/rate first year of period segment

Figure 2.e-1 Age distribution in the Netherlands in men and women in 1998 and 2010






3

LOCATION SPECIFIC RISK FACTORS FOR INTRACEREBRAL HEMORRHAGE: SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Objective

To conduct a systematic review and meta-analysis of studies reporting on risk factors according to the location of the intracerebral hemorrhage.

Methods

We searched PubMed and Embase for cohort and case-control studies reporting on ≥100 patients with spontaneous intracerebral hemorrhage, that specified the location of the hematoma and reported associations with risk factors published until June 27th 2019. Two authors independently extracted data on risk factors. Estimates were pooled with the generic variance-based random effects method.

Results

After screening 10 013 articles, we included 42 studies totaling 26 174 patients with intracerebral hemorrhage (9 141 lobar and 17 033 non-lobar). Risk factors for non-lobar intracerebral hemorrhage were hypertension (risk ratio 4.25, 95% confidence interval 3.05-5.91, I^2=92%), diabetes (RR 1.35, 1.11-1.64, I^2=37%), male sex (RR 1.63, 1.25-2.14, I^2=61%), alcohol overuse (RR 1.48, 1.21-1.81, I^2=19%), underweight (RR 2.12, 1.12-4.01, I^2=31%), and being black (RR 2.19, 1.21-3.96, I^2=96%) or Hispanic (RR 2.95,1.69-5.14, I^2=71%) in comparison with being white. Hypertension, but not any of the other risk factors, was also a risk factor for lobar intracerebral hemorrhage (RR 1.83, 1.39-2.42, I^2=76%). Smoking, hypercholesterolemia and obesity were associated with neither non-lobar nor lobar intracerebral hemorrhage.

Conclusions

Hypertension is a risk factor for both non-lobar and lobar intracerebral hemorrhage, although with double the effect for non-lobar intracerebral hemorrhage. Diabetes, male sex, alcohol overuse, underweight, and being black or Hispanic are risk factors for non-lobar intracerebral hemorrhage only. Hence, the term "hypertensive intracerebral hemorrhage" for non-lobar intracerebral hemorrhage is not appropriate.

INTRODUCTION

Intracerebral hemorrhage (ICH) is still the deadliest subtype of stroke.[1-3] One-month case fatality is approximately 40% and the reported proportion of patients with poor outcome at 12-50 months varies between 61 and 88%.[4] Although the overall incidence of ICH has remained stable over the last decades, there are important differences in time trends according to age and ICH location.[5, 6,e1] ICH incidence remains stable[5] or increases among people ≥75 years due to increase in lobar ICH and rise in use of antithrombotic medication.[e1] In contrast, in younger patients ICH incidence decreases[5, 6, e1] as a result of a decrease in non-lobar ICH, probably because of an improved control of hypertension.[e1]

In a previous review and meta-analysis of risk factors for spontaneous ICH, we identified hypertension and high alcohol intake as important modifiable risk factors besides the non-modifiable factors male sex and higher age.[7] Subsequent systematic reviews have suggested additional risk factors, including ApoE genotype and diabetes.[8-15] However, these reviews did not assess risk factors for ICH according to its location. Pathogenesis of spontaneous ICH varies according to location, as cerebral amyloid angiopathy (CAA) plays a role in a significant proportion of lobar ICH but not in non-lobar ICH. Some studies have suggested that risk factor profiles vary according to location,[16, 17] which may have implications for secondary prevention. The aim of this systematic review and meta-analysis was to estimate associations of risk factors and ICH according to location.

METHODS

Search strategy and selection criteria

We registered our protocol in PROSPERO (CRD42019117543). We searched PubMed and Embase for cohort, case-crossover, and case-control studies on risk factors for ICH published until June 27th 2019 according to the PRISMA statement methodology.[18] We used different combinations of the keywords intracerebral hemorrhage and synonyms; cohort, case-control, case-crossover or longitudinal study; and potential risk factors and synonyms (data available from Dryad; see e-1 for detailed search strategy). For this review we did not assess use of (antithrombotic) medication as a risk factor nor did we study genetic risk factors. We used the studies selected in our previous systematic review and meta-analysis for studies published before 2001. We checked reference lists of all included publications and the citation list of our previous systematic review and meta-analysis for additional articles,[7] and repeated this until no further studies were found. We applied no language restrictions.

Titles and abstracts and subsequently full-text versions were screened independently by two investigators (WMTJ and KW) using the following inclusion criteria: 1) Included patients were 18 years or older; 2) ICH had to be confirmed by CT, MRI, or autopsy in 100% of cases, not only based on International Classification of Diseases (ICD) codes; 3) ICH location had to be specified; 4) A cohort, case-crossover or case-control design; 5) ICH had to be analysed as a separate entity, not in combination with subarachnoid hemorrhage; 6) Reporting on at least 100 patients with ICH. If studies included patients with ICH caused by a vascular malformation, tumour, coagulation disorder (use of antithrombotic medication was allowed), or hemorrhagic transformation of infarction, data extraction needed to allow exclusion of these patients; if not the study was excluded. Conflicts regarding inclusion were resolved by consensus with a third reviewer (CJMK). We used Covidence (www.covidence.org) for standardized screening of articles.

Data extraction

Data were extracted independently by two reviewers (WMTJ and KW) using a prespecified and piloted extraction form (data available from Dryad; e-2). Discrepancies in extracted data were resolved by discussion, and if necessary a third reviewer (CJMK) was consulted. In case of multiple publications on overlapping cohorts, we included the study that best matched our inclusion criteria and with the largest amount of data relevant to the review. We extracted data on study period, study design, country of study, in- and exclusion criteria, number of cases and controls, mean or median age, proportion of males, and risk factors. Risk factors were assessed according to lobar and non-lobar (deep and infratentorial) ICH location and if possible, for deep (basal ganglia, thalamus and intraventricular) and for infratentorial hemorrhages (brainstem and cerebellum) separately. We assessed methodological quality, including risk of bias, of the included studies according to the Newcastle-Ottawa Scale (NOS) for cohort and case-control studies.[19]

Statistical analysis

Estimates of cohort and case-control studies were first analysed separately and then combined if 95% confidence intervals (CIs) of the pooled estimates from cohort studies overlapped with those of case-control studies. We also combined maximally adjusted estimates, when available, with unadjusted estimates, if 95% CIs of the pooled unadjusted estimates overlapped with pooled adjusted estimates. If studies used different definitions for risk factors, we standardised risk factors across studies whenever possible or otherwise we accepted the criteria used in the studies. Risk factors reported in at least three studies were combined in meta-analyses; for the different subgroups of a risk factor we accepted two studies for meta-analyses. For the included studies odds ratios (ORs), relative risks (RRs) and hazard ratios (HRs) with corresponding 95% CIs, whichever were available, were obtained for the various risk factors and pooled with the

generic variance-based method, weighing individual study results by the inverse of their variance. Heterogeneity was assessed using I^2 statistics.[20] We used a random-effects model because of the heterogeneous study characteristics. Because ORs accurately estimate RRs when risks of disease are small, we combined ORs with RRs and HRs from the longitudinal studies.[21, 22] We performed a sensitivity analysis for studies with a high-quality, defined as studies with >5 points (arbitrarily chosen) on the NOS. Meta-analyses were performed in R (R programming, version 1.1.456), using the meta package (version 4.9-4).[23]

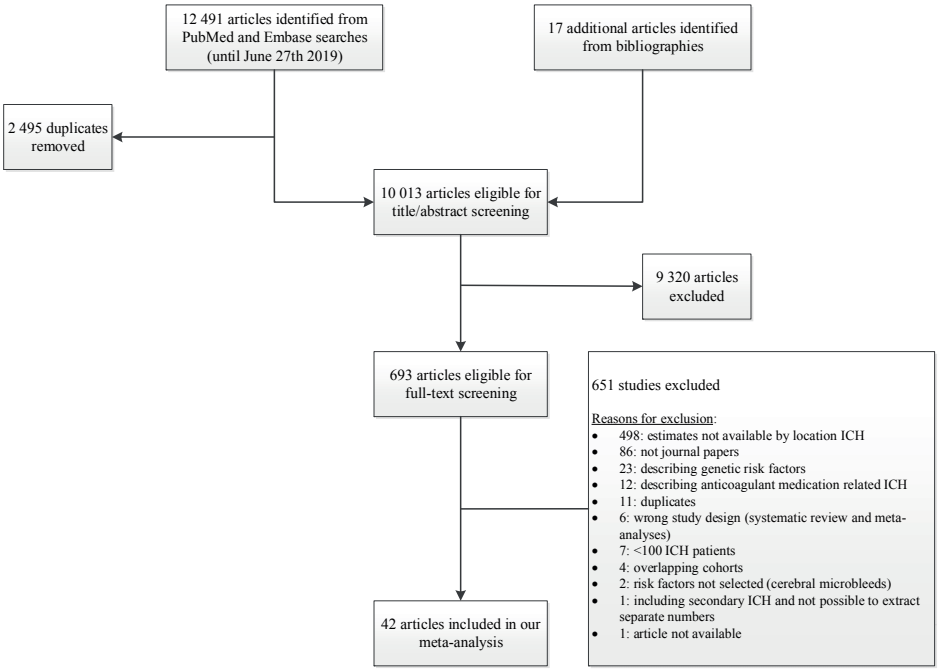
DATA AVAILABILITY

Data used in this study are available to qualified investigators on request to the corresponding and senior authors.

RESULTS

We identified 42 studies (references indicated with *e-* are available from Dryad),[e1-e42] of which 24 were cohort studies,[e1-e4, e8-e11, e13, e14, e17-e21, e24, e25, e28, e30, e31, e33, e37, e38, e41] 17 case-control studies,[e5-7, e12, e15,e16, e22, e23, e26, e27, e29, e32, e34-e36, e39, e42] and one had a case-crossover design[e40] (figure 3.1).

Figure 3.1 flowchart of literature search



Study characteristics are summarized in the data supplement available from Dryad (e-3). The 42 studies included 26 174 patients (mean age 68.2 years, standard deviation 14.1 and 57% men) with ICH, of which 9 141 had lobar ICH (35%) and 17 033 non-lobar ICH (65%). Twenty-three studies further stratified non-lobar ICH into 7 758 patients with deep ICH (82%), of whom 78 had intraventricular hemorrhage (1%), and 1 649 infratentorial ICH (17%). Eighteen studies were population-based (43%) and 24 were hospital-based (57%). Eleven of the 17 (65%) case-controls studies adjusted for potential confounders. Of the 42 included studies, 29 (69%) fulfilled the criteria for high quality studies (NOS >5). Definitions of the risk factors in the included studies, adjustment factors for maximally adjusted estimates, and all separate analyses of cohort and case-control studies with unadjusted and adjusted estimates are available from Dryad (tables e-4 - e27). To investigate the possible influence of publication bias we made funnel plots for the risk factor with the most studies (hypertension), which showed that publication bias has not played an important role in our meta-analyses (data not shown).

We report pooled RRs combining unadjusted and maximally adjusted estimates and estimates from cohort and case-control studies, since the pooled estimates had overlapping 95% CIs. For all risk factors, 95% CIs of the pooled estimates of the sensitivity analyses of high quality studies overlapped with those including all studies (data available from Dryad; tables e-5, e-7, e-10, e-12, e-14, e-15, e-17, e-19, e-20, e23 – e-25 and e-27).

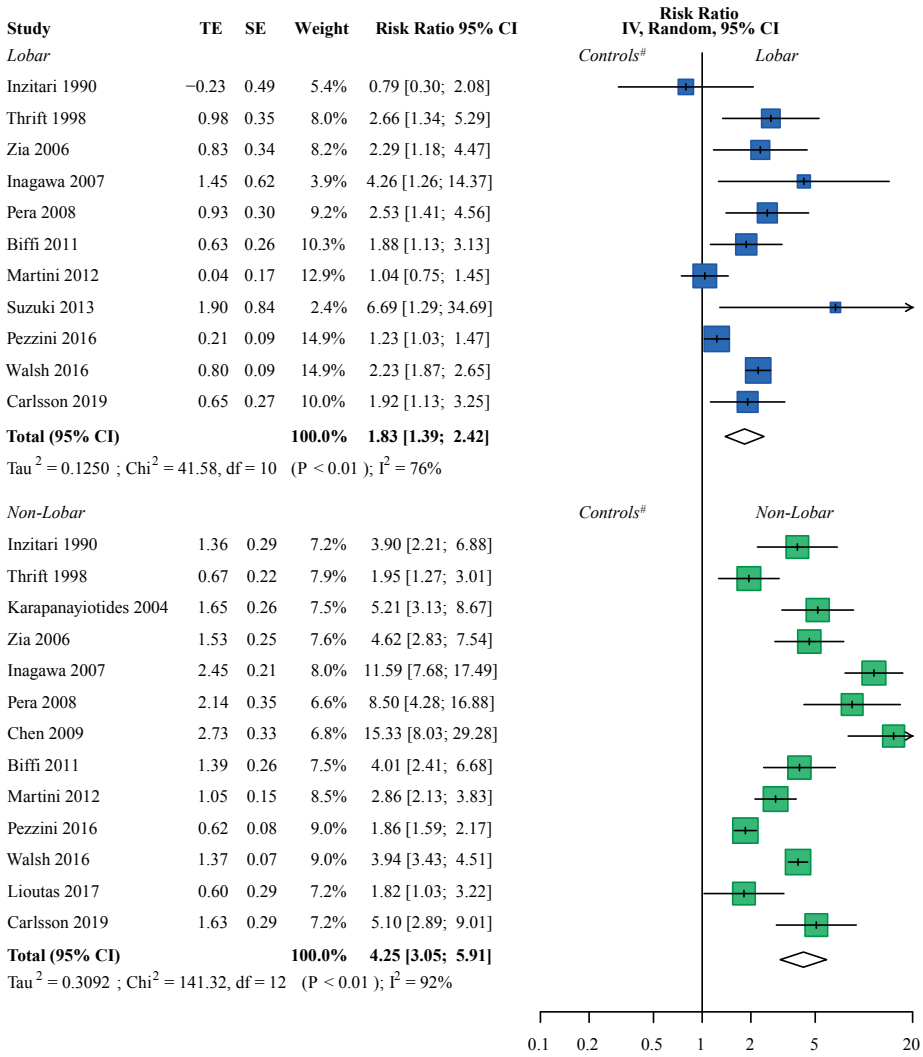
In table 3.1 we summarized the main results. Twenty-five studies [e1, e3, e6, e8, e13, e15, e16, e18, e20, e22-e31, e33-e35, e39, e41, e42] provided data on hypertension (figure 3.2), resulting in a pooled RR for lobar ICH versus controls without ICH of 1.83 (95% CI 1.39-2.42, I^2=76%, 11 studies) and for non-lobar ICH versus controls without ICH of 4.25 (95% CI 3.05-5.91, I^2=92%, 13 studies). The pooled RR for non-lobar ICH versus lobar ICH was 1.96 (95% CI 1.59-2.41, I^2=79%, 20 studies). Within the group of non-lobar ICH, we found no difference between deep and infratentorial ICH.

Table 3.1 Pooled risk ratios of risk factors for ICH according to location

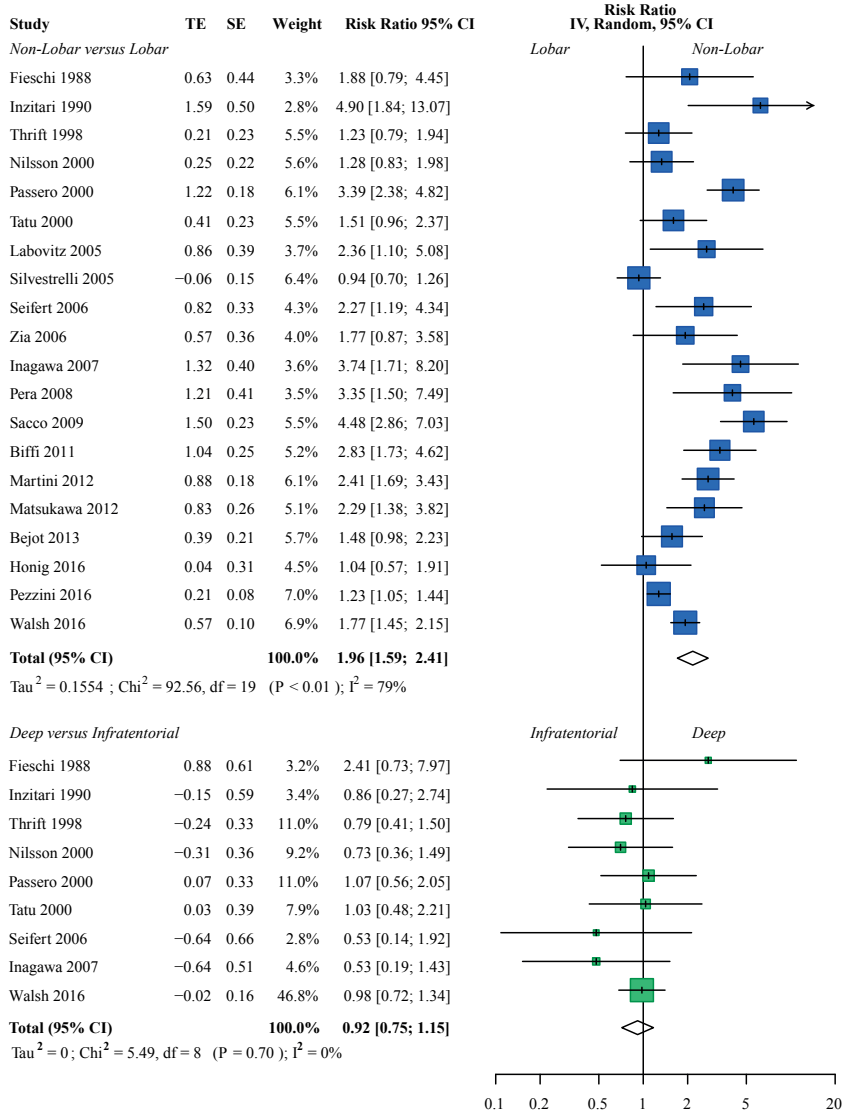
	Lobar ICH versus controls without ICH			Non-lobar ICH versus controls without ICH			Non-lobar versus lobar ICH			Deep versus infratentorial ICH		
Risk factors	**Number of studies**	**Pooled RR (95% CI)**	**I^2 %**	**Number of studies**	**Pooled RR (95% CI)**	**I^2 %**	**Number of studies**	**Pooled RR (95% CI)**	**I^2 %**	**Number of studies**	**Pooled RR (95% CI)**	**I^2 %**
Hypertension	11	1.83 (1.39-2.42)	76	13	4.25 (3.05-5.91)	92	20	1.96 (1.59-2.41)	79	9	0.92 (0.75-1.15)	0
Diabetes mellitus	9	1.13 (0.94-1.36)	0	12	1.35 (1.11-1.64)	37	16	1.28 (1.06-1.55)	32	4	1.85 (0.95-3.59)	0
Male sex	6	0.98 (0.82-1.16)	26	5	1.63 (1.25-2.14)	61	10	1.32 (1.02-1.71)	72	2	1.00 (0.61-1.63)	0
Smoking	7	1.04 (0.76-1.41)	57	9	1.09 (0.92-1.31)	39	8	1.24 (0.98-1.58)	46	-	-	-
Alcohol overuse	4	1.01 (0.78-1.29)	16	5	1.48 (1.21-1.81)	19	8	1.50 (1.31-1.72)	0	2	1.08 (0.78-1.49)	0
Alcohol consumption	2	0.83 (0.39-1.77)	0	-	-	-	4	0.98 (0.55-1.76)	72	2	1.63 (0.88-3.04)	0
Hypercholesterolemia	4	0.82 (0.66-1.01)	18	4	0.78 (0.46-1.32)	90	7	0.99 (0.79-1.23)	42	-	-	-
Obesity	2	1.66 (0.66-4.17)	89	3	1.39 (0.85-2.26)	77	5	1.43 (0.87-2.34)	74	-	-	-
Underweight	-	-	-	2	2.12 (1.12-4.01)	31	2	0.34 (0.09-1.23)	81	-	-	-
Black[a]	2	1.93 (0.98-3.78)	84	2	2.83 (1.02-7.84)	96	4	1.72 (0.99-2.96)	52	2	1.08 (0.80-1.44)	0
Asian[a]	-	-	-	-	-	-	2	3.86 (1.65-9.04)	0	-	-	-
Hispanic[a]	-	-	-	2	2.95 (1.69-5.14)	71	3	2.01 (1.68-2.41)	0	2	0.93 (0.55-1.56)	43

CI = confidence interval, ICH = intracerebral hemorrhage, RR = risk ratio
[a] compared with whites

Figure 3.2 Forest plot of risk ratios of hypertension for ICH according to location

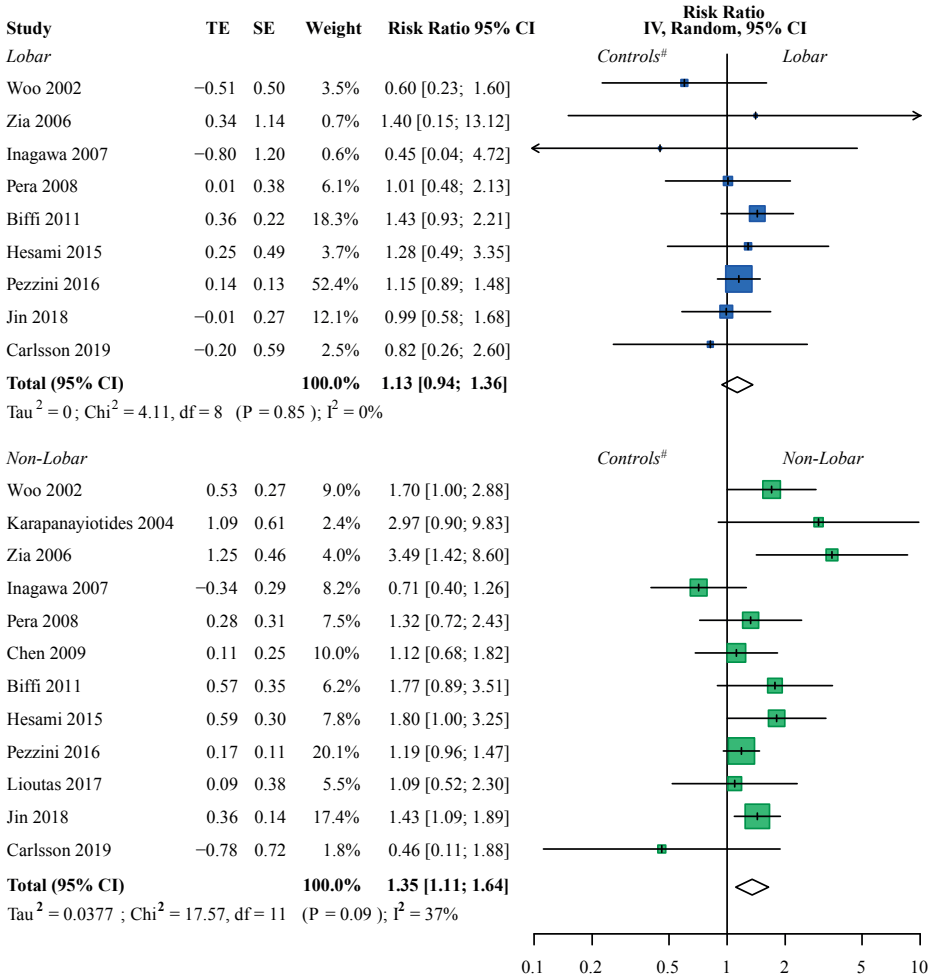


Association between hypertension and the occurrence of ICH by location. Size of rectangle is proportional to the weight of the study; ICH=intracerebral hemorrhage; TE=treatment effect; SE=standard error; CI=confidence interval; IV=inverse variance. # Controls without ICH.

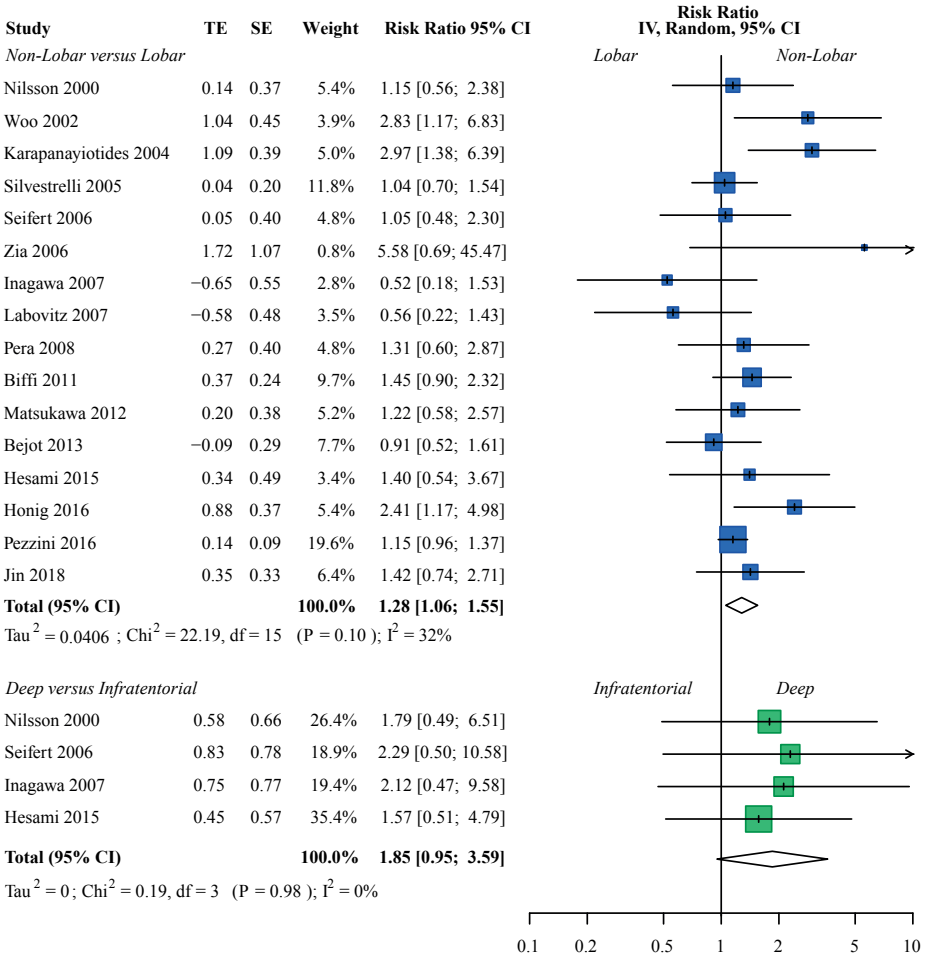


Nineteen studies [e1, e3, e6, e12, e13, e15, e17, e18, e21, e22, e24, e26, e2, e29, e30, e36, e39, e41, e42] provided data on diabetes mellitus (figure 3.3), resulting in a pooled RR for lobar ICH versus controls without ICH of 1.13 (95% CI 0.94-1.36, I^2=0%, nine studies) and for non-lobar ICH versus controls without ICH of 1.35 (95% CI 1.11-1.64, I^2=37%, 12 studies). The pooled RR for non-lobar ICH versus lobar ICH was 1.28 (95% CI 1.06-1.55, I^2=32%, 16 studies) and for deep versus infratentorial ICH 1.85 (95% CI 0.95-3.59, I^2=0%, four studies).

Figure 3.3 Forest plots of risk ratios of diabetes mellitus for ICH according to location



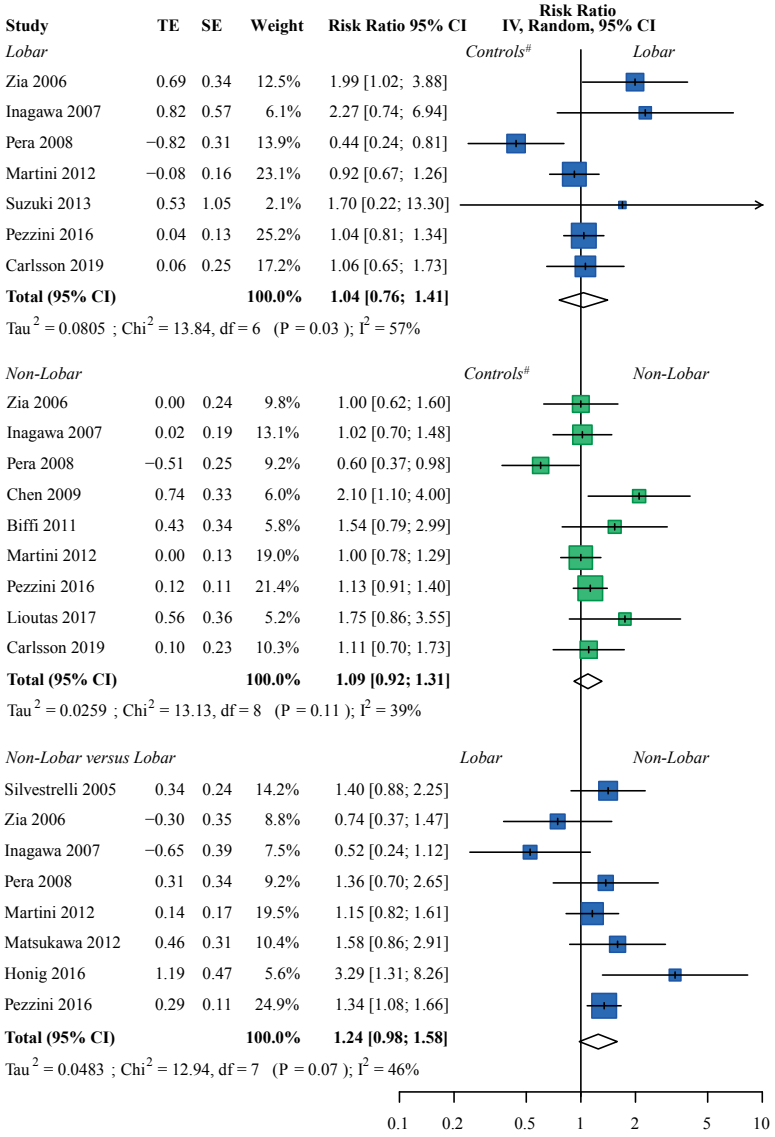
Association between diabetes mellitus and the occurrence of ICH by location. Size of rectangle is proportional to the weight of the study; ICH=intracerebral hemorrhage; TE=treatment effect; SE=standard error; CI=confidence interval; IV=inverse variance. # Controls without ICH.



Fourteen studies[e3, e6, e9, e13, e15, e20, e24, e26, e27, e30, e31, e39, e41, e42] provided data on male sex (data available from Dryad; e-9), resulting in a pooled RR for lobar ICH versus controls without ICH of 0.98 (95% CI 0.82-1.16, I^2=26%, six studies) and for non-lobar ICH versus controls without ICH of 1.63 (95% CI 1.25-2.14, I^2=61%, five studies). The pooled RR for non-lobar versus lobar ICH was 1.32 (95% CI 1.02-1.71, I^2=72%, ten studies).

Thirteen studies[e3, e6, e13, e15, e22, e23, e26, e27, e30, e31, e39, e41,e42] provided data on (current or ever) smoking (figure 3.4), resulting in a pooled RR for lobar ICH versus controls without ICH of 1.04 (95% CI 0.76-1.41, I^2=57%, seven studies) and for non-lobar ICH versus controls without ICH of 1.09 (95% CI 0.92-1.31, I^2=39%, nine studies). The pooled RR for non-lobar ICH versus lobar ICH was 1.24 (95% CI 0.98-1.58, I^2=46%, eight studies).

Figure 3.4 Forest plots of risk ratios of smoking for ICH according to location



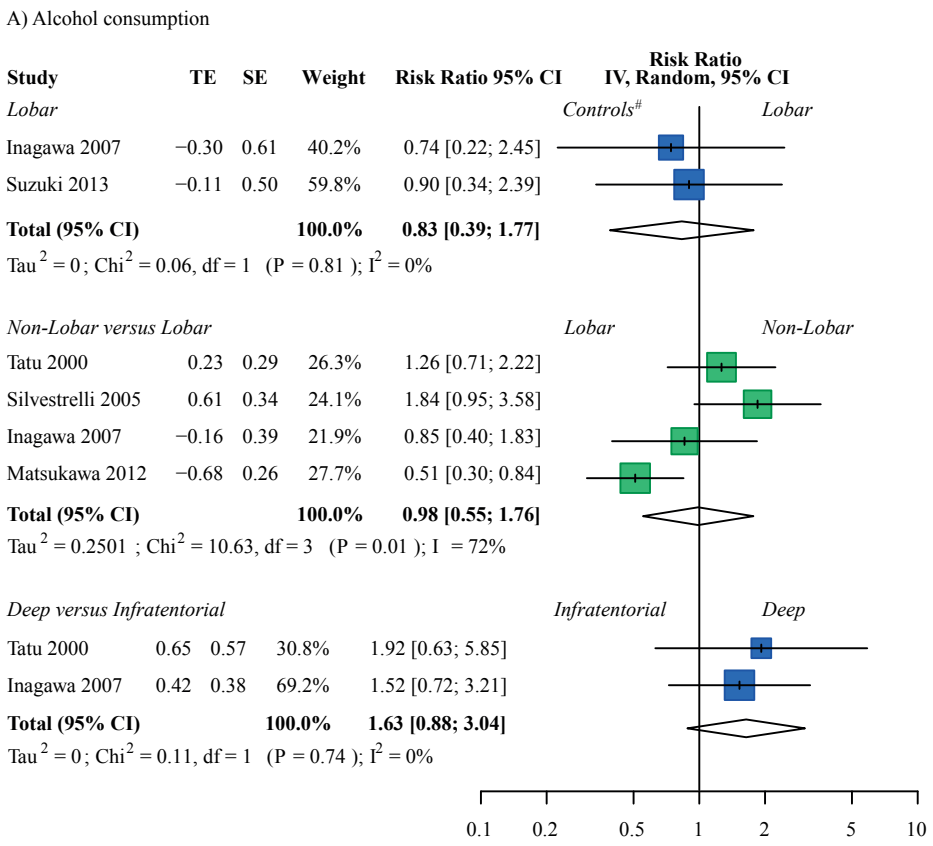
Association between smoking and the occurrence of ICH by location. Size of rectangle is proportional to the weight of the study; ICH=intracerebral hemorrhage; TE=treatment effect; SE=standard error; CI=confidence interval; IV=inverse variance. # Controls without ICH.

Thirteen studies[e2, e4-e6, e15, e20, e23, e27, e30, e31, e33, e39, e41] provided data on alcohol (figure 3.5). Alcohol overuse was not a risk factor for lobar ICH (versus controls without ICH; RR 1.01, 95% CI 0.78-1.29, I^2=16%, four studies), whereas it increased the risk of non-lobar ICH (versus controls without

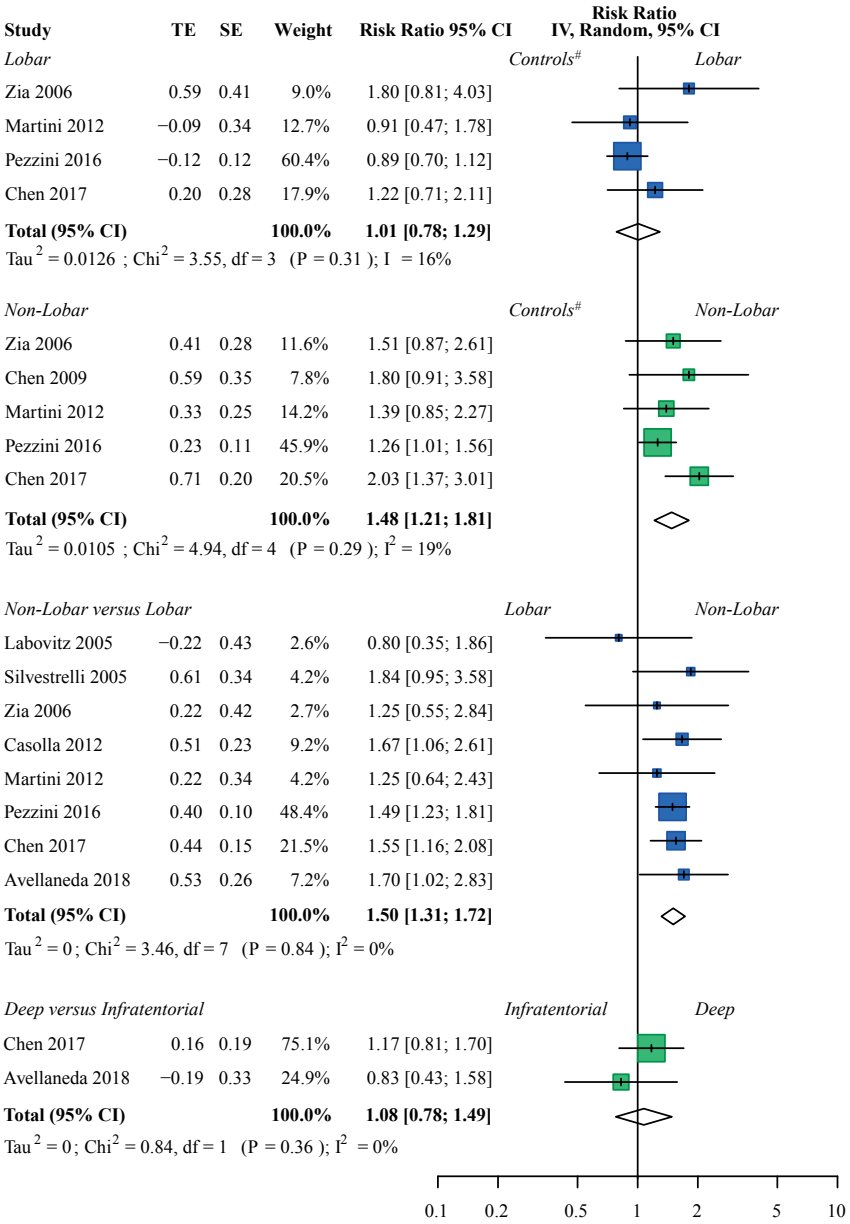
ICH; RR 1.48, 95% CI 1.21-1.81, I^2=19%, five studies). The pooled RR for non-lobar ICH versus lobar ICH was 1.50 (95% CI 1.31-1.72, I^2=0%, eight studies) and for deep versus infratentorial ICH 1.08 (95% CI 0.78-1.49, I^2=0%, two studies). Alcohol consumption was not associated with lobar ICH and for non-lobar ICH we did not have enough data to pool.

Nine studies[e1, e15, e22, e23, e27, e30, e39, e41, e42] provided data on cholesterol (figure 3.6), resulting in a pooled RR of hypercholesterolemia for lobar ICH versus controls without ICH of 0.82 (95% CI 0.66-1.01, I^2=18%, four studies) and for non-lobar ICH versus controls without ICH of 0.78 (95% CI 0.46-1.32, I^2=90%, four studies). When we excluded the one study performed in an Asian population,[e15] hypercholesterolemia was a protective factor for both lobar ICH (versus controls without ICH; pooled RR 0.81, 95% CI 0.68-0.97, I^2=0%, three studies) and for non-lobar ICH (versus controls without ICH; pooled RR 0.59, 95% CI 0.42-0.82, I^2=72%, three studies). The pooled RR for non-lobar ICH versus lobar ICH was 0.99 (95% CI 0.79-1.23, I^2=42%, seven studies).

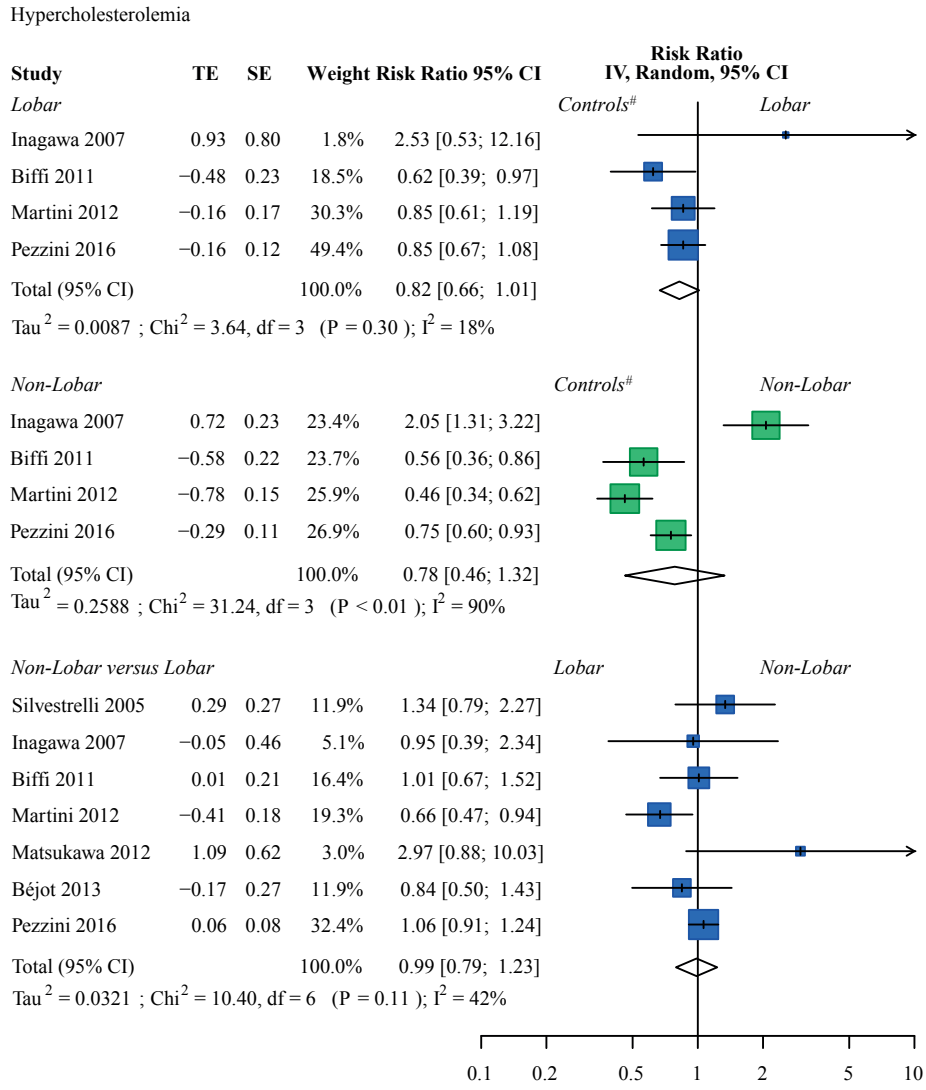
Figure 3.5 Forest plot of risk ratios of alcohol consumption and overuse for ICH according to location



B) Alcohol overuse



Association between alcohol overuse and consumption and the occurrence of ICH by location. Size of rectangle is proportional to the weight of the study; ICH=intracerebral hemorrhage; TE=treatment effect; SE=standard error; CI=confidence interval; IV=inverse variance. # Controls without ICH.



Association between hypercholesterolemia and the occurrence of ICH by location. Size of rectangle is proportional to the weight of the study; ICH=intracerebral hemorrhage; TE=treatment effect; SE=standard error; CI=confidence interval; IV=inverse variance. # Controls without ICH.

Nine studies[e3, e6, e7, e14, e22, e30, e39, e41, e42] provided data on weight (data available from Dryad e-21), of which six used obesity as risk factor and three used underweight. The pooled RR of obesity with lobar ICH versus controls without ICH was 1.66 (95% CI 0.66-4.17, I^2=89%, two studies) and for non-lobar ICH versus controls without ICH 1.39 (95% CI 0.85-2.26, I^2=77%, three studies). The pooled RR for non-lobar ICH versus lobar ICH was 1.43 (95% CI 0.87-2.34, I^2=74%, five studies). For the association of underweight with lobar ICH we did not have enough data to pool, the

pooled RR for non-lobar ICH versus controls without ICH was 2.12 (95% CI 1.12-4.01, I^2=31%, two studies). The pooled RR for non-lobar ICH versus lobar ICH was 0.34 (95% CI 0.09-1.23, I^2=81%, two studies).

Six studies[e9, e10, e19, e20, e35, e38] provided data on ethnicity (data available from Dryad; e-26), of which five studies reported on blacks, three studies on Hispanics and two studies on Asians. With whites as a reference group, the pooled RR of blacks with lobar ICH versus controls without ICH was 1.93 (95% CI 0.98-3.78, I^2=84%, two studies) and for non-lobar ICH versus controls without ICH 2.83 (95% CI 1.02-7.84, I^2=96%, two studies). The pooled RR for non-lobar versus lobar ICH was 1.72 (95% CI 0.99-2.96, I^2=52%, four studies) and for deep versus infratentorial ICH 1.08 (95% CI 0.80-1.44, I^2=0%, two studies). For the association of Asians with lobar or non-lobar ICH we did not have enough data to pool. The pooled RR for non-lobar versus lobar ICH was 3.86 (95% CI 1.65-9.04, I^2=0%, two studies). For the association of Hispanics with lobar ICH we did not have enough data to pool and the pooled RR for non-lobar ICH versus controls without ICH was 2.95 (95% CI 1.69-5.14, I^2=71%, two studies). The pooled RR for non-lobar ICH versus lobar ICH was 2.01 (95% CI 1.68-2.41, I^2=0%, three studies) and for deep versus infratentorial ICH 0.93 (95% CI 0.55-1.56, I^2=43%, two studies).

A total of thirteen risk factors were reported in only one or two studies and for three risk factors (age, history of cardiac disease and history of cerebrovascular disease) definitions were too divergent to allow pooling of data (data available from Dryad; e-27). Of these risk factors, school education less than high school showed an association with lobar ICH.[e23] School education less than high school,[e23] a first degree relative with ICH,[e23] a lower intake of dietary saturated fatty acids,[e37] tumour necrosis factor receptor 1 and 2,[e32] and air pollution (fine particulate matter ≤2.5 μm)[e40] were associated with non-lobar ICH (versus controls without ICH). In the direct comparison of non-lobar versus lobar ICH atherosclerotic peripheral arterial disease,[e27] a family history of stroke,[e30] atmospheric pressure, and air temperature[e11] were associated with non-lobar ICH and hepatic disorder was reported as a risk factor for deep versus infratentorial ICH.[e15]

DISCUSSION

Our data indicate that hypertension is a risk factor for both non-lobar and lobar ICH, whereas diabetes, male sex, alcohol overuse, underweight, and being black or Hispanic compared with being white are risk factors for non-lobar ICH only. Smoking, hypercholesterolemia and obesity were not a risk factor for either lobar or non-lobar ICH. We found no differences in risk factors between deep and infratentorial ICH.

In a previous review that compared patients with lobar and deep ICH,[24] prevalence of hypertension was higher in patients with deep ICH, which supports our finding that hypertension is a stronger risk factor for non-lobar than for lobar ICH. The exact mechanism through which hypertension causes ICH in both lobar and non-lobar location is unclear. Hypertension is thought to cause arteriolosclerosis with lipohyalinosis, microatheromas, and microaneurysms in the small, deep, perforating intracranial arteries,[25] which increases the probability of vessel rupture in deep and infratentorial locations.[26] In pathology studies similar hypertension-related changes have been described in leptomeningeal and cortical vessels, which suggest that similar mechanisms play a role in lobar ICH.[27-29] These changes in leptomeningeal and cortical vessels can be found both in patients with and without CAA. A recent study found that 42% of participants with lobar ICH had moderate or severe arteriolosclerosis in addition to moderate or severe CAA on pathological examination.[30] Moreover, 39% of participants with lobar ICH had moderate or severe arteriolosclerosis alone.[30] Leptomeningeal and cortical vessels might simply be less affected by hypertension than deep perforating arteries, but this needs further investigation.

In addition to hypertension, other vascular risk factors, including diabetes and hypercholesterolemia, affect the small, deep, perforating intracranial arteries,[31] resulting in an increased risk of non-lobar ICH.[32] Our results show that the previously reported increased risk of ICH with diabetes,[9] is driven by the increased risk of non-lobar ICH. Abnormal glucose metabolism causes impaired endothelial function leading to arteriolosclerosis,[32] and subsequently the likelihood of non-lobar ICH.[25]. It has been suggested that small (deep or leptomeningeal) arteries might be susceptible to hemorrhage because of low cholesterol, which is hypothesized to induce necrosis of arterial smooth muscle cells, making the endothelium vulnerable to development of microaneurysms.[10, 33-35] However, we found the higher risk of lobar and non-lobar ICH only in non-Asians, which may be explained by differences in genetic backgrounds and dietary pattern between Asians and non-Asians.[36, e15] Low serum total cholesterol, might be a result of a low BMI.[10] Our finding that underweight is associated with ICH has been shown in several other studies.[37,38] These studies also showed an increased risk of ICH, but did not take location of the ICH into account. Furthermore, weight loss might be associated with alcohol overuse, which could confound the association with a higher risk of ICH.[38, e41] Of the three articles describing underweight included in our meta-analysis, only one adjusted for alcohol overuse. We showed that the previously found increased risk of alcohol overuse for ICH in general,[39] is predominantly caused by its effect on the risk of non-lobar ICH. As alcohol overuse is a risk factor for hypertension, it may act as a mediator for the relationship between hypertension and non-lobar ICH.[40] Three of the five studies included in our meta-analysis adjusted for hypertension as a confounder; in two of these alcohol remained a risk factor for non-lobar ICH. Moreover, alcohol intake may cause impaired platelet function and

additional effects on hemostasis,[41,42,e4] resulting in a higher bleeding propensity. In line with a previous systematic review and meta-analysis in ICH not taking into account its location,[39] we found that light and moderate alcohol consumption was not associated with either lobar or non-lobar ICH.

Men have a higher prevalence of hypertension, diabetes and alcohol overuse,[43-45] which may explain our finding that male sex is a risk factor for non-lobar ICH but not for lobar ICH. Our finding that blacks, Asians and Hispanics compared with whites have an increased risk of non-lobar ICH, but not lobar ICH, may also be explained by a higher prevalence of these risk factors in these populations.[46, e35] This probably plays also a role in the two times higher incidence of ICH in general in Asians.[4] In the current meta-analysis we did not find any studies from Eastern Asia that investigated risk factor for ICH by location.

In contrast to our previous review,[7] we did not find (current) smoking to be a risk factor for either lobar or non-lobar ICH. This may be explained by the different categorization of never, previous or current smoker, in the included studies in our meta-analysis, and the absence of data on burden of smoking, such as numbers of packyears.

Our results show a similar risk factor profile in patients with deep ICH (basal ganglia and thalamus), and those with infratentorial ICH (brainstem or cerebellum), suggesting a common underlying small vessel disease. Previous pathology studies showed that most cerebellar ICHs are likely caused by arteriolosclerosis, similar to changes seen in deep perforating vessels, although a small proportion of cerebellar hematomas is related to vascular amyloid deposition.[47, 48]

Strengths of our systematic review and meta-analysis include the comprehensive literature search without language restrictions, and inclusion of studies based on predefined selection criteria. A further strength is our diagnostic accuracy, because we did not include studies based solely on ICD codes, and diagnosis and location had to be confirmed by imaging. We were able to quantify the associations between risk factors and lobar and non-lobar ICH in many studies that were not specifically designed to examine these associations, resulting in a meta-analysis of a high number of patients. In addition, we checked consistency between estimates from cohort and case-control studies, and unadjusted and adjusted estimates, before pooling data. Finally, we performed sensitivity analyses including high quality studies only, in which we found similar results as in our main analyses, which strengthens the validity of our results.

Our study also has limitations. First, different sources of bias may have influenced the results of the included observational studies. Selection bias in case-control studies may influence external validation of the results. Recall and misclassification bias because of different and

changing definitions for risk factors may have resulted in both over- and underestimation of the true effect. Investigation bias, because of varying methods and extent to which patients were investigated for the presence of risk factors may have been different between patients with lobar ICH and non-lobar ICH. For example, patients who are younger or had lobar ICH may have been investigated more extensively for a secondary cause,[49, 50] which may have resulted in over- or underestimation of a risk factor. However, since macrovascular lesions are the cause in only a minority of ICH patients, we do not think this potential bias has influenced our results to a large extent. Second, although we included a total of 42 studies, the number of studies per risk factor by location was relatively small with a maximum of 13 studies. Third, misclassification of hematoma location in included studies might have occurred.[24] Although some studies defined hemorrhages that could not be classified as lobar or non-lobar as mixed ICH and excluded these patients from analysis.[e7, e22, e27, e42] If investigators were not blinded for risk factors such as hypertension, this knowledge may have resulted in an incorrect classification of a hematoma as non-lobar and subsequently an over- or underestimation of risk factors.[24] Fourth, not all included studies adjusted for age or other risk factors and there was variation in number and type of adjustment factors between studies.

The differences in risk factor profiles between lobar and non-lobar ICH suggest that with regard to secondary prevention spontaneous ICH should not be treated as one single disease. In contrast to traditional beliefs, hypertension is not the only cause for non-lobar ICH, as also diabetes, alcohol overuse, underweight, and being male, black or Hispanic are risk factors for non-lobar ICH. For lobar ICH we could only identify the risk factor hypertension. Based on our findings, we consider the term “hypertensive ICH” to distinguish deep from lobar ICH inappropriate. Since non-lobar ICH shares more risk factors with ischemic stroke than lobar ICH, future studies should investigate whether this difference in risk factor profiles should lead to distinct secondary prevention strategies for lobar and non-lobar ICH.

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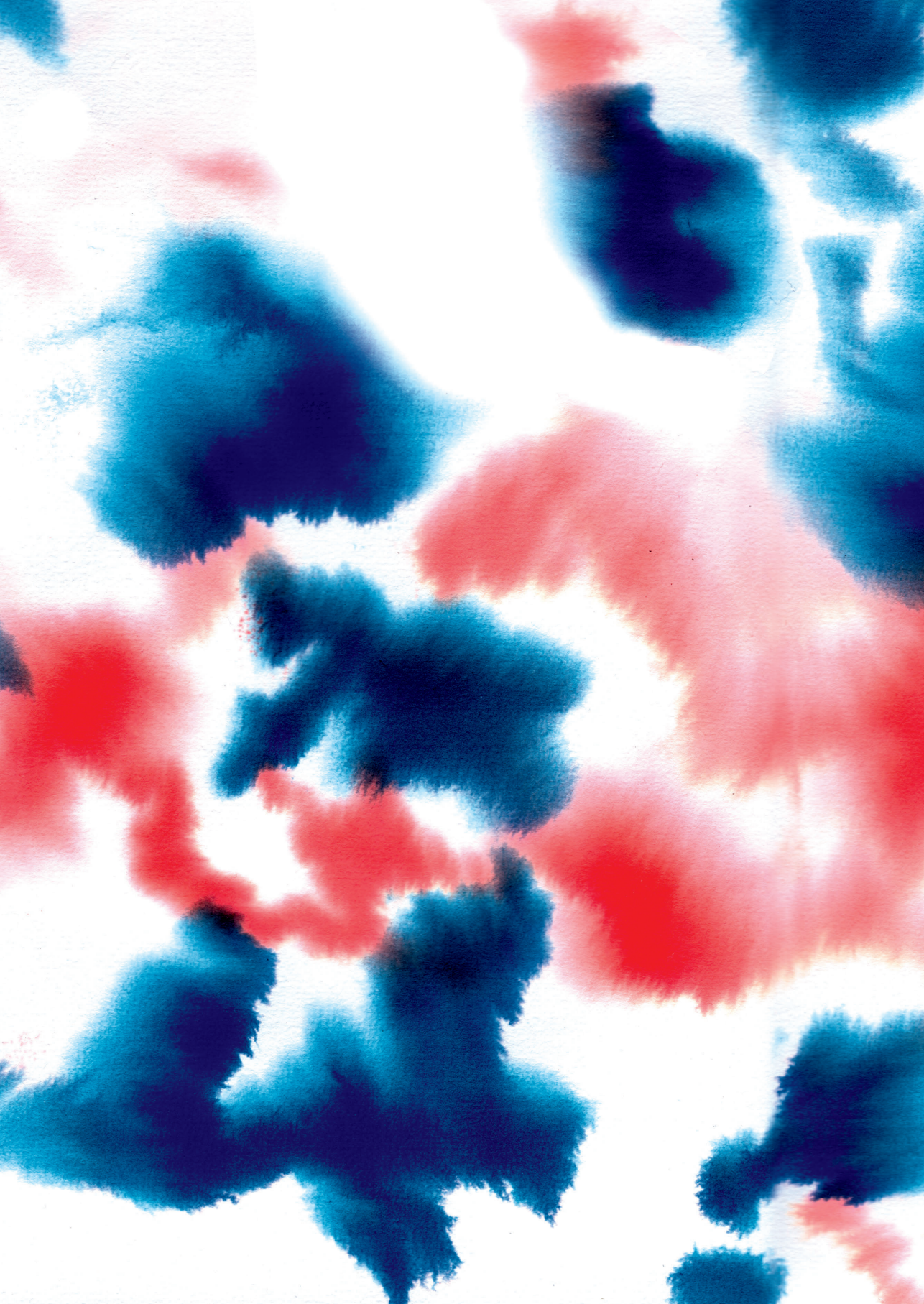
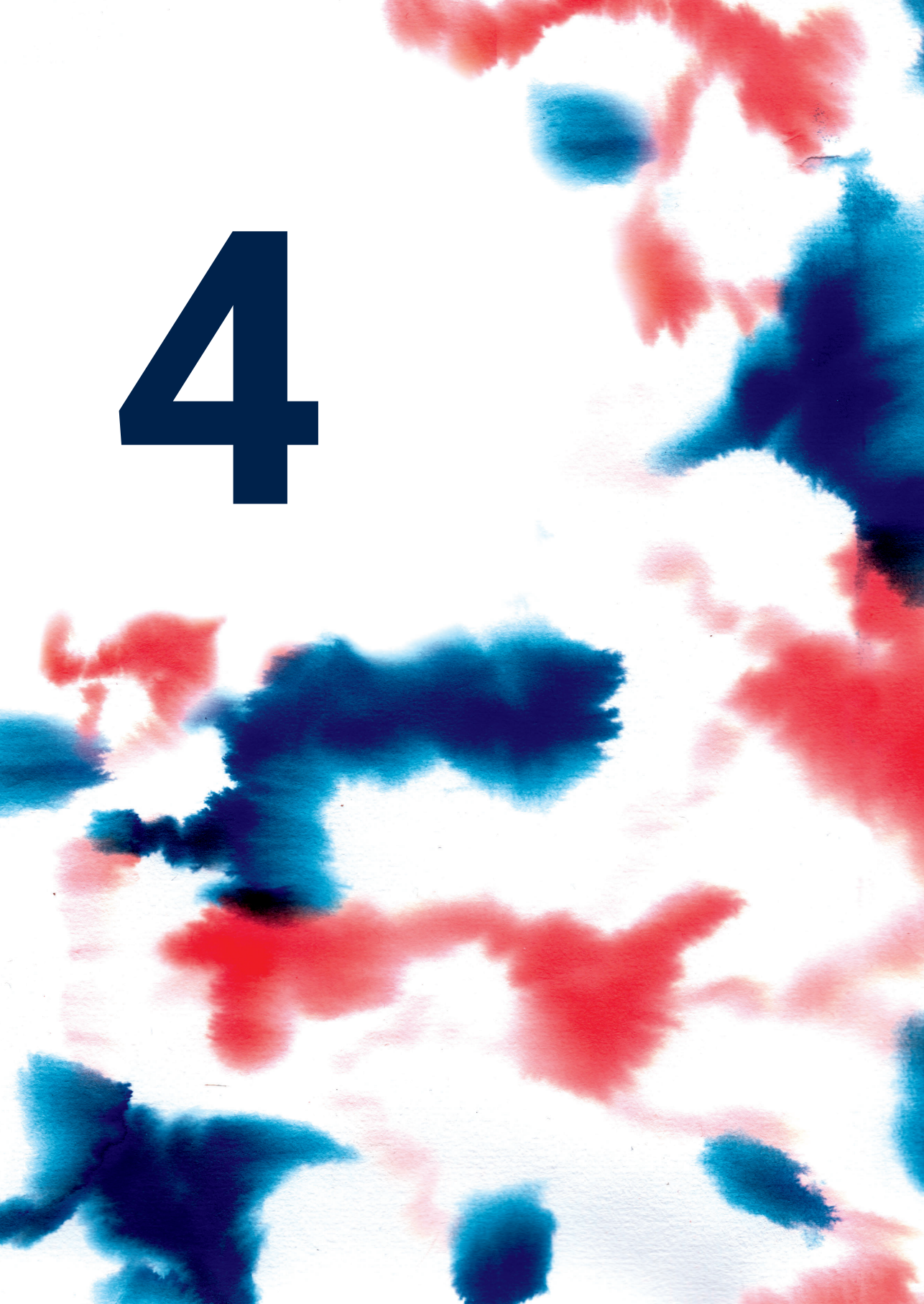


Part II

CHARACTERIZATION OF SMALL VESSEL DISEASE IN SPONTANEOUS INTRACEREBRAL HEMORRHAGE

4



CORTICAL MICROINFARCTS ON 7 T MRI IN PATIENTS WITH SPONTANEOUS INTRACEREBRAL HEMORRHAGE

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ABSTRACT

In patients with spontaneous intracerebral hemorrhage (ICH) coexisting abnormalities on brain imaging can provide clues on the etiology of the underlying small vessel disease. We examined cortical cerebral microinfarcts as a novel marker of coexistent vascular damage in ICH. Twelve patients with spontaneous ICH and 15 controls underwent 7 tesla MRI. Microinfarcts were present in 9 of 12 patients with spontaneous ICH, and in 5 of 15 controls. This explorative study shows, for the first time, that microinfarcts appear to be a very common vascular comorbidity in spontaneous ICH. Future larger studies should further assess the etiological significance of these lesions.

INTRODUCTION

Spontaneous, or primary, intracerebral hemorrhage (ICH) is the deadliest subtype of stroke with a 30-day mortality of around 40%.[1] In contrast with ischemic stroke and subarachnoid hemorrhage, advances in the field have not resulted in a decrease in case fatality over the past 25 years.[1] One factor that may have delayed progress is that the etiology of spontaneous ICH is heterogeneous and still poorly understood. As long as we cannot pinpoint the specific cause in an individual patient we cannot provide targeted treatment. Typically, deep ICH (i.e. located in the basal ganglia or thalamus) has been associated with hypertension, whereas lobar ICH in elderly patients is considered to be caused by cerebral amyloid angiopathy (CAA). This distinction is likely to be an oversimplification as many patients with deep ICH do not have hypertension,[2] and pathological studies found CAA in only one-third of patients with lobar ICH.[3]

Apart from the ICH itself, coexisting brain imaging abnormalities can provide clues on the potential cause of spontaneous ICH. Lobar cerebral microbleeds (CMBs) and superficial siderosis on T_2*-weighted gradient-echo magnetic resonance imaging (MRI), for example, have been identified as helpful biomarkers of CAA. Cerebral microinfarcts (CMIs) are common manifestations of small vessel disease that have been linked to other vascular pathologies and dementia in autopsy studies.[4] On pathological examination, CMIs are characterised by microscopic regions of cellular death or tissue necrosis with gliosis, sometimes accompanied by cavitation.[5] Whether the occurrence of CMIs may help to differentiate the type of small vessel diseases underlying spontaneous ICH is unclear. Recent advances in high field MRI now allow detection of cortical CMIs in vivo.[6] In this explorative study, we assessed whether cortical CMIs on 7 tesla MR images can be observed in patients with deep or lobar spontaneous ICH and whether they are more frequent in patients than in controls.

MATERIAL AND METHODS

Study population

We performed 7 tesla MRI scanning in 12 patients with spontaneous ICH under the age of 70, who presented to the University Medical Center Utrecht (UMCU), the Netherlands, who had no contraindications for 7 tesla MRI and in whom the clinical condition was stable enough to undergo MRI. In all patients secondary causes of ICH were excluded by CTA (5 patients), MRI/MRA (all), or angiogram (7 patients). Fifteen healthy controls of similar age, without a history of neurological or psychiatric disorders, recruited via their general practitioners as part of the PREDICT-MR study[7], served as a reference group. The study was approved by the medical ethics committee of the UMCU and all subjects gave written informed consent. The guidelines according to the Declaration of Helsinki were followed.

MRI scanning protocol

Scans were acquired on a whole-body 7 tesla MR system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16 or 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). The standard scanning protocol included a fluid attenuated inversion recovery (FLAIR) image (repetition time 8,000 ms; nominal echo time 300 ms; inversion time 2,250 ms; matrix size 312 x 304; voxel size 0.8 x 0.8 x 0.8 mm^3; scan duration 12 minutes 48 seconds) and a T_2*-weighted image (repetition time 37 ms; echo time 1 8.5 ms; echo time 2 19.1 ms; matrix size 444 x 353; voxel size 0.5 x 0.5 x 0.5 mm^3; scan duration 9 minutes 15 seconds), which were used to assess small vessel pathology in these subjects.

MRI rating

Rating was performed by two independent raters (SvV and WJ). Cortical CMIs were assessed on the FLAIR image and defined as hyperintense lesions, restricted to the cortex, distinct from perivascular spaces, and ≤3mm.[6] CMBs were assessed on the dual echo T_2* image, using the Microbleed Anatomical Rating Scale (MARS). CMBs directly adjacent to the ICH were not scored. Consensus was established between both raters in case of disagreement. The inter-rater agreement for both CMIs (intraclass correlation coefficient (ICC) = 0.96) and CMBs (ICC = 0.96) was good. Volume of the intraparenchymal ICH was assessed on CT on admission using an in-house developed tool.[8]

Statistical analysis

Between group differences in median numbers of CMBs and CMIs between patients and controls were analysed with Mann-Whitney U tests.

RESULTS

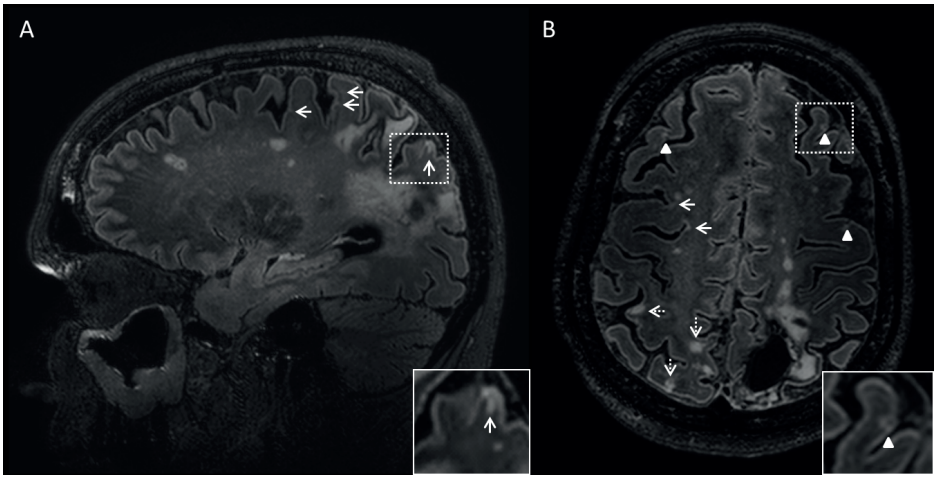
Patient characteristics are provided in table 4.1. The 12 patients with ICH had a mean age of 52 years (SD 8), and 8 were male. Mean age of the 15 healthy controls was 55 years (SD 7), and 6 were male. Eight out of 12 patients had hypertension versus 5 out of 15 control subjects. Three out of 12 patients and 1 out of 15 control subjects had diabetes. Two patients had hyperlipidemia compared to 6 control subjects, one patient was a current smoker compared to none of the control subjects, and one patient had a history of alcohol abuse (not known for control subjects).

Cortical CMIs were found in all 6 patients with lobar ICH and in 3 of 6 patients with deep ICH. The location of CMIs was not restricted to the hemisphere of the macrohemorrhage. CMIs were found in 5 of 15 control subjects. Median number of CMIs was 2.5 (range 0 to >100) in the patient group, compared to 0 (range 0 to 2) in the reference group (p = 0.01). This difference remained

significant when the patient with >100 CMIs was excluded from the analysis (p = 0.02). CMBs were observed in 8 of 12 patients and in 4 of 15 controls. Patients had a higher number of CMBs (median number 1.5, range 0 to 26) than the reference group (median number 0, range 0 to 4; p = 0.03). In 4 of the patients CMIs were observed in the absence of CMBs.

The patient with >100 CMIs not only had CMIs with gliosis, without cavitation, but also several cavitated cortical CMIs (i.e. hypointense on FLAIR with a hyperintense rim, figure 4.1).

Figure 4.1



Multiple cortical cerebral microinfarcts on the fluid-attenuated inversion recovery (FLAIR) scan of a 52-year old woman (patient 8) with a left occipito-parietal ICH. Depicted are a sagittal view (A) and transversal view (B) of the brain. Numerous cortical cerebral microinfarcts (arrows, insert A) were present throughout the brain, including multiple with cavitation (arrowheads, insert B). In the cavitated cortical cerebral microinfarcts (insert B), note the interruption of the cortex and the hyperintense rim surrounding the hypointense cavity. Also many cortical infarcts (>3mm) were present (dashed arrows).

Table 4.1 Patient characteristics and MRI findings in 12 patients with spontaneous ICH

Patient	Sex (M/F)	Age at time of ICH (years)	Interval between ICH and MRI (days)	ICH location (volume)	Hyper-tension[a]	Diabetes	Current smoking	Alcohol abuse	Hyper-lipidemia[b]	Microbleeds N (location)	Microinfarcts N
1	M	43	102	Deep (16.1 mL)	Yes	Yes	No	No	No	0	4
2	M	48	6	Deep (21.6 mL)	Yes	Yes	No	No	No	1 (infratentorial)	0
3	F	59	346	Deep (0.3 mL)	Yes	No	No	No	No	4 (deep, lobar, infratentorial)	0
4	M	46	68	Deep (4.3 mL)	Yes	No	No	No	No	2 (deep, lobar)	2
5	M	56	2	Deep (22.0 mL)	Yes	No	No	No	No	3 (deep)	0
6	M	49	32	Deep (0.2 mL)	No	No	No	No	No	0	1
7	M	42	205	Lobar (35.3 mL)	Yes	No	Yes	Yes	No	1 (deep)	2
8	F	42	339	Lobar (44.5 mL)	Yes	No	No	No	Yes	2 (deep, lobar)	>100
9	F	57	103	Lobar (89.7 mL)	No	No	No	No	No	0	5
10	M	57	265	Lobar (19.7 mL)	Yes	No	No	No	No	26 (deep, lobar, infratentorial)	10
11	F	68	477	Lobar (43.5 mL)	No	No	No	No	No	0[c]	3
12	M	51	1127	Lobar (15.8 mL)	No	Yes	No	No	Yes	2 (lobar)[c]	4

Abbreviations: F, female; FLAIR, fluid attenuated inversion recovery; ICH, intracerebral hemorrhage; M, male; MRI, magnetic resonance imaging;

[a] Patients were considered to have hypertension, if they received drug treatment for this condition prior to ICH or showed left ventricle hypertrophy on their electrocardiogram.

[b] Patients were considered to have hyperlipidemia if their cholesterol levels were > 6.5 mmol/L or if they received drug treatment for this condition.

[c] multiple microbleeds were observed in close proximity to the ICH (6 in patient 11, and >100 in patient 12)

DISCUSSION

Multiple CMIs were found on 7 tesla MRI in the majority of patients with ICH, despite their relatively young age. CMIs occurred in patients with and without CMBs.

The occurrence of CMIs in the majority of patients with spontaneous ICH in this small series of relatively young patients is remarkable. As expected, the occurrence of CMBs and CMIs in the control group is lower than previously reported for older (mean age 72 and 68 years) healthy individuals at 7 tesla MRI.[6,9,10] To the best of our knowledge, CMIs have never been examined before in patients with spontaneous ICH, either by neuropathology or by MRI. Neuropathological studies reported a prevalence of CMIs of 24% in healthy elderly and 43% in patients with Alzheimer's disease.[5] The few available neuropathological studies in patients with different forms of vascular brain damage report frequencies ranging from 26 to 78%.[5] Our small case-series suggests that CMIs may be more frequent in patients with lobar ICH than in those with ICH in a deep location, but this observation requires further study in larger cohorts. Although 5 of the 6 patients with lobar ICH do not fulfil the Boston criteria for probable CAA, as they had deep CMBs or no CMBs, CAA cannot be excluded as contributing factor. Neuropathological studies have shown that severe CAA is associated with presence of CMIs in patients with Alzheimer's disease.[11]

Our findings suggest that the underlying small vessel diseases in patients with spontaneous ICH not only result in macrobleeds and CMBs, but also in ischemic brain injury. In the majority of our patients, there was a substantial interval between the time of the ICH and the 7 tesla MRI. Hence, we cannot determine whether the ischemic injury was elicited by the acute event of the ICH or its treatment or an expression of the underlying vasculopathy with both hemorrhagic and ischemic insults. Recently, multiple prospective studies have shown that around one-third of patients have signs of acute ischemic brain injury on diffusion weighted imaging (DWI) on regular field strength MRI early after spontaneous ICH, and that their presence is associated with poor outcome.[12,13] A recent letter discussed whether some supposedly ischemic DWI lesions could in fact represent CMBs in evolution.[14] With 7 tesla FLAIR not only acute micro-ischemic lesions can be detected, but also persistent ones. Our study suggests that patients with spontaneous ICH have indeed a high load of ischemic brain injury in addition to the hemorrhagic burden of macro- and microhemorrhages. Cavitation of some of the CMIs, as identified in one patient, is indicative of the chronic aspect of these ischemic lesions. It should be noted that 7 tesla FLAIR currently allows us to detect only the largest cortical CMIs which are likely to represent only the tip of the iceberg of total CMI load.[6] Also, further histopathological validation of cortical CMIs as detected with 7 tesla FLAIR is needed.

This study has three important limitations. First, the number of patients is small. Nonetheless, we found a significant difference in number of CMIs between patients and controls. Second, we only had a FLAIR image to our disposal for the rating of CMIs, without an additional T_2- or T_1-weighted image. This may have affected CMI detection. Finally, it was impossible to perform rating of both CMIs and CMBs blinded to patient versus control status.

Further studies are needed to determine whether CMIs can be used as a biomarker of underlying etiology in ICH and whether they predict functional and cognitive outcome.

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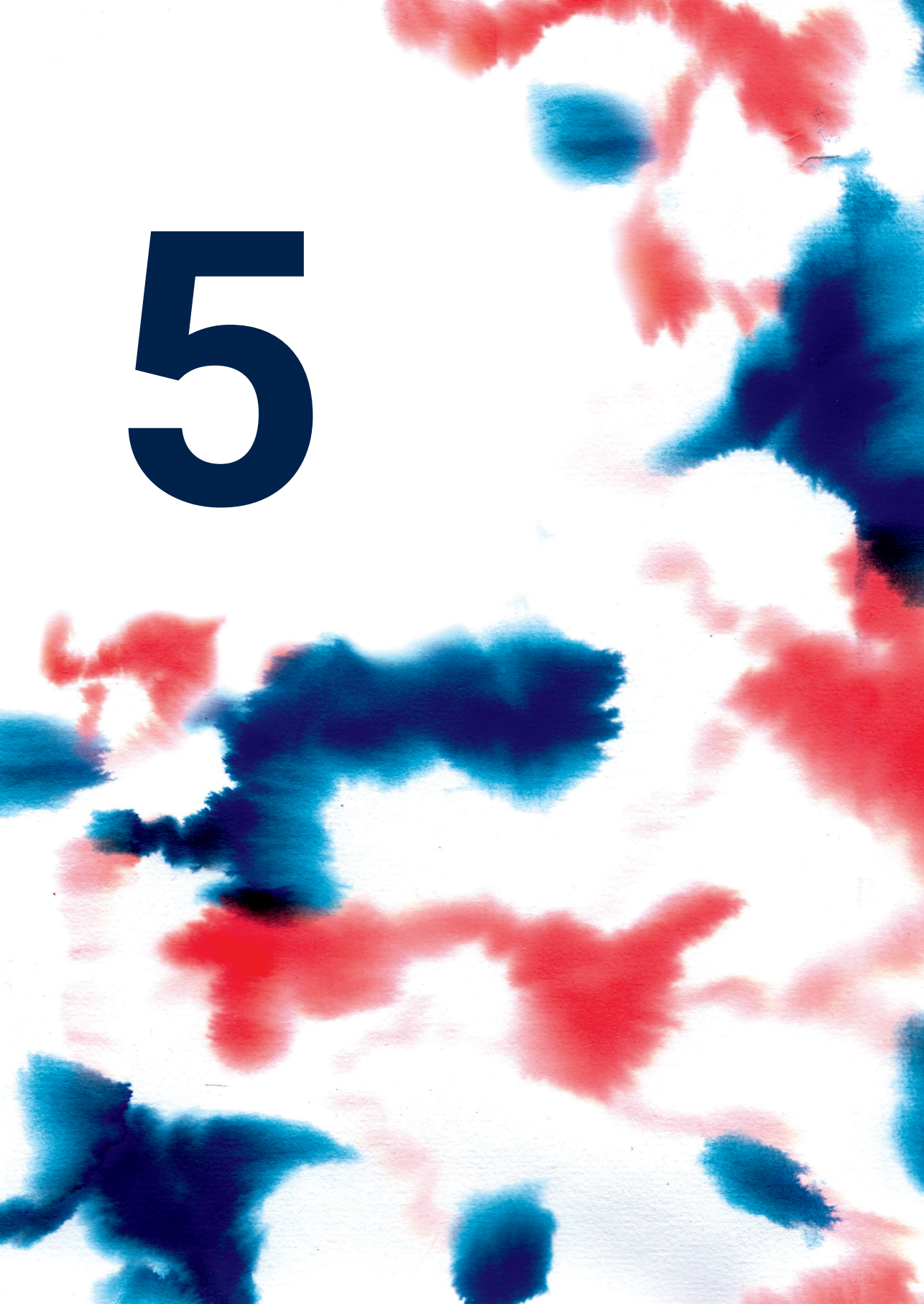
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HISTOPATHOLOGY OF CEREBRAL MICROINFARCTS AND MICROBLEEDS IN SPONTANEOUS INTRACEREBRAL HEMORRHAGE

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ABSTRACT

Objective

To explore the histopathological substrate of cerebral microinfarcts (CMIs) and cerebral microbleeds (CMBs) in patients with lobar and non-lobar intracerebral hemorrhage (ICH).

Methods

We studied 20 patients (11 women, median age at death 77 years) who died from ICH (9 lobar, 11 non-lobar). From each patient, we scanned three formalin-fixed coronal brain slabs with 7 tesla MRI. Images were examined for CMIs and CMBs. Per patient at least one (if present) CMI and CMB was sampled and processed for histopathological analysis. We assessed lesion location within the cortex, CAA severity, concentric vessel wall splitting, loss of vascular smooth muscle cells, and leakage of fibrin.

Results

We identified 132 CMIs and 204 CMBs on MRI, with higher numbers of CMIs in lobar ICH (median 6, IQR 1-33) compared to non-lobar ICH patients (median 1, IQR 0-2; p=0.03), and similar numbers of CMBs per patient (lobar ICH median 1, IQR 0-42; non-lobar ICH median 2, IQR 0-8; p=0.83). We examined 62 CMIs and CMBs in detail on histopathology. CMIs and CMBs were in lobar ICH more often located in the superficial than in the deep layers of the cortex, and in non-lobar ICH more often located in the deeper layers of the cortex. Other histopathological characteristics were comparable between lobar and non-lobar ICH patients.

Conclusions

Although CMIs and CMBs were found in different segments of the cortex in lobar ICH patients compared to non-lobar ICH patients, otherwise similar histopathological features of cortical CMIs and CMBs distant from the ICH suggest shared pathophysiological mechanisms in CAA-related and arteriolosclerotic vasculopathy-related ICH.

INTRODUCTION

The etiology of spontaneous intracerebral hemorrhage (ICH) is still poorly understood. Classically, non-lobar (deep and infratentorial) ICH has been associated with arteriolosclerotic vasculopathy of the deep penetrating blood vessels caused by longstanding high blood pressure, diabetes and alcohol overuse,[1](Jolink et al; accepted Neurology 2020) and lobar ICH in elderly patients with cerebral amyloid angiopathy (CAA) in the leptomeningeal and cortical blood vessels.[2] However, recent studies found that arteriolosclerotic vasculopathy and CAA often exist together, and clinical and imaging phenotypes may overlap.[3, 4]

High blood pressure is not only an important risk factor for non-lobar ICH, but also for lobar ICH, although with a smaller effect.(Jolink et al. accepted Neurology 2020) On MRI, lobar and non-lobar ICH share manifestations of cerebral small vessel disease (cSVD), including white matter hyperintensities, enlarged perivascular spaces, lobar and deep CMBs, and cortical superficial siderosis.[5, 6] Recent studies have also identified focal ischemic lesions in both patients with lobar and patients with non-lobar ICH.[7, 8] Small diffusion-weighted imaging (DWI) positive lesions, indicative of acute cerebral microinfarcts (CMIs), are found in both lobar and deep regions of the brain in approximately 20% of patients with lobar or non-lobar ICH.[8, 9] With high field MRI, chronic CMIs can be visualized in the cortex.[10, 11] This method recently revealed that chronic cortical CMIs are common in patients with lobar and in those with non-lobar ICH and that they co-occur with (deep and lobar) CMBs.[12]

In patients with spontaneous ICH caused by different vasculopathies, CMIs and CMBs in different locations have the same aspect on MRI. It is unclear what vascular changes underlie these MRI-visible CMIs and CMBs. The aim of this study was to determine whether the histopathological characteristics of cortical CMIs and CMBs found on post-mortem 7 tesla (T) MRI scans differ between patients with lobar and non-lobar ICH.

MATERIALS AND METHODS

Patients

From the database of autopsy cases of the University Medical Center Utrecht (UMCU), we included 12 consecutive patients who died as a consequence of an ICH and underwent autopsy between 2008 and 2015, including six lobar, four deep, one cerebellar, and one pons hemorrhage. One of the six lobar ICH patients was a patient with hereditary Dutch-CAA (D-CAA; also known as hereditary cerebral hemorrhage with amyloidosis – Dutch type, a hereditary form of CAA), which had been diagnosed during life. We selected eight additional consecutive patients from

the Netherlands Brain Bank (NBB) of patients who died as a consequence of ICH between 1997 and 2013, including three lobar, four deep, and one pons hemorrhage. The presumed cause (*i.e.* CAA or arteriolosclerotic vasculopathy) of the ICH was extracted from the pathology reports from routine pathological examination, which included screening for arteriolosclerotic changes and Congo red stain and/or immunohistochemistry against amyloid β (Aβ). Hypertension was defined as a reported history of hypertension or use of antihypertensive medication as registered in the pathology reports and/or the medical record for the patients from the UMCU. We based presence of large vessel atherosclerosis on the pathology reports.

The brain samples obtained from The NBB, Netherlands Institute for Neuroscience, Amsterdam (open access: www.brainbank.nl), had been collected from donors that had provided written informed consent for the use of autopsy material and clinical information for research purposes. For patients from the UMCU, informed consent was obtained prior to autopsy, according to local ethical guidelines. The study was approved by the medical research ethics committee of the UMCU.

MRI acquisition and analysis

From each patient, we selected three 10-mm-thick formalin-fixed coronal brain slabs from the frontal, parieto-temporal and occipital regions of the brain. Per patient we submerged the three slabs in 10% formalin in a purpose-built Perspex container that fitted in the head coil of the MR scanner. We specifically removed any air bubbles, because gradient echo sequences are susceptible to artifacts caused by air bubbles.

Scans were acquired on a whole body 7 T MRI system (Philips, Best, the Netherlands) with a dual transmit and 32-channel receive head coil (Nova Medical, USA). The protocol included a 3D fluid attenuated inversion recovery (FLAIR) sequence (acquired isotropic resolution of 400×400×400 μm^3, repetition time (TR) = 8,000 ms, nominal echo time (TE) = 164 ms, scan duration 5 hours 20 minutes 8 seconds), a 3D T_2-weighted turbo spin echo (TSE) (acquired isotropic resolution of 400×400×400 μm^3, TR = 3,500 ms, nominal TE = 164 ms, scan duration 1 hour 52 minutes 3 seconds), a 3D T_1-weighted sequence (acquired isotropic resolution of 400×400×400 μm^3, TR = 7.7 ms, TE = 3.5 ms, TR between inversion pulses 2,000 ms, flip angle 6°, scan duration 1 hour 9 minutes 36 seconds) and a 3D T_2*-weighted sequence (acquired isotropic resolution of 180×180×180 μm^3, flip angle 25°, TR = 75 ms, TE = 20 ms, scan duration 4 hours 59 minutes 31 seconds).

The acquired MR images were rated for cortical CMIs and CMBs by two trained readers (WMTJ, SJvV) independently and blinded to hemorrhage location and diagnosis. Discrepancies were resolved in a consensus meeting. CMIs were defined as small (≤5 mm) cortical lesions,

hyperintense on T_2, isointense on T_2* and hypointense on T_1-weighted sequences.[10, 11] We only looked at cortical CMIs, since the current detection criteria do not discriminate CMIs in deeper areas of the brain from other pathologies, such as enlarged perivascular spaces and white matter hyperintensities. CMBs were defined as small (≤10 mm) round or ovoid lesions of signal void on T_2-weighted sequences with associated blooming on T_2*-weighted sequence.[13, 14] For CMBs we screened for both cortical and deep CMBs. Lesions were annotated in MeVisLab (MeVis Medical Solutions, Bremen, Germany).

Tissue sampling and histopathological analysis

From each patient, if present, we sampled at least one CMI and CMB. In addition, from the D-CAA patient we took a total of eight samples to allow a more extensive comparison of this patient with the sporadic CAA patients.

All samples were dehydrated, embedded in paraffin and cut in 6-µm-thick sections on a microtome. Guided by the MR images we attempted to retrieve the lesions; based on tissue architecture we estimated the depth of the lesion in the tissue block. We took ten sections around the estimated lesion location at three depths with a slice gap of approximately 500 µm. Standard hematoxylin & eosin (H&E) staining was performed on the first sections of the three series. After successful retrieval of lesions, adjacent sections underwent Perls' Prussian Blue staining (for Iron) and immunohistochemistry against Aβ (clone 6F/3D, Agilent, Dako), smooth muscle cells (SMC; Agilent, Dako) and fibrin(ogen) (Agilent, Dako), using methods that have previously been described in detail.[15]

Microscopic analysis

All sections were imaged with brightfield microscopy using the Hamamatsu NanoZoomer Digital Pathology-HT scanner (C9600-12, Hamamatsu Photonics KK, Japan) and examined by two observers (WMTJ and SJvV), using the viewing platform NDP.View (version 2.6.13.0). If needed, sections were discussed with two experienced neuropathologists (AJMR, MPF). CMIs and CMBs were evaluated on H&E stained sections. CMI identification was based on areas of tissue pallor with evidence of eosinophilic necrosis or "red" neurons (acute CMIs) or cell loss with cavitation or "puckering" (chronic CMIs). CMBs were identified by the presence of erythrocyte extravasation (acute CMBs) or blood breakdown products, such as hematoidin or hemosiderin (chronic CMBs). Vessels near the lesions and on adjacent sections were examined to identify the involved vessel. We used a piloted assessment form to score characteristics of each identified lesion. We determined the location of the lesion in the cortex (superficial/along penetrating cortical arteriole [layers I-III], deeper in cortex [layers IV-VI] or subcortical) and scored the severity of CAA (as described by Vonsattel),[16, 17] presence of capillary CAA (CAA type 1 = capillary CAA present, type 2 = no capillary CAA),[18] presence of concentric vessel wall

splitting (as a marker of severity of vasculopathy),[16, 19] and loss of SMCs in the walls of vessels in close proximity to the lesions and for vessels present in the surrounding area (*i.e.* average impression of the whole section containing the lesion) of the CMI and CMB, using a 4-point scale (0 = absent, 1 = mild, 2 = moderate, and 3 = severe) for each marker. In addition, we determined the presence of fibrin(ogen) in the walls of the involved vessel and in the surrounding cells around a lesion, also on a 4-point scale (0 = absent, 1 = mild, 2 = moderate, and 3 = severe), as a measure for blood-brain barrier leakage.[15]

Statistical analysis

We used the χ^2 test, Fisher's exact test and Mann-Whitney U test, as appropriate, to analyze group differences in number of MRI-observed CMIs and CMBs, lesion location in the cortex, CAA severity (score ≥2 vs <2), severity of vessel wall splitting (score ≥2 vs <2), loss of SMCs (score ≥2 vs <2) and presence of fibrin in vessel wall and surrounding cells (score ≥2 vs <2) between patients with lobar and non-lobar ICH, and between sporadic CAA and D-CAA.

Data availability

Data used in this study are available to qualified investigators upon request to the corresponding and senior authors.

RESULTS

Table 5.1 summarizes the characteristics of the 20 included patients. Median age at time of death was 77 years (IQR 51-83), 55% were females and 35% of the patients had a known history of hypertension. Location of the ICH was lobar in nine patients and non-lobar in 11 patients (eight deep and three infratentorial ICH). Median age at death in patients with lobar ICH was 78 years (IQR 75-82) and in non-lobar patients 55 years (IQR 45-85). Eleven percent of patients with lobar ICH and 55% of patients with non-lobar ICH had a known history of hypertension. Based on the routine pathological examination six of the nine patients with lobar ICH had sporadic CAA, one patient had D-CAA (genetically proven during life), one patient showed mixed pathology (abnormalities consistent with both arteriolosclerotic vasculopathy and CAA), and one patient showed neither arteriolosclerotic vasculopathy nor CAA. Of the 11 patients with non-lobar ICH, five had abnormalities consistent with arteriolosclerotic vasculopathy in the basal ganglia, two patients had mixed pathology (abnormalities indicating both arteriolosclerotic

Table 5.1. Patient characteristics

Case no.	Age at death (years)	Sex	Location ICH	Hemisphere	Hypertension	Diagnosis (based on PA reports)	Large vessel atherosclerosis[a]
1	56	M	Lobar	Right	No	D-CAA[b]	Mild
2	76	M	Lobar	Right	No	CAA	Mild
3	78	F	Lobar	Left	No	Lobar ICH, no CAA	Mild to moderate
4	80	M	Lobar	Unknown	Unknown	CAA	Unknown
5	85	M	Lobar	Right	No	CAA and arteriolosclerotic vasculopathy	Moderate to severe
6	78	M	Lobar	Both	Yes	CAA	None
7	83	F	Lobar	Right	No	CAA	Moderate
8	81	F	Lobar	Left	No	CAA	Severe
9	73	F	Lobar	Left	No	CAA	Unknown
10	81	M	Deep	Right	Yes	Arteriolosclerotic vasculopathy	Moderate to severe
11	89	F	Deep	Left	Yes	Arteriolosclerotic vasculopathy	Moderate to severe
12	89	F	Deep	Left	Yes	Arteriolosclerotic vasculopathy and CAA	Severe
13	49	F	Deep	Left	Yes	Arteriolosclerotic vasculopathy	Mild to moderate
14	44	F	Deep	Left	No	Arteriolosclerotic vasculopathy	Mild to moderate
15	46	M	Deep	Right	Yes	Arteriolosclerotic vasculopathy	Severe
16	40	F	Deep	Right	No	Coagulation disorder in hepatic insufficiency	Mild
17	85	F	Deep	Right	Yes	Arteriolosclerotic vasculopathy and CAA	Severe
18	55	M	Cerebellum	Left	No	Minimal arteriolosclerotic vasculopathy	Moderate
19	75	F	Pons	NA	No	Arteriolosclerotic vasculopathy and use of oral anticoagulants	Severe
20	45	M	Pons	NA	No	Atherosclerosis large vessels, lacunae, no other abnormalities small vessels	Moderate

CAA = cerebral amyloid angiopathy, D-CAA = Dutch-type CAA, F = female, ICH = intracerebral hemorrhage, M = male, [a] based on PA report; [b] Diagnosed during life by genetic testing vasculopathy and CAA), one patient had arteriolosclerotic vasculopathy and used a vitamin-K antagonist at the time of ICH, one patient had a coagulation disorder due to hepatic insufficiency and heavy alcohol intake, and two patients showed moderate atherosclerosis in the large cerebral arteries, but no abnormalities in the small vessels.

Ex vivo 7 T MRI findings

On the ex vivo 7 T MR images, four patients had no microvascular lesions, including one patient with CAA (no. 6), one patient with deep ICH associated with a coagulation disorder in hepatic insufficiency (no. 16), one patient with cerebellar ICH (no. 18), and one patient with a pons hemorrhage (no. 15). In the patient with D-CAA we found a total of seven CMIs and 29 CMBs (all cortical) on ex vivo 7 T MRI, which we analyzed separately as described below. See figure 5.1 for a flow chart of the identified, retrieved and evaluated CMIs and CMBs.

In the remaining 15 patients, we found a total of 125 cortical CMIs, 170 cortical CMBs and 5 deep CMBs. Of the 125 CMIs, 32 (26%) were found in the frontal slabs, 38 (30%) parieto-temporal and 55 (44%) occipital and of the 175 CMBs, 26 (15%) were located in the frontal slabs, 94 (54%) parieto-temporal and 55 (31%) occipital. In patients with lobar ICH (n=7; six with CAA) compared to non-lobar ICH (n=8; two with mixed pathology), we found more CMIs (lobar ICH: median 6 (IQR 1-33), non-lobar ICH: median 1, (IQR 0-2), p=0.03). We also found a higher total number of CMBs in lobar ICH patients, but the median number per patient was not significantly different between lobar (median 1 (IQR 0-42)) and non-lobar ICH (median 2 (IQR 0-8)) patients (p=0.83). We found a higher median number of CMIs in parieto-temporal regions in lobar ICH patients (median 2, IQR 1-8), compared to non-lobar ICH patients (median 0, IQR 0-1, p=0.01), but not in other areas. After evaluation of the pathology slides, we excluded one patient with lobar ICH and CAA (no. 9) from further analysis, because of the presence of extensive hypoxic-ischemic changes throughout the tissue. Notably, the exclusion of this patient did not change the finding of more CMIs on MRI in the lobar ICH patients (median 3, IQR 0-54), compared to the non-lobar ICH patients (median 1, IQR 0-2, p=0.05).

We selected 71 of the lesions that we identified on MRI (30 CMIs and 41 CMBs) for histopathological analysis. On the corresponding sections, we could retrieve 27 lesions (38%; 20% of the CMIs and 51% of the CMBs). The lesions we could not retrieve were mostly small. We found 35 additional lesions after screening each section included in the MRI-targeted analysis, of which 18 could be identified on MRI in retrospect. Histopathology of the targeted MRI lesions confirmed the lesions as CMI or CMB in all (figure 5.2). In three patients (1 lobar [no. 6] and two deep ICH [no. 10 and 14]) we could not retrieve the targeted lesions and found no additional lesions. We could also not retrieve the targeted lesions in deep areas (case no. 15); hence we limited the analysis to the 62 cortical lesions, 40 lesions in 10 patients with lobar or non-lobar ICH, and 22 lesions in the patient with D-CAA.

Figure 5.1 Flow chart of CMIs and CMBs identified on 7 T MRI, retrieved on histopathology and evaluated in detail.

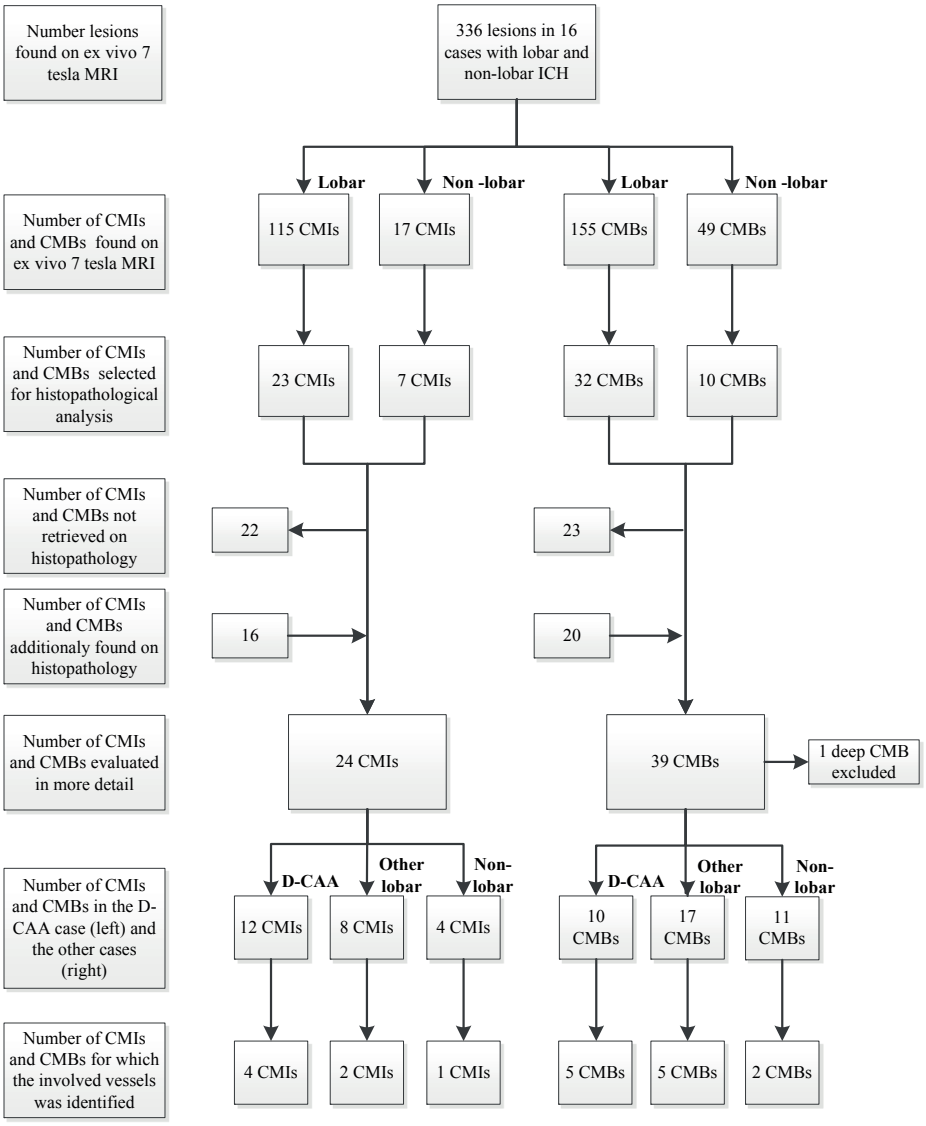
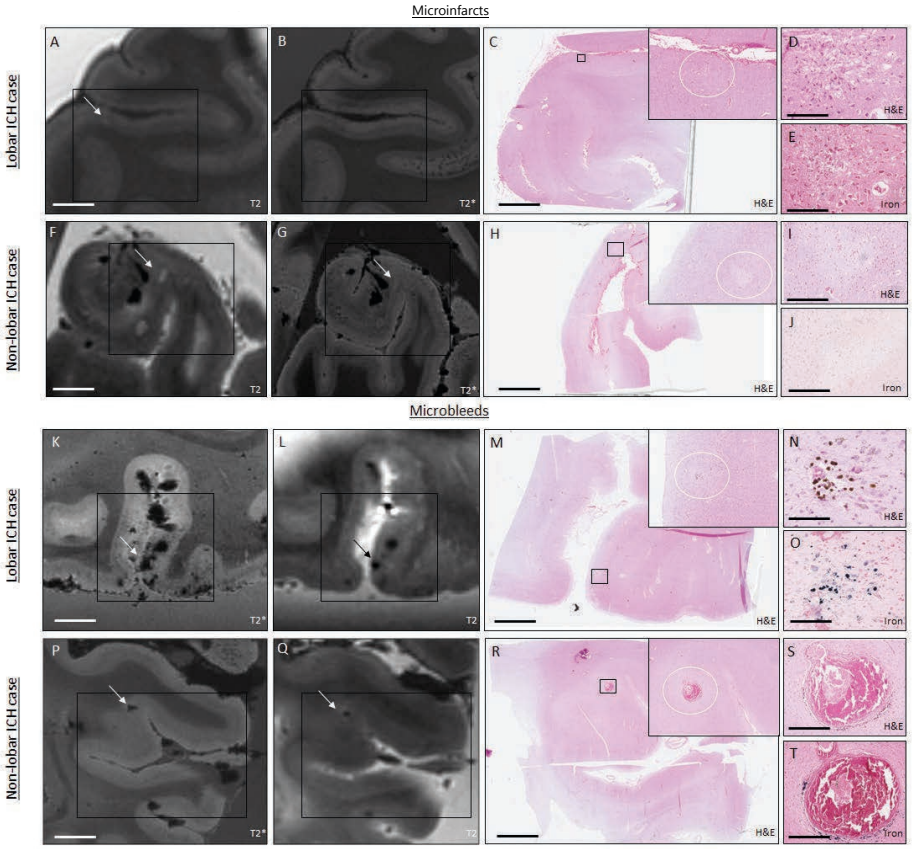


Figure 5.2 Representative examples of microbleeds and microinfarcts and their location in the cortex on ex vivo 7 T MRI and histopathology in lobar and non-lobar ICH patients



Representative examples of microinfarcts (A-J) and microbleeds (K-T) in patients with lobar ICH (A-E [case no. 1] and K-O [case no. 8]) and non-lobar ICH (F-J [case no.17] and P-T [case no. 11]) indicated with the black and white arrows. From each lesion we show ex vivo 7 T T_2 (A,F, L and Q) and T_2* (B, G, K and P) sequences, an overview of the H&E section (C, H, M and R) and the identified lesion on H&E (D, I, N and S) and iron staining (E, J, O and T).
The inset in panels C, H, M and R shows in more detail the location of the CMIs and CMBs in the superficial layers of the cortex in lobar ICH (C and M) and in the deeper layers of the cortex in non-lobar ICH (H and R). Scale bars in A, B, F, G, K, L, P and Q are 10 mm; in C, H, M and R 5 mm; in D, E, N and O 200 µm; and I, J, S and T 400 µm.

HISTOPATHOLOGICAL CHARACTERISTICS OF AREAS AROUND CMBS AND CMIS IN LOBAR AND NON-LOBAR ICH PATIENTS

In five patients with lobar ICH (four with CAA, one with mixed pathology) we found eight cortical CMIs and 17 CMBs and in five patients with non-lobar ICH (three with arteriolosclerotic vasculopathy and two with mixed pathology), we found a total of four cortical CMIs and 11 CMBs.

In patients with lobar ICH the cortical CMIs and CMBs were on histopathology more frequently located in the superficial layers of the cortex (layers I-III; lobar ICH: 63% of the CMIs and 71% of the CMBs, non-lobar ICH 9% of the CMBs and 25% of the CMIs; p=0.001), whereas in non-lobar ICH patients CMIs and CMBs were mostly located in the deeper layers of the cortex (layers IV-VI; lobar ICH: 38% of the CMIs and 6% of the CMBs, non-lobar ICH 50% of the CMIs and 55% of the CMBs; p=0.03; table 5.2 and figure 5.2). When we evaluated the corresponding CMIs and CMBs on the 7 T MR images in retrospect, differences in cortical lesion location between patients were not significant (p<0.20). This was due to five lesions (3 CMIs and 2 CMBs) that were not identified on MRI in retrospect and four lesions (1 CMI and 3 CMBs) that were classified as located in the deep cortical layers on the section and superficial on MRI.

Table 5.2 Histopathological characteristics of identified lesions in lobar and non-lobar ICH

Lobar vs non-lobar ICH	Lobar ICH[a] (n = 25 lesions)[b]	Non-lobar ICH (n = 15 lesions)[c]	p-value
Type of lesion[d]			
CMI, n (%)	8 (32)	4 (26.7)	
CMB, n (%)	17 (68)	11 (73.3)	
Location of lesion			
Occipital, n (%)	6 (24)	5 (33.3)	
Parieto-temporal, n (%)	13 (52)	7 (46.7)	
Frontal, n (%)	6 (24)	3 (20)	
Location in the cortex			
Superficial cortex (layers I-III)	17 (68)	2 (13.3)	0.001
Deep cortex (layers IV-VI)	4 (16)	8 (53.3)	0.03
Subcortical	4 (16)	5 (33.3)	0.26
Presence of microaneurysms, n (%)	0 (0)	1 (6.7)	0.38
Presence of fibrinoid necrosis, n (%)	8 (32)	4 (26.7)	0.72
CAA score ≥2	19 (76)	7 (46.7)	0.09
VWS score ≥2	19 (76)	8 (53.3)	0.18
Loss of SMCs score ≥2[e]	14/21 (56)	8/14 (57.1)	0.72
Fibrin leakage score ≥2			
Vessel walls[f]	5/10 (50)	4/10 (40)	0.65
Surrounding cells[g]	10/19 (52.6)	11/15 (73.3)	0.30

CAA = cerebral amyloid angiopathy, CMB = cerebral microbleed, CMI = cerebral microinfarct, ICH = intracerebral hemorrhage, SMC = smooth muscle cell, VWS = vessel wall splitting

[a] Excluding the patient with D-CAA

[b] Cases: no.2 (2 CMIs, 2 CMBs), no 3. (1 CMI, 3 CMBs), no. 7 (4 CMBs), no. 8 (1 CMI, 8 CMBs), no. 9(4 CMIs)

[c] Cases: no. no 11. (4 CMBs), no. 12 (2 CMBs), no. 13 (2 CMIs), 17 (2 CMIs, 4 CMBs), no. 19 (CMB)

[d] We performed no statistical test for this variable, because it is a selection of lesions based on MRI

[e] In 5 lesions (4 in lobar ICH patients and 1 a non-lobar ICH patient) the vascular smooth muscle cells stained section was not available

[f] In 14 lesions (9 in lobar ICH patients and 5 in non-lobar ICH patients) the involved vessels were not identified and in 6 lesions (all in lobar ICH patients) no fibrin(ogen) stained section was available

[g] In 6 lesions (all in lobar ICH patients) the fibrin(ogen) stained section was not available

Patients with lobar ICH tended to have more often moderate or severe CAA (score ≥2) in the surrounding areas of CMIs and CMBs compared to non-lobar ICH patients (p=0.09; figure 5.3 and table 5.2). Lobar and non-lobar ICH patients were otherwise comparable in terms of presence of moderate or severe vessel wall splitting (score ≥2) in the surrounding area (p=0.18), moderate or severe loss of SMCs (score ≥2) (p=0.72) and moderate or severe leakage of fibrin (score ≥2) in the surrounding vessel walls (p=0.64) and the surrounding cells (p=0.30).

Exploratory comparisons between sporadic CAA and D-CAA

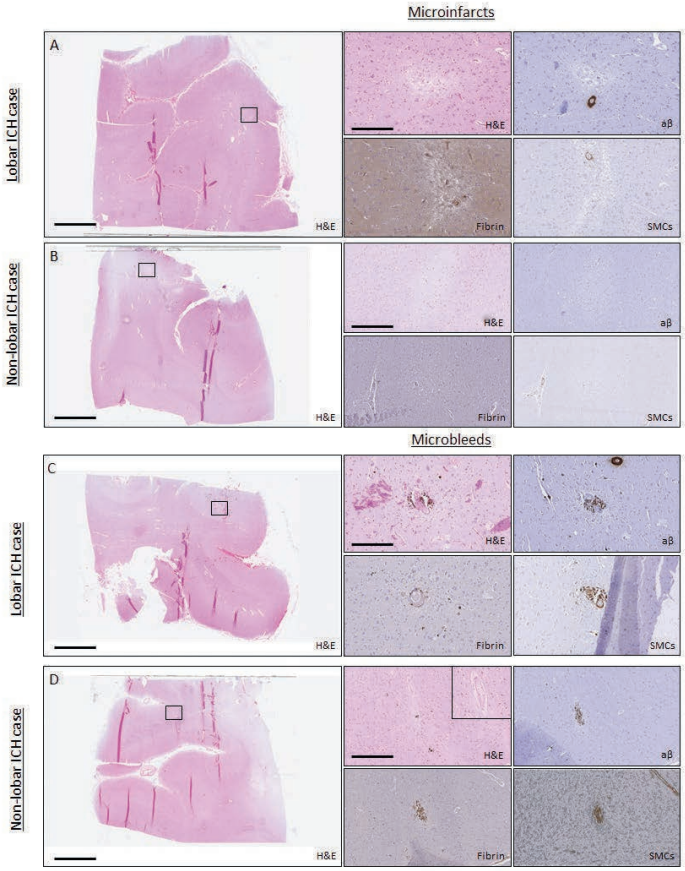
In the patient with D-CAA (age at death 56 years) we found a total of 22 lesions with the combined MRI-targeted and untargeted approach. Twelve of the 22 lesions were CMIs and ten were CMBs. We compared these lesions to 21 lesions found in four patients with lobar ICH and sporadic CAA (median age at death 80, IQR 75-82), including seven CMIs and 14 CMBs,

The location of the lesions in the cortex was in the superficial layers (layers I-III) in the majority of lesions in sporadic CAA patients (61.9%) and the D-CAA patient (77.3%; supplementary table 5.e-1). Presence of moderate or severe CAA (score ≥2) in the surrounding areas in sporadic CAA patients (90.5% of lesions) was comparable to the D- CAA patient (100%). All patients with sporadic CAA had CAA type 2 (i.e. no capillary CAA) and the D-CAA patient had CAA type 1 (i.e. presence of capillary CAA). Between sporadic CAA and D-CAA we also found similar percentages of lesions with moderate or severe vessel wall splitting (score ≥2) of the vessels in the surrounding areas (sporadic CAA patients: 71%; D-CAA patients: 59%), similar percentages of lesions with moderate or severe loss of SMCs (score ≥2) of the vessels in the surrounding areas (sporadic CAA patients: 62%; D-CAA patient: 77%), and similar percentages of lesions with fibrin leakage in the vessel walls (sporadic CAA patients: 50%; D-CAA patient: 55%) and surrounding cells (sporadic CAA patients: 53%; D-CAA patient: 50%). See figure 5.4 for representative examples.

Descriptive findings regarding the involved vessels

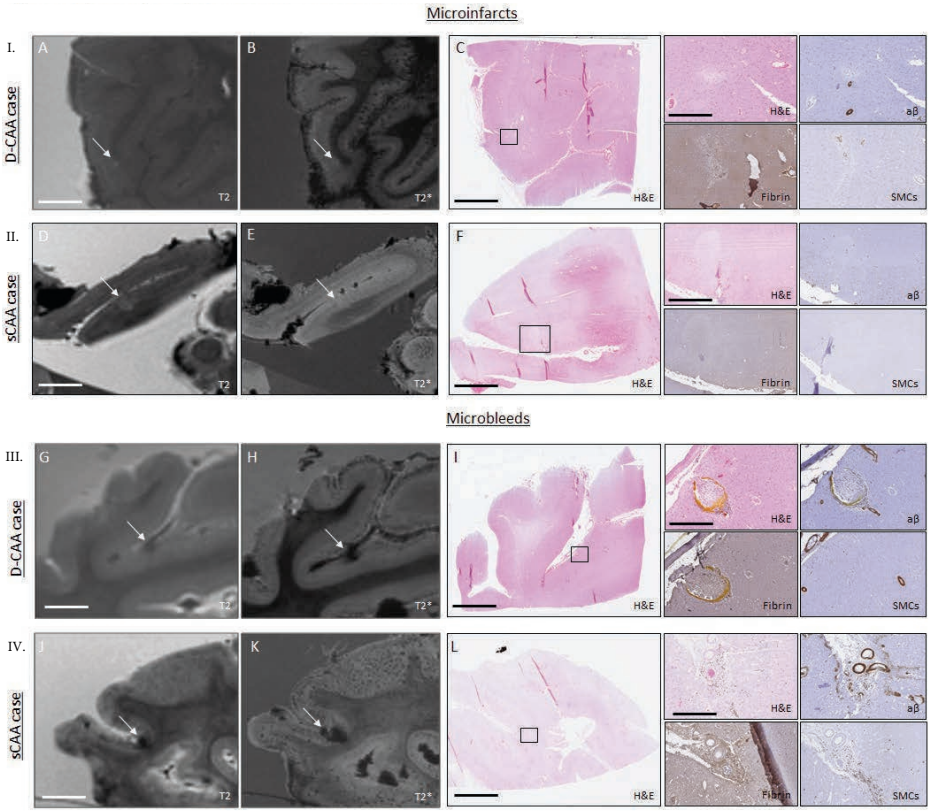
From the 40 lesions in lobar and non-lobar ICH patients, we were able to identify the involved cortical vessel for three CMIs and seven CMBs (table 5.e-2), allowing only a qualitative assessment of these "culprit" vessels involved in CMBs. Notably, even though the vessel walls appeared abnormal (see for example figure 5.2S), severe CAA (score ≥2) of the involved vessels was infrequently observed in both non-lobar (1 of 3 CMBs) and lobar ICH patients (0 of 4 CMBs) (see for example figure 5.3C). In comparison, we identified the involved vessel for five CMBs in the D-CAA patient. Interestingly, 4/5 vessels involved in CMBs showed severe CAA (see for example figure 5.4I).

Figure 5.3 Histopathological characteristics of microbleeds and microinfarcts in lobar and non-lobar ICH patients



A. A chronic CMI in a patient with D-CAA (case no 1.), located in the deeper layers of the cortex, visible as an area with cell loss and gliosis on H&E, a severe CAA score of the section, a moderate vessel wall splitting score, severe uptake of fibrin in the surrounding cells, and moderate loss of SMCs of the vessels in the section.
B. An acute CMI in a patient with a deep ICH due to arteriolosclerotic vasculopathy on histopathology (case no. 13), located in the deeper layers of the cortex, visible as tissue pallor and red neurons on H&E, no presence of CAA, a moderate vessel wall splitting score in the section, mild uptake of fibrin in the surrounding vessel walls and a moderate uptake of fibrin in the surrounding cells, and mild loss of SMCs of the vessels in the section.
C. A chronic CMB in a patient with D-CAA (case no. 1) located in the deeper layers of the cortex visible as hemosiderin deposits on H&E, a moderate CAA score of the section but no amyloid β in the direct vicinity of the CMB, absence of vessel wall splitting on the section, mild uptake of fibrin in the vessel walls but moderate uptake in the surrounding cells, and mild loss of SMCs in the surrounding vessels and the rest of the section.
D. A chronic CMB in a patient with a deep ICH due to arteriolosclerotic vasculopathy on histopathology (case no. 11), located in the deeper layers of the cortex, visible as hemosiderin deposits on H&E, a mild CAA score of the section, a severe vessel wall splitting score in the section, no uptake of fibrin in the vessel walls and moderate uptake of fibrin in the surrounding cells, and moderate loss of SMCs of the vessels in the section. The inset shows the changes in the involved vessel with mild vessel wall splitting.
Scale bars in the section overviews are 5 mm. The scale bars in the stained sections zoomed in on lesions are 200 µm for lesions A and C and 400 µm for B and D.

Figure 5.4 Exploratory comparison between sporadic CAA and D-CAA



I. A CMI in a patient with D-CAA (case no. 1) on ex-vivo 7 T MRI T_2-weighted (A) and T_2*-weighted (B) images visible as an old CMI on histopathology (C) in the deeper layers of the cortex, visible as cell loss and cavitation on H&E, a severe CAA score on the amyloid stain, a moderate vessel wall splitting score in the section, moderate uptake of fibrin in the vessel walls and severe uptake of fibrin in the surrounding cells, and moderate loss of SMCs of the vessels in the section.
II. A CMI in a patient with sporadic CAA (case no. 17) on ex-vivo 7 T MRI T_2-weighted (D) and T_2*-weighted (E) images visible as an acute CMI on histopathology (F) in the deeper layers of the cortex, visible as tissue pallor and presence of red neurons on H&E, a moderate CAA score on the amyloid stain, no presence of vessel wall splitting in the section, mild uptake of fibrin in the vessel walls and no uptake of fibrin in the surrounding cells, and severe loss of SMCs of the vessels in the section
III. A CMB in a patient with D-CAA (case no. 1) on ex-vivo 7 T MRI T_2-weighted (G) and T_2*-weighted (H) images visible as an old CMB on histopathology (I) in the superficial layers of the cortex, visible as hemosiderin and hematoidin deposit on H&E, a severe CAA score on the amyloid stain, a moderate vessel wall splitting score in the section, mild uptake of fibrin in the vessel walls and no uptake of fibrin in the surrounding cells, and moderate loss of SMCs of the vessels in the section.
IV. A CMB in a patient with sporadic CAA (case no. 7) on ex-vivo 7 T MRI T_2-weighted (J) and T_2*-weighted (K) images visible as an old CMB on histopathology (L) in the superficial layers of the cortex, visible as hemosiderin deposits on H&E, a severe CAA score on the amyloid stain, a mild vessel wall splitting score in the section, mild uptake of fibrin in the vessel walls and no uptake of fibrin in the surrounding cells, and severe loss of SMCs of the vessels in the section.
Scale bars A, D, G and J are 10 mm; in section overviews C, F, I and L 5 mm, in the stained sections zoomed in on lesions are 400 µm for lesions in C, I and L and 2,5 mm for F.

DISCUSSION

This study found more CMIs, but a similar number of CMBs on 7 T MRI in lobar ICH patients compared to non-lobar ICH patients, while on histopathology, the underlying histopathological signature of the CMIs and CMBs in the cortex was comparable in lobar and non-lobar ICH. We found a tendency towards more severe CAA scores in lobar ICH patients, but severity of vessel wall splitting, loss of smooth muscle cells and fibrin leakage around CMIs and CMBs was similar in lobar and non-lobar ICH. CMIs and CMBs in lobar ICH were located predominantly in the superficial layers of the cortex whereas in non-lobar ICH these lesions occur mostly in the deeper layers of the cortex. Severity of CAA, vessel wall splitting, loss of smooth muscle cells and fibrin leakage associated with CMIs and CMBs appeared similar between sporadic CAA, arteriolosclerosis and D-CAA etiologies.

The characteristic histopathological features of CAA include accumulation of amyloid β in the media of parenchymal arterioles with progressive loss of smooth muscle cells and secondary changes consisting of fibrinoid necrosis, vessel wall thickening, microaneurysm formation, and perivascular deposition of blood break down products.[2, 18, 19] Arteriolosclerotic vasculopathy is characterized by changes in small, perforating vessels with collagenous vessel wall thickening with lumen narrowing and also progressive loss of smooth muscle cells, exudation of fibrin and other serum proteins, microaneurysms, fibrinoid necrosis and lipohyalinosis.[1, 2] We found similar histopathologic characteristics in lobar and non-lobar ICH, which is in line with accumulating evidence suggesting that the histopathological features of the two types of small vessel disease often co-exist in patients.[3] A recent study found that 42% of participants with lobar ICH had moderate or severe arteriolosclerosis in addition to moderate or severe CAA on pathological examination.[3] Moreover, in that study 39% of participants with lobar ICH had moderate or severe arteriolosclerosis alone and 13% of participants with non-lobar ICH had moderate or severe CAA.[3] Another study describing a neuropathological cohort of ICH patients showed that 11 of the 38 (28.9%) patients with hypertension and lobar or non-lobar ICH had CAA and that 61% of ICH patients with CAA had hypertension, suggesting an interaction between CAA and arteriolosclerotic vasculopathy in the pathophysiology of ICH. However, they did not describe the presence of arteriolosclerotic changes in the vessels.[4] In contrast to traditional conceptualization, CAA and arteriolosclerotic vasculopathy may more commonly co-occur than previously thought and may share some pathophysiological pathways.[4, 20, 21] It is possible that drainage around vessel with arteriolosclerosis is hampered and that this can lead to more CAA.

We found that in patients with lobar ICH, CMIs and CMBs were mainly located in the superficial layers of the cortex. This is in line with a recent study of 12 patients with definite CAA, which found more than 50% of the described CMIs (49%) and CMBs (77%) in the more superficial layers

of the cortex.[22] We also found this percentage to be higher for CMBs (71%) than CMIs (63%). In our study we were able to study patients with non-lobar ICH as well. We found that in non-lobar ICH patients CMIs (55%) and CMBs (50%) were more often found in the deeper layers of the cortex. This finding suggests that different types of vasculopathy affect distinct categories of arterioles. Superficial perforating cortical arterioles are potentially more vulnerable to CAA and deep perforating cortical arterioles more to arteriolosclerosis, but the histopathological consequences of the impingement appear similar.

We also focused on individual vessels involved in CMIs and CMBs. The abnormal appearance of vessels involved in CMBs in both lobar and non-lobar ICH patients in our study suggests reduced vessel integrity including vessel wall breakdown. The absence of severe CAA in these involved vessels suggests that the presence of vascular Aβ is not necessarily required to induce bleeding, but that complex pathways – potentially involving vascular remodeling – likely play a role.[13] Regarding CMIs, a shared underlying pathophysiology in lobar and non-lobar ICH may include thickening of the vessel wall with increased CAA severity (in lobar ICH), and loss of SMCs, although this notion requires further experimental investigations.[13]

In lobar ICH compared with non-lobar ICH patients, but also in sporadic CAA patients compared with the D-CAA patient, we found in 40-70% of lesions blood-brain barrier leakage, expressed by the presence and severity of fibrin in the vessel walls and surrounding cells of CMIs and CMBs. This is in line with a recent systematic review of 10 animal studies and 16 studies in humans showing indications of blood-brain barrier dysfunction in both ICH related to CAA (9 of 12 studies) and arteriolosclerotic vasculopathy related ICH (4 out of 5 studies).[23]

Despite the difference in age at death between the sporadic CAA patients (median age at death 80 years) and the D-CAA patient (age at death 56 years), overall CAA burden and individual histopathological features of CMIs and CMBs were comparable,[24] except for the increased deposition of vascular Aβ in walls of vessels involved in CMBs.

Strengths of our study are the combination of post-mortem 7 T MRI and histopathological examination to identify and characterize microvascular lesions in patients with spontaneous ICH. On routine autopsy these lesions are often missed. Using 7 T MRI to target lesions for sampling, we were able to substantially increase the number of lesions available for detailed histopathological analysis.

Our study also had limitations. First, our sample size was relatively small. Nevertheless, for spontaneous ICH, in which autopsy is not performed routinely, it is one of the larger samples currently described. Second, of all lesions identified by MRI, we could retrieve less than half of the lesions for histopathological analysis, which is consistent with previous experiences.[25]

Lesions may have been missed because we did not perform serial sectioning. For the same reason, we were not able to retrieve the "culprit" vessel for all lesions, limiting our analysis to a descriptive examination. Third, patients in our study might not be representative of the whole spectrum of ICH, because autopsy is not routinely performed in patients who die of ICH. Fourth, due to the retrospective nature of our study we had some missing data concerning baseline characteristics. In addition, we did not have a CT or MRI of the acute ICH in all patients. Finally, although we screened for both cortical and deep CMBs, we retrieved only one deep CMB on microscopic examination.

CONCLUSIONS

Although CMIs and CMBs were found in different segments of the cortex in lobar ICH compared to non-lobar ICH patients, otherwise similar histopathological features suggest shared pathophysiological mechanisms in arteriolosclerotic vasculopathy-related ICH and CAA-related ICH. Thickening of vessel walls, vascular remodeling and blood brain barrier dysfunction may be triggered by accumulation of amyloid or arteriolosclerotic vasculopathy, or both, and cause CMIs and CMBs.

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SUPPLEMENTARY TABLES

Table 5.e-1 Histopathological characteristics of identified lesions in sporadic CAA and Dutch-type hereditary CAA

Sporadic CAA vs D-CAA	Sporadic CAA[a] (n=21 lesions)	D-CAA[b] (n=22 lesions)	p-value
Type of lesion[c]			
CMI, n (%)	7 (33.3)	12 (54.5)	
CMB, n (%)	14 (66.7)	10 (45.5)	
Location of lesion			
Occipital, n (%)	3 (14.3)	13 (59.1)	
Parieto-temporal, n (%)	12 (57.1)	5 (22.7)	
Frontal, n (%)	6 (28.6)	4 (18.2)	
Location in the cortex			
Superficial cortex (layers I-III)	13 (61.9)	17 (77.3)	0.33
Deep cortex (layers IV-VI)	4 (19)	4 (18.2)	0.94
Subcortical	4 (19)	1 (4.5)	0.19
Presence of microaneurysms, n (%)	0 (0)	1 (4.5)	0.32
Presence of fibrinoid necrosis, n (%)	7 (33.3)	3 (13.6)	0.16
CAA score ≥2	19 (90.5)	22 (100)	0.23
VWS score ≥2	15 (71.4)	13 (59.1)	0.53
Loss of SMCs score ≥2	13 (61.9)	17 (77.3)	0.27
Not available	2 (9.5)	2 (9.1)	
Fibrin leakage score ≥2			
Vessel walls[d]	4/8 (50)	11/20 (55)	0.81
Surrounding cells[e]	9/17 (52.9)	11/22 (50)	0.86

CAA = cerebral amyloid angiopathy, CMB = cerebral microbleed, CMI = cerebral microinfarct, D-CAA = Dutch-type hereditary CAA, ICH = intracerebral hemorrhage, SMC = smooth muscle cell, VWS = vessel wall splitting

[a] Cases: no.2 (2 CMIs, 2 CMBs), no. 7 (4 CMBs), no. 8 (1 CMI, 8 CMBs), no. 9(4 CMIs)

[b] Case: no 1. (12 CMIs, 10 CMBs)

[c] We performed no statistical test for this variable, because it is a selection of lesions based on MRI

[d] In 11 lesions (9 in sCAA patients and 2 in the D-CAA patient) no surrounding (presumably) involved vessels were identified and in 4 lesions (all in sCAA patients) the fibrin(ogen) stained sections were not available

[e] In 4 lesions (all sCAA patients) fibrin(ogen) stained sections were not available

Table 5.e-2 Histopathological characteristics of presumed involved vessels in lobar and non-lobar ICH stratified by CMIs and CMBs

Lobar vs non-lobar ICH	Lobar ICH[a] (n = 25 lesions)	Non-lobar ICH (n = 15 lesions)	p-value
CAA score ≥2			
Number of involved vessel (% of involved vessels with CAA score ≥2)	1/6 (16.7)	1/4 (25)	0.89
CMI, n (% of CMIs of whom we found the involved vessel and CAA score ≥2)	1/2 (50.0)	0/1 (0)	0.39
CMB, n (% of CMBs of whom we found the involved vessel and CAA score ≥2)	0/4 (0)	1/3 (33.3)	0.43
Involved vessel not identified	19 (76)	11 (73.3)	
VWS score ≥2			
Number of involved vessel (% of involved vessels with VWS score ≥2)	1/6 (16.7)	1/4 (25)	0.89
CMI, n (% of CMIs of whom we found the involved vessel and VWS ≥2)	0/2 (0)	0/1 (0)	NA
CMB, n (% of CMBs of whom we found the involved vessel and VWS ≥2l)	1/4 (25)	1/3 (33.3)	0.81
Involved vessel not identified	19 (76)	11 (73.3)	
Loss of SMCs score ≥2			
Number of involved vessel (% of involved vessels with loss of SMCs score ≥2)	1/2 (50)	3/3 (100)	0.40
CMI, n (% of CMBs of whom we found the involved vessel and loss of SMCs score ≥2)	1/1 (100)	0/0 (0)	NA
CMB, n (% of CMBs of whom we found the involved vessel and loss of SMCs score ≥2)	0/1 (0)	3/3 (100)	0.25
Involved vessel not identified	23 (92)	12 (80)	
Fibrin leakage score ≥2			
Vessel wall (% of involved vessels with fibrin leakage score ≥2)	5/10 (50)	4/10 (40)	0.67
CMI, n (% of CMBs of whom we found the involved vessel and fibrin leakage score ≥2)	2/5 (40)	0/2 (0)	0.29
CMB, n (% of CMBs of whom we found the involved vessel and fibrin leakage score ≥2)	3/5 (60)	4/8 (50)	0.73
Involved vessel not identified	15 (60)	5 (33.3)	

CAA = cerebral amyloid angiopathy, CMB = cerebral microbleed, CMI = cerebral microinfarct, ICH = intracerebral hemorrhage, NA = not applicable, SMC = smooth muscle cells, VWS = Vessel wall splitting

[a] Excluding the case with D-CAA

Table 5.e-3 Histopathological characteristics of the presumed involved vessels in sporadic CAA and D-CAA stratified by CMIs and CMBs

Sporadic CAA vs D-CAA	Sporadic CAA (n = 21 lesions)	D-CAA (n = 22 lesions)	p-value
CAA score ≥2			
Number of involved vessel (% of involved vessels with CAA score ≥2)	1/5 (20)	7/9 (77.8)	0.09
CMI, n (% of CMIs of whom we found the involved vessel and CAA score ≥2)	1/2 (50)	3/4 (75)	0.54
CMB, n (% of CMBs of whom we found the involved vessel and CAA score ≥2)	0/3 (0)	4/5 (80)	0.14
Involved vessel not identified	16 (76.2)	13 (59.1)	
VWS score ≥2			
Number of involved vessel (% of involved vessels with VWS score ≥2)	1/5 (20)	4/9 (44.4)	0.58
CMI, n (% of CMIs of whom we found the involved vessel and VWS ≥2)	0/2 (0)	2/4 (50)	0.47
CMB, n (% of CMBs of whom we found the involved vessel and VWS ≥2l)	1/3 (33.3)	2/5 (40)	0.85
Involved vessel not identified	16 (76.2)	13 (59.1)	
Loss of SMCs score ≥2			
Number of involved vessel (% of involved vessels with loss of SMCs score ≥2)	1/2 (50)	6/7 (85.7)	0.42
CMI, n (% of CMBs of whom we found the involved vessel and loss of SMCs score ≥2)	1/1 (100)	3/3 (100)	NA
CMB, n (% of CMBs of whom we found the involved vessel and loss of SMCs score ≥2)	0/1 (0)	3/4(75)	0.40
Involved vessel not identified	19 (90.5)	15 (68.2)	
Fibrin leakage score ≥2			
Vessel wall (% of involved vessels with fibrin leakage score ≥2)	4/8 (50)	11/20 (55)	0.27
CMI, n (% of CMBs of whom we found the involved vessel and fibrin leakage score ≥2)	2/4 (50)	5/10 (50)	1.00
CMB, n (% of CMBs of whom we found the involved vessel and fibrin leakage score ≥2)	2/4 (50)	6/10 (60)	0.73
Involved vessel not identified	13 (61.9)	2 (9.1)	

CAA = cerebral amyloid angiopathy, CMB = cerebral microbleed, CMI = cerebral microinfarct, ICH = intracerebral hemorrhage, NA = not applicable, SMC = smooth muscle cell, VWS = Vessel wall splitting

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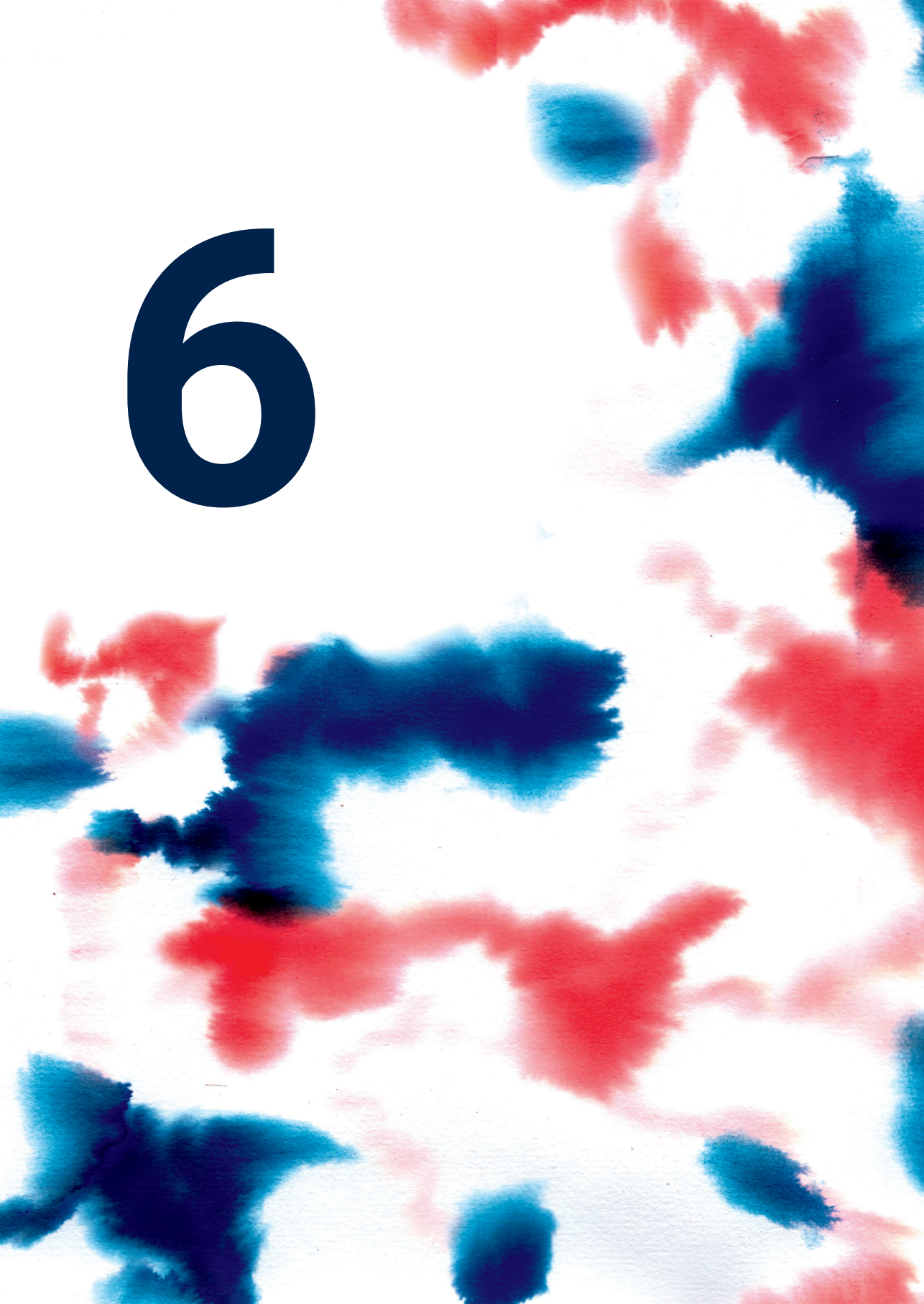


CEREBRAL MICROINFARCTS IN SPONTANEOUS INTRACEREBRAL HEMORRHAGE: A 7 T MRI STUDY

In preparation

ABSTRACT

Objective

The etiology of spontaneous intracerebral hemorrhage (ICH) remains poorly understood. MRI markers of cerebral small vessel disease (cSVD) might provide insight in the underlying vascular disease. Cerebral microinfarcts (CMIs) are a novel marker of cSVD in patients with ICH. The aim of this study was to assess presence and numbers of CMIs and determine the location in the cortex in a cohort of ICH patients.

Methods

From a multicenter prospective observational cohort study, we included consecutive patients with spontaneous ICH, who underwent 7 T MRI <3 months after the ICH. CMIs were defined as lesions ≤3 mm restricted to the cortex, hypointense on T_1 and hyperintense on T_2-FLAIR. We determined the location of the CMIs in the cortex and assessed associations with other markers of cSVD.

Results

Of 40 included patients (median age 59 years, 30% women, 12 lobar ICH), 28 had CMIs (70%, median 2, IQR 1-3). Patients with CMIs more often had cerebral microbleeds (CMBs) than those without (aOR 6.2, 95% CI 1.1-33.8). Presence of CMIs was not associated with vascular risk factors, DWI lesions, severity of white matter hyperintensities, enlarged perivascular spaces, lacunes or cortical superficial siderosis. In patients with lobar ICH, CMIs were more often located in the superficial part of the cortex than in patients with non-lobar ICH (RR 2.7, 95% CI 1.5-5.0).

Interpretation

In patients with lobar and non-lobar ICH, more than two-thirds have CMIs. Associations with CMBs suggest a relationship with more severe cSVD. Superficial CMIs may be a marker of cerebral amyloid angiopathy.

INTRODUCTION

Spontaneous intracerebral hemorrhage (ICH) is a devastating disease affecting over four million people worldwide each year.[1] The etiology remains poorly understood. Classically, lobar ICH in elderly patients has been associated with cerebral amyloid angiopathy (CAA), but pathology studies found CAA in only 30% of patients with lobar ICH.[2] In non-lobar ICH vascular risk factors play an important role.[3,4] In a recent systematic review and meta-analysis, we showed that risk factor profiles differ for lobar and non-lobar ICH. However, hypertension is an important risk factor both in lobar and non-lobar ICH suggesting also overlapping disease mechanisms (chapter 3).

MRI markers of cerebral small vessel disease (cSVD) might provide insight in the underlying vascular disease in lobar and non-lobar ICH. Strictly lobar cerebral microbleeds (CMBs),[5] cortical superficial siderosis (cSS),[6] multiple subcortical spots of white matter hyperintensities (WMH) with a relative posterior distribution,[7,8] and severe enlarged perivascular spaces (EPVS) in the centrum semiovale[9] are associated with CAA. Predominantly deep CMBs,[5] peri-basal ganglia WMH,[7] and EVPS more pronounced in the basal ganglia[9] are more commonly found in arteriolosclerotic vasculopathy associated with vascular risk factors. Recent advances in high-field MRI have made it possible to visualize submillimeter lesions. In a post-mortem study combing 7 tesla (7 T) MRI and histopathology (chapter 5), we found that cortical cerebral microinfarcts (CMIs) were more prevalent in lobar ICH cases compared with non-lobar ICH cases. In addition, in lobar ICH CMIs were predominantly located in the superficial layers of the cortex, whereas in non-lobar ICH CMIs were more often located in the deeper layers of the cortex. In a small case-series, we previously found CMIs on 7 T MRI in around three-quarter of patients with lobar and non-lobar ICH.[10]

In this study, we aimed to further investigate CMIs as biomarker of cSVD in spontaneous ICH by determining their presence, spatial distribution, and associations with other markers of cSVD on 7 T MRI in patients with lobar and non-lobar ICH.

MATERIALS AND METHODS

Study population

We included consecutive adult patients, who presented to the University Medical Centers of Utrecht, Leiden or Nijmegen between October 1st 2013 and December 31st 2018, and who underwent 7 T MRI within three months of the ICH. This study was part of a multicenter prospective observational cohort study in the Netherlands, the Finding the ETiology in

spontaneous Cerebral Hemorrhage (FETCH) study. In FETCH we included a total of 221 patients with spontaneous ICH confirmed by computed tomography (CT) who were able to undergo 3 T and/or 7 T MR imaging. We excluded patients with a known cause of the ICH on CTA, such as a vascular malformation, bleeding disorder or tumor.

The FETCH study was approved by the Medical Ethics Review Committee of the University Medical Center Utrecht. Each patient gave written informed consent for participation in the study and we followed the guidelines according to the Declaration of Helsinki.

Vascular risk factors

Demographics and vascular risk factors were recorded on admission. We defined history of hypertension as use of antihypertensive medication, a documented systolic blood pressure higher than 140 mm Hg or a diastolic blood pressure higher than 90 mm Hg on two independent measurements prior to the ICH, or left ventricular hypertrophy on ECG. History of a transient ischemic attack, ischemic stroke, atrial fibrillation and cardiac disease was extracted from the medical history. Diabetes was defined as diabetes in past medical history or two fasting glucose measurements above 7 mmol/L and hypercholesterolemia as total cholesterol above 6.2 mmol/L or use of lipid-lowering drugs. We defined smoking as current or past tobacco use. Patients with possible or probable CAA were identified using the modified Boston criteria.[11]

Imaging protocol and analysis

The imaging protocol and analysis have been described in detail before.[12] In summary, on admission CT we assessed ICH location and hematoma volume using an in-house developed tool.[13] Lobar ICH was defined as ICH in the cortex with or without involvement of subcortical white matter and non-lobar ICH as deep (thalamus and basal ganglia) or infratentorial (brainstem and cerebellum) ICH.[14]

7 T MRI (Philips, Best, The Netherlands) scans were acquired by a standardized protocol including 3D T_2-weighted images, 3D T_1-weighted images, dual echo susceptibility weighted images (SWI) and 3D fluid-attenuated inversion recovery (FLAIR). We administered gadolinium-containing contrast agent in a single intravenous injection of 0.1 mL Gadovist/kg body weight with a maximum of 10 mL Gadovist or 0.2 mL Dotarem/kg body weight with a maximum of 30 mL Dotarem. Post-gadolinium FLAIR images were acquired at least 10 minutes after contrast injection.

We used 7 T MRI to assess presence, number and location of CMIs, CMBs, presence and severity of contrast leakage, WMH, cortical superficial siderosis (cSS) and lacunes. We assessed presence and severity of EPVS and diffusion weighted imaging (DWI) lesions on 3 T MRI.

One trained reader (WMTJ) annotated the presence and location of CMIs, CMBs, WMHs, cSS, lacunes, contrast leakage, EPVS and DWI lesions, blinded for patient information. A random sample of at least 20% was assessed independently by a second reader. Discrepancies were discussed in a consensus meeting and if necessary discussed with an experienced neuroradiologist (MAAvW, JH) and neurologist (CJMK).

CMIs were assessed on FLAIR and T_1-weighted images and defined as hypointense on T_1, hyperintense, isointense or cavitated on FLAIR, strictly intracortical and ≤ 3 mm. They had to be distinct from enlarged perivascular spaces, microbleeds and leptomeningeal vessels and visible in at least two planes (sagittal, transversal, coronal).[15] We determined the location of the CMIs in the cortex as superficial (touching the outer border of the cortex and predominantly in the upper half of the cortex), deep (not touching the cortex and predominantly in the lower half of the cortex) or transcortical (covering more than 50% of the cortex). Contrast leakage was defined as a hyperintense signal in normal appearing brain or CSF on delayed post-gadolinium FLAIR images, while absent on pre-contrast images. The signal had to be visually distinct and anatomically non-contiguous with the hematoma. We used the 5-point HARM rating scale to rate the extent of the contrast leakage.[12,16] CMBs were rated in accordance with the Standards for Reporting Vascular changes on nEuroimaging (STRIVE) definitions and the Microbleed Anatomical Rating Scale.[17,18] We assessed the WMH volume by measuring the WMH volume in the ICH-free hemisphere and subsequently doubling this value and we assessed WMH severity by the Fazekas rating scale.[19] DWI lesions were defined as small, hyperintense lesions on DWI with low intensity in the corresponding region on apparent diffusion coefficient maps remote from ICH and the area of perihematomal edema.[20] EPVS were rated in basal ganglia and centrum semiovale regions on axial T_2-weighted sequences using a validated rating scale.[21] For lacunes we used the STRIVE criteria,[18] cSS was defined as a homogeneous hypointense curvilinear signal intensity on SWI-images in the superficial layers of the cerebral cortex, within the subarachnoid space or both.[6]

Statistical analysis

We analyzed group differences in presence, frequency and distribution of baseline characteristics, CMBs (presence [yes/no], number and categories [0:0-1; 1:2-5; 2:6-10; 3:>10] of CMBs), contrast leakage (presence (yes/no) and HARM scores [0-4]), DWI lesions (yes/no), categories of EPVS in basal ganglia and centrum semiovale (0: no EPVS, 1: 1-10, 2: 11-20, 3:21-40, 4: >40 EPVS) and WMH (Fazekas ≥2 vs <2) between patients with and without CMIs on 7 T MRI, using the Student t-test, χ^2 test, Mann-Whitney U test and logistic regression, as appropriate. Multivariable logistic regression analysis was performed to look for independent associations with presence of CMIs; we included variables that had a statistically significant ($p < 0.05$) association in univariable analysis.

RESULTS

We included 40 of 45 patients who underwent a 7 T MRI. In 5 patients quality of the FLAIR or T_1-weighted images was insufficient due to movement artefacts. Excluded patients were similar to included patients with respect to age, sex, and presence of vascular risk factors (data not shown).

Baseline characteristics of included patients are listed in table 6.1. The median time interval between ICH and 7 T MRI was 36 days (IQR 9-71 days) and the median time interval between 3 T and 7 T MRI 9 days (IQR 1-42 days). Location of the ICH was lobar in 12 patients (30%) and non-lobar in 28 patients (70%; 22 deep, 6 infratentorial). Six of 12 patients (58%) with lobar ICH fulfilled the modified Boston criteria for probable CAA.[11] Of these six patients with probable CAA, four patients also had a history of hypertension.

We found a total of 59 CMIs in 28 patients (70%; 8 in lobar and 20 in non-lobar ICH). The number of CMIs per patients ranged from 1 to10; 14 patients had more than one CMI, (median 2, IQR 1-3). Of the 59 CMIs, 28 (47%) were found in the frontal cortex, 18 parietal (31%), five temporal (8%) and eight occipital (14%). Illustrative examples are provided in figure 6.1. The percentage of patients with CMIs was similar in patients with lobar ICH (67%) and non-lobar ICH (71%; RR 0.9, 95% CI 0.6-1.5), as well as the median number of CMIs in each group (both median 1.5, IQR 1-2). Patients with CMIs were older (mean 62 years, SD 12, for patients with CMIs versus 52 years, SD 14, for patients without, p=0.04). Patients with CMIs appeared to have higher mean systolic blood pressure at presentation (177 mm Hg, p=0.07) and less often subarachnoid extension on admission CT (7% in patients with CMIs compared to 33% in patients without CMIs, p=0.06), but these differences were not statistically significant. Sex, other vascular risk factors and other hematoma characteristics were comparable in patients with and without CMIs.

Table 6.1 Baseline characteristics for patients with and without presence of CMIs

Characteristics	All N = 40	Presence of CMIs N = 28 (70%)	No CMIs N = 12 (30%)	Risk ratio (95% CI)	p-value
Patient characteristics					
Mean age, years (SD)	59 (13)	62 (12)	52 (14)		0.04*
Female sex, n (%)	12 (30)	10 (35)	2 (17)	2.1 (0.6-8.3)	0.29#
GCS at presentation, median (IQR)	15 (12-15)	15 (13-15)	14 (12-15)		0.46^
NIHSS at presentation, median (IQR)	4 (2-9)	4 (2-10)	4 (1-6)		0.42^
Mean systolic BP at admission, mm Hg (SD)	171 (33)	177 (31)	156 (33)		0.07*
Mean diastolic BP at admission, mm Hg (SD)	96 (22)	97 (21)	94 (23)		0.65*
History of hypertension, n (%)	27 (68)	20 (71)	7 (58)	1.5 (0.6-3.8)	0.48#
History of TIA, n (%)	4 (10)	4 (14)	0 (0)	1.4 (0.9-2.0)	0.30#
History of ischemic stroke, n (%)	4 (10)	3 (11)	1 (8)	1.2 (0.2-7.2)	0.82#
History of cardiac disease, n (%)	2 (5)	1 (4)	1 (8)	0.6 (0.1-2.5)	0.52#
Diabetes mellitus, n (%)	2 (5)	2 (7)	0 (0)	1.2 (0.7-2.1)	0.34#
Atrial fibrillation, n (%)	5 (13)	4 (14)	1 (8)	1.6 (0.3-9.7)	0.60#
Smoking (current or past), n (%)	18 (45)	13 (45)	5 (42)	1.2 (0.5-3.1)	0.74#
Anticoagulants, n (%)	7 (18)	6 (21)	1 (9)	2.3 (0.4-15.3)	0.65#
Antiplatelets, n (%)	8 (20)	6 (21)	2 (17)	1.3 (0.3-4.6)	0.73#
Hematoma characteristics					
Location, n (%)					
Lobar	12 (30)	8 (28)	4 (36)		0.59#
Deep	22 (55)	17 (59)	5 (46)		
Infratentorial	6 (15)	4 (14)	2 (18)		
Median hematoma volume, mL (IQR)	11 (4-19)	11 (4-15)	19 (3-28)		0.31^
Subarachnoid extension, n (%)	6 (15)	2 (7)	4 (33)	0.4 (0.1-1.4)	0.06#
Intraventricular extension, n (%)	11 (28)	5 (18)	6 (50)	0.6 (0.3-1.1)	0.11#
Presence of CMBs[a]					
CMBs (yes, %)	29 (73)	24 (86)	5 (42)	3.5 (1.4-8.9)	0.02#
0-1	16 (40)	9 (32)	7 (58)		ref
2-5	8 (20)	6 (21)	2 (17)		0.38@
6-10	7 (18)	6 (21)	1 (8)		0.45@
>10	8 (20)	7 (25)	1 (8)		0.54@
Any lobar CMBs (yes, %)	22 (55)	18 (64)	4 (33)	2.3 (0.8-6.5)	0.16#
Strictly lobar CMBs (yes, %)	5 (13)	4 (14)	1 (8)	1.5 (0.2-9.2)	0.66#
Number of CMBs					
Number of lobar CMBs, median (IQR)	1 (0-6)	2 (0-8)	0 (0-2)		0.12^
Number of non-lobar CMBs, median (IQR)	1 (0-5)	2 (0-8)	0 (0-1)		0.13^
Total number of CMBs, median (IQR)	3 (0-8)	3 (1-10)	0 (0-3)		0.03^

Table 6.1 Baseline characteristics for patients with and without presence of CMIs (continued)

Characteristics	All N = 40	Presence of CMIs N = 28 (70%)	No CMIs N = 12 (30%)	Risk ratio (95% CI)	p-value
Contrast leakage[b]					
Presence, n (%)	16 (40)	13 (46)	3 (25)	2.0 (0.6-6.4)	0.29#
HARM scale					
0: None	19 (48)	12 (43)	7 (58)		ref
1: Punctate	4 (10)	4 (14)	0 (0)		0.99@
2: Multipunctate	5 (13)	5 (18)	0 (0)		0.99@
3: Focal	3 (8)	1 (4)	2 (17)		0.35@
4: Generalized	4 (10)	3 (11)	1 (8)		0.65@
White Matter Hyperintensities					
Fazekas score ≥2, n (%)	20 (50)	17 (61)	3 (25)	3 (0.95-9.5)	0.08#
Median WMH volume, mL (IQR)	6 (2-13)	10 (4-13)	2 (1-5)		0.054^
DWI lesions[c]					
Presence, n (%)	5 (13)	4 (14)	1 (8)	1.9 (0.3-11.6)	0.64#
EPVS[d]					
Basal ganglia					
<10	13 (33)	8 (29)	5 (42)		ref
10-20	17 (43)	12 (43)	5 (42)		0.60@
20-40	5 (13)	4 (14)	1 (8)		0.47@
>40	0 (0)	0 (0)	0 (0)		-
Centrum semiovale					
<10	3 (8)	2 (7)	1 (8)		ref
10-20	14 (35)	11 (39)	3 (25)		0.66@
20-40	18 (45)	11 (39)	7 (58)		0.86@
>40	0 (0)	0 (0)	0 (0)		-
Cortical superficial siderosis					
Presence, n (%)	4 (10)	4 (14)	0 (0)	1.4 (0.9-2.0)	0.30#
Lacunes					
Presence, n (%)	11 (28)	9 (32)	2 (17)	1.9 (0.5-7.3)	0.45#
Number of lacunes, median (IQR)	3 (2-5)	2 (2-5)	4 (3-4)		0.34^
Modified Boston criteria					
Probable CAA, n (%)	7 (18)	5 (18)	2 (17)	1.1 (0.3-3.8)	0.93#
Probable CAA (including patients <55 years), n (%)	10 (25)	7 (25)	3 (25)	1.0 (0.3-3.0)	1.00#

BP = blood pressure, CI= confidence interval, CMB = cerebral microbleed, CMI = cerebral microinfarct, DWI = diffusion weighted imaging, EPVS = enlarged perivascular spaces, GCS= Glasgow coma scale, ICH = intracerebral hemorrhage, IQR = interquartile range, NIHSS = national institutes of health stroke scale, SD = standard deviation, TIA = transient ischemic attack.

[a] In one patient CMBs were not assessable due to movement artifacts; [b] In five patients FLAIR images after gadolinium were not available or assessable; [c] In six patients 3 T MRI was not available

* t-test; ^ Mann-Whitney U test; # chi square @ logistic regression

Patients with CMIs more often had CMBs (86%) than those without CMIs (42%; risk ratio (RR) 3.5, 95% confidence interval (CI) 1.4-8.9) as well as a higher total number of CMBs (patients with CMIs: median 3, IQR 1-10 versus patients without CMIs: median 0, IQR 0-3; p=0.03). We found more severe WMH in patients with CMIs compared to patients without CMIs with a higher median WMH volume (patients with CMIs: median 10 mL, IQR 4-13 versus patients without CMIs: median 2 mL, IQR 1-5, p=0.054), although this difference did not reach statistical significance. We found no differences between patients with and without CMIs in the percentage of patients with presence of contrast leakage, categories of HARM, percentage of patients with DWI lesions, categories of EPVS, percentage of patients with presence of cortical superficial siderosis or presence and number of lacunes.

In a multivariable model, including age and presence of CMBs, presence of CMBs (adjusted odds ratio (aOR) 6.2, 95% CI 1.1-33.8), but not age (aOR 1.06, 95% CI 0.99-1.14) was independently related to presence of CMIs.

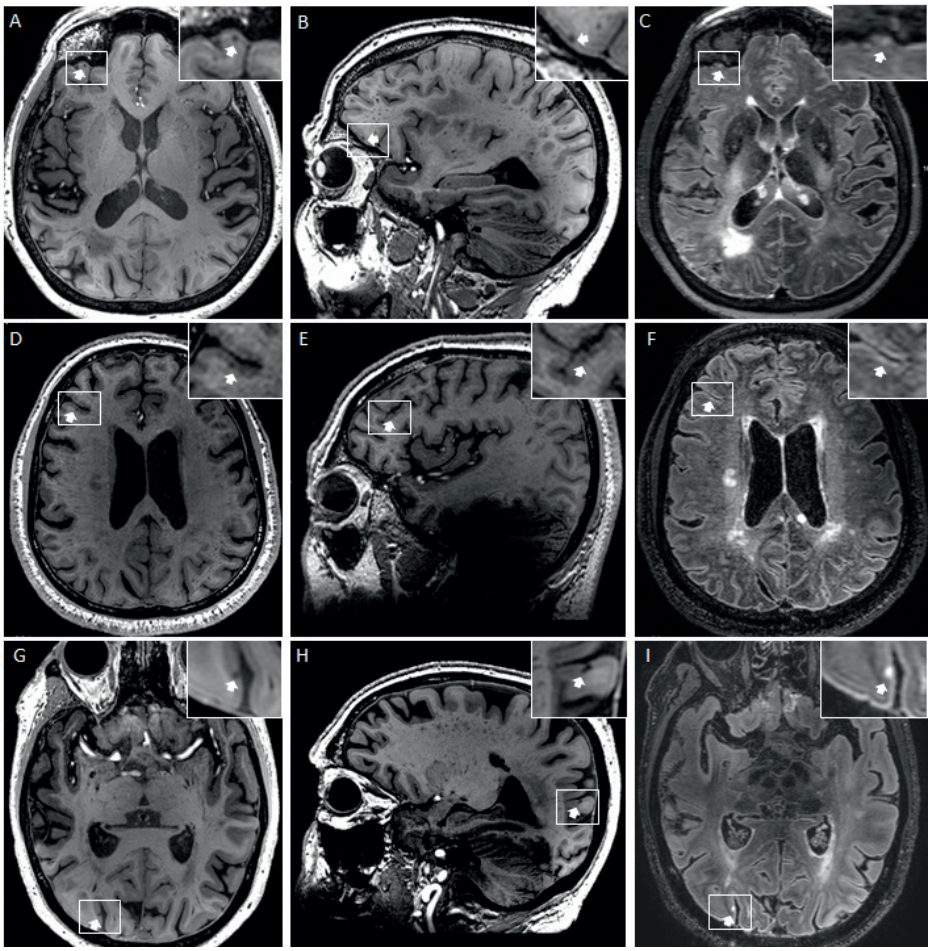
The location of the CMIs in the cortex was more often superficial in patients with lobar ICH (30%) compared to patients with non-lobar ICH (5%; RR 2.7, 95% CI 1.5-5.0; table 6.2 and figure 6.1). Percentage of CMIs with deep (p=0.29) or transcortical (p=0.06) location in the cortex was similar in lobar and non-lobar ICH patients. Within the group of patients with lobar ICH, we found no difference in the location of the CMIs in the cortex between those who fulfilled the modified Boston criteria for probable CAA and those who did not (RR for superficial location 0.6, 95% CI 0.1-3.0).

Table 6.2 Location of cerebral microinfarcts in the cortex in patients with lobar and non-lobar intracerebral hemorrhage

Location of CMIs in the cortex	Lobar ICH 12 patients; 20 CMIs	Non-lobar ICH 28 patients, 39 CMIs	Risk ratio (95% CI)[#]	p-value
Superficial, no (%)	6 (30)	2 (5)	2.7 (1.5-5.0)	0.001
Deep, no (%)	6 (30)	16 (41)	0.8 (0.6-1.2)	0.40
Transcortical , no (%)	8 (40)	21 (54)	0.9 (0.6-1.2)	0.58

CMI = cerebral microinfarct, ICH = intracerebral hemorrhage
[#] Chi square

Figure 6.1. Examples of cerebral microinfarcts in patients with spontaneous intracerebral hemorrhage



Panels A-C show the images of a 71 year old woman with a right lobar ICH, fulfilling the criteria of probable cerebral amyloid angiopathy, and a CMI (white arrows) in the superficial part of the cortex (A: axial T_1-weighted image; B: sagittal T_1-weighted image; C: axial fluid attenuated inversion recovery (FLAIR) image).

Panels D-F depict a 72 year old man with a left deep ICH and a CMI (white arrows) in the deeper part of the cortex (D: axial T_1-weighted image; E: sagittal T_1-weighted image; F: axial FLAIR image).

Panels G-I show images of a 70 year old man with a right deep ICH and a transcortical CMI (white arrows; G: axial T_1-weighted image; H: sagittal T_1-weighted image; I: axial FLAIR image).

The inserts show the CMIs in more detail.

DISCUSSION

In patients with lobar or non-lobar ICH, more than two-thirds have CMIs. Presence of CMIs was related to presence and number of CMBs, but not to vascular risk factors and other MRI markers of cSVD. In lobar ICH compared to non-lobar ICH, CMIs were more often located in the superficial part of the cortex.

This study provides corroborating evidence that CMIs are indeed present in the majority of patients with spontaneous ICH patients on 7 T MRI as described in a previous small case-series.[10] In comparison with the previous study, patients in this study were somewhat older (mean 59 years, SD 13, versus 52 years, SD 8), but the number of CMIs per patient (median 1.5) was comparable to that in the case-series (median 2.5).[10] The small case-series suggested that CMIs may be more frequent in patients with lobar ICH than in patients with deep ICH, but in this larger study we found CMIs in similar percentages in both groups.[10] CMIs have also been described in patients with other types of vascular disease with frequencies varying from 15% in patients with TIA or ischemic stroke (231 patients, mean age 67 years) to 30% in patients with cardiac disease (243 patients, mean age 73 years).[22,23] CMIs are also a common finding in patients with CAA with and without lobar ICH with percentages ranging from 40% in a cohort of 102 CAA patients (mean age 70 years, 61% previous ICH with a median time-gap of 1.5 years between ICH occurrence and enrollment) that underwent 1.5 or 3 T MRI,[24] to 60% in a series of 35 CAA patients (mean age 75 years, number of patients with ICH unknown) that underwent 3 T MRI.[25] We showed that CMIs can be found not only in patients with lobar ICH, but also in those with non-lobar ICH. Based on our observations and those of others, presence of CMIs does not appear to be specific for ICH, or for stroke, but CMIs appear to be related to older age.

In our study, we showed an association of occurrence of CMIs with CMBs. We found the link for presence and number of CMBs irrespective of their location, and not specifically for strictly lobar CMBs. This is in line with a recent meta-analysis that showed an association between prevalence of acute CMIs visualized with DWI and presence and number of CMBs in any location.[26] In the studies of CAA patients with or without previous ICH, no associations were found for presence or number of lobar CMBs.[24,25] Our findings suggest that CMIs are not only related to CAA, but also to other types of cSVD-related ICH.

We found no associations between other MRI markers of cSVD and CMIs. This is in contrast to two population based studies (n=194 patients, mean age 77 years; n=861 patients, mean age 70 years) and a memory clinic cohort (238 patients, mean age 73 years) that showed a relation with lacunar infarcts, with higher volume of white matter hyperintensities and with cerebral atrophy.[27–29] We did find a higher median WMH volume in patients with CMIs compared to

patients without CMIs, but this difference did not reach statistical significance (p=0.054). In one of the studies in CAA patients an association was found with cSS.[24] We could not confirm this relation in the CAA patients in our cohort, but in our study the number of patients with probable CAA was small (n=6) and only three had cSS.

We found no associations with vascular risk factors for presence of CMIs in patients with ICH. Another 7 T MRI study found no differences in presence and number of CMIs between 48 patients (mean age 70 years) with type 2 diabetes mellitus and controls.[30] In the study in patients attending a memory clinic, investigated with 3 T MRI, no associations were found for presence of CMIs with diabetes or hypertension, but they did find an association with hyperlipidemia, a history of ischemic stroke and a history of cardiovascular disease.[27] In this study no association was found between older age and presence of CMIs, so associations with risk factors were not adjusted for age.[27] In the cohort of patients with TIA and ischemic stroke and 3 T MRI no association was found with older age and also none of the investigated risk factors showed an association with presence of CMIs.[31] In the population based study with 861 patients that underwent 3 T MRI, CMIs were associated with hypertension.[29] In this study, presence of CMIs was related to older age and associations were adjusted for age.[29] It seems that the disease setting is important in interpreting associations of vascular risk factors with CMIs.[15] Due to our selection of patients with ICH, risk factor patterns were different than in the general population or a memory clinic setting. It remains unclear what the influence is of vascular risk factors on the development of CMIs, but older age seems to be an important modifying factor.

In line with our post-mortem study combing 7 T MRI and histopathology (chapter 5), we found CMIs more often in the superficial part of the cortex in lobar ICH patients compared to non-lobar ICH patients. This is consistent with a recent study using ex vivo 3 T MRI and histopathology in 12 CAA cases, which showed that half of the CMIs were located in the superficial layers of the cortex, in the perfusion area of a penetrating cortical arteriole.[32] Another neuropathological study including 113 consecutive brain autopsies, showed that CAA predominantly affected the superficial cortical branches of pial arteries.[33] Combined with our study, these findings suggests that CAA might specifically damage the superficial branches of cortical arteries with superficial cortical CMIs as a consequence. Superficial cortical CMIs might therefore be a specific marker for CAA.

Strengths of our study include the unique cohort of spontaneous ICH patients that underwent ultra-high field 7 T MRI. 7 T MRI has a high signal-to-noise ratio and better contrast-to-noise ratio than conventional (1.5 or 3 T) MRI scanners. This results in submillimeter voxel sizes and subsequently a better yield, because this allows detection of smaller lesions.[34]

Our study also has limitations. Selection bias has likely played a role. It is challenging to include severely affected ICH patients and they are not always able to undergo scanning in 7 T MRI. This may have resulted in including less affected patients with lower NIHSS scores and possibly smaller hematomas. Information bias might have been introduced, because we were not able to blind assessment of MRI markers for ICH location. Another limitation is the relatively small number of included patients.

Further studies are needed to confirm our findings, preferably in a larger cohort of ICH patients. Because 7 T MRI is not routinely used in clinical practice, confirmation of presence and number of CMIs on conventional 3 T MRI in patients with ICH is necessary. To further elucidate the meaning of presence of CMIs in ICH, it would be of interest to investigate associations of presence and number of CMIs with functional outcome and with cognitive function and decline both in patients with lobar and with non-lobar ICH.

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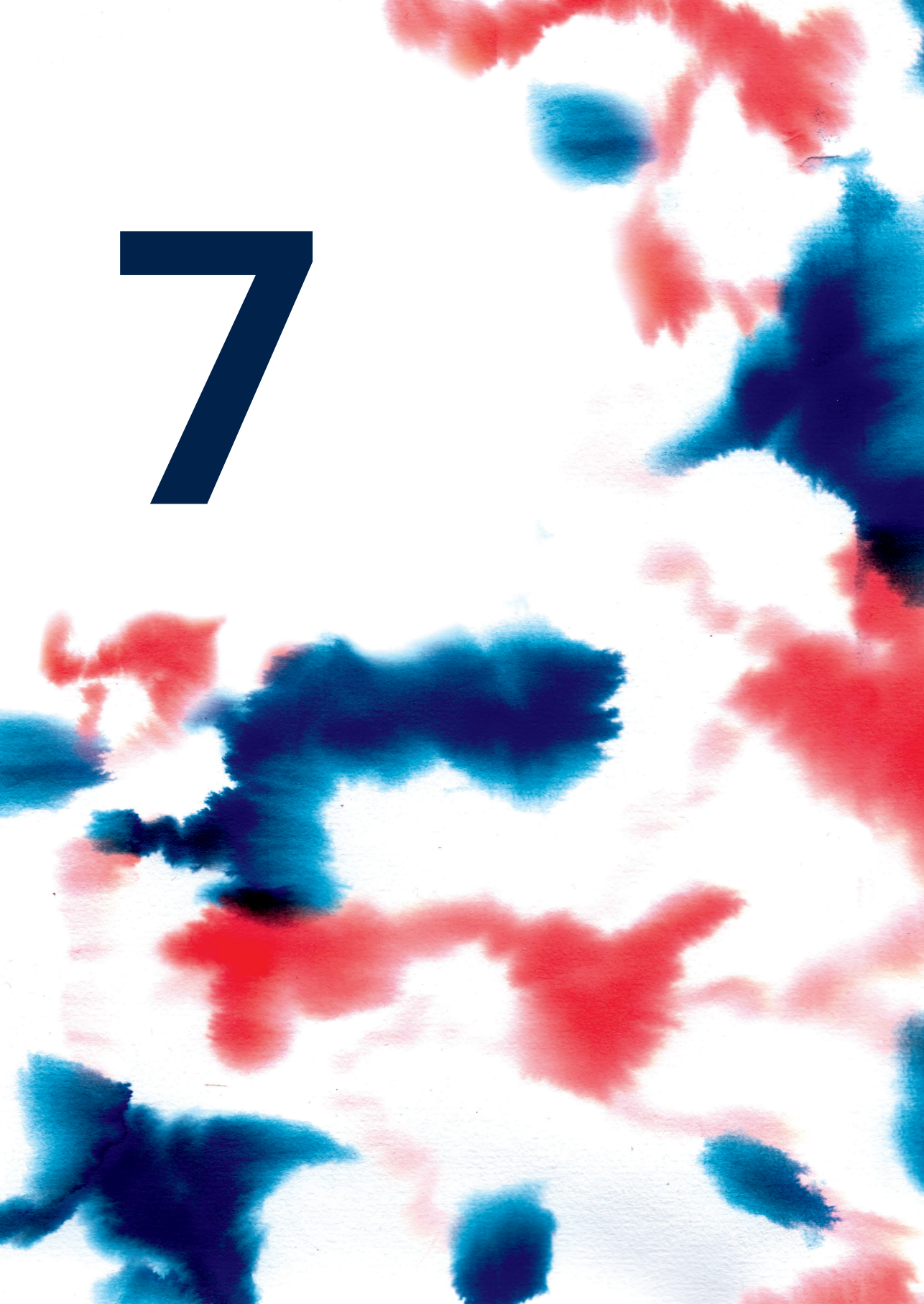
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CONTRAST LEAKAGE DISTANT FROM THE HEMATOMA IN PATIENTS WITH SPONTANEOUS ICH: A 7 T MRI STUDY

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ABSTRACT

Disruption of the blood-brain barrier (BBB) might play a role in the pathophysiology of cerebral small vessel disease related ICH. The aim of this study was to assess presence and extent of contrast agent leakage distant from the hematoma as a marker of BBB disruption in patients with spontaneous ICH. We prospectively performed 7 tesla MRI in adult patients with spontaneous ICH and assessed contrast leakage distant from the hematoma on 3D FLAIR images. Thirty-one patients were included (mean age 60 years, 29% women). Median time between ICH and MRI was 20 days (IQR 9-67 days). Seventeen patients (54%; seven lobar, nine deep, one infratentorial ICH) had contrast leakage, located cortical in 16 and cortical and deep in one patient. Patients with contrast leakage more often had lobar cerebral microbleeds (CMBs; 77%) than those without (36%; RR 2.5, 95% CI 1.1-5.7) and a higher number of lobar CMBs (patients with contrast leakage: median 2, IQR 1-8 versus those without: median 0, IQR 0-2; p=0.02). This study shows that contrast leakage distant from the hematoma is common in days to weeks after spontaneous ICH. It is located predominantly cortical and related to lobar CMBs and therefore possibly to cerebral amyloid angiopathy.

INTRODUCTION

Intracerebral hemorrhage (ICH) related to cerebral small vessel disease (cSVD) is a detrimental disease resulting in high case-morbidity and -fatality.[1,2] Despite ongoing advances in imaging and supportive treatment, incidence has not declined and outcome after ICH has at most marginally improved.[2-4] An important contributing factor may be that the underlying pathophysiology is incompletely understood. cSVD related ICH is mostly attributed to hypertensive vasculopathy or cerebral amyloid angiopathy (CAA),[5] but the exact sequence of events and mechanisms that lead to vessel rupture remain unknown. Accumulating evidence suggests that disruption of the blood-brain barrier (BBB) plays a role in cSVD related ICH.[6-8]

Contrast agent leakage (further referred to as contrast leakage) on Dynamic Contrast Enhanced (DCE) MRI as a biomarker of BBB disruption, has been demonstrated in patients with (lacunar) stroke,[9-11] mild cognitive impairment and Alzheimer's disease.[12,13] Using dedicated fluid attenuated inversion recovery (FLAIR) sequences after gadolinium contrast agent injection, hyperintense foci can be found in the normal appearing brain parenchyma or cerebrospinal fluid (CSF) space: the so-called Hyperintense Acute Reperfusion Marker (HARM).[14-17] In the acute phase after ICH (median interval between ICH and MRI 11 hours), HARM was found in 85% of patients.[18] We hypothesized that contrast leakage as a marker of BBB disruption is a marker of the underlying cSVD in patients with ICH. Therefore, we assessed the presence and extent of contrast leakage in normal appearing brain and CSF distant from the hematoma in patients with spontaneous ICH on 7 tesla (T) MRI in the subacute phase after ICH. Additionally, we related the presence and extent of contrast leakage to classical markers of cSVD.

MATERIALS AND METHODS

Study population

Patients were enrolled from an ongoing multicenter prospective observational cohort study in the Netherlands, the Finding the ETiology in spontaneous Cerebral Hemorrhage (FETCH) study. In this study we included 31 consecutive adult patients who presented to the University Medical Centers of Utrecht, Leiden or Nijmegen, since October 1st 2013, with spontaneous ICH confirmed by computed tomography (CT) and were able to undergo 3 T and/or 7 T MR imaging. Patients with a known cause of ICH, such as a vascular malformation, tumor or trauma, are excluded. For this study we included all patients who underwent 7 T MRI with a gadolinium-containing contrast agent within three months of the ICH.

The FETCH study was approved by the Medical Ethics Review Committee of the University Medical Center Utrecht, and by all participating centers. Each patient gave written informed consent for participation in the study. The guidelines according to the Declaration of Helsinki were followed.

We categorized the ICH according to location as lobar, deep or infratentorial. Patients with possible or probable CAA were identified using the modified Boston criteria.[19] Hypertension was defined as use of antihypertensive medication, a systolic blood pressure greater than 140 mm Hg, a diastolic blood pressure greater than 90 mm Hg on two documented independent measurements prior to the ICH or left ventricular hypertrophy on ECG.

Imaging protocol and analysis

On admission CT we assessed hematoma volume using an in-house developed tool,[20] and ICH location. Lobar ICH was defined as ICH isolated to the cortex (with or without involvement of subcortical white matter) and non-lobar ICH as deep (thalamus and basal ganglia) or infratentorial (brainstem and cerebellum) ICH. We used 7 T MRI to assess presence of contrast leakage, CMBs and white matter hyperintensities (WMH). Presence of enlarged perivascular spaces (EPVS) and diffusion weighted imaging (DWI) lesions was assessed on 3 T MRI.

7 T MRI (Philips, Best, The Netherlands) scans were acquired by a standardized protocol; 3D T_2-weighted (repetition time (TR)/equivalent echo time (TE) = 3158/60 ms; voxel size = acquired: 0.70x0.70x0.70 mm^3, reconstructed: 0.35x0.35x0.35 mm^3), 3D T_1-weighted (TR/TE = 4.8/2.2 ms; voxel size = acquired: 1.00x1.01x1.00 mm^3, reconstructed: 0.66x0.66x0.50 mm^3), dual echo 3D T_2^*-weighted (TR/first TE/second TE = 20/6.9/15.8 ms; voxel size = acquired: 0.50x0.50x0.70 mm^3, reconstructed: 0.39x0.39x0.35 mm^3) and 3D FLAIR images were acquired (TR/TE/inversion time (TI) = 8000/300/2325 ms; voxel size = acquired: 0.80x0.82x0.80 mm^3, reconstructed: 0.49x0.49x0.40 mm^3). A gadolinium-containing contrast agent was administered in a single intravenous injection of 0.1 mL Gadovist/kg body weight with a maximum of 10 mL Gadovist or 0.2 mL Dotarem/kg body weight with a maximum of 30 mL Dotarem. Post-gadolinium FLAIR images were acquired at least 10 minutes after contrast injection.

3 T unenhanced MRI (Philips, Best, The Netherlands) scans were acquired by a standardized protocol including DWI with apparent diffusion coefficient (ADC) map, an axial T_2*, T_2-Proton Density-weighted sequence, inversion recovery and FLAIR, all with 48 contiguous slices and 0.96x0.95x3.00 mm^3 voxels. Also a 3D T_1-weighted sequence was acquired.

Contrast leakage, CMBs, EPVS, WMH and DWI lesions were annotated by two trained readers (WMTJ and AL) independently and blinded for patient information. Discrepancies were resolved

in a consensus meeting with an experienced neuroradiologist (JH) and neurologist (CJMK). Contrast leakage was defined as a hyperintense signal in normal appearing brain or CSF on delayed post-gadolinium FLAIR images, while absent on pre-contrast images. The signal had to be visually distinct and anatomically non-contiguous with the hematoma. We used the 5-point HARM rating scale to rate the extent of the contrast leakage as HARM 0: no contrast leakage, HARM 1: punctate lesions of contrast leakage, HARM 2: multiple punctate lesions of contrast leakage, HARM 3: focal sulcal contrast enhancement, and HARM 4: bilateral and diffuse contrast leakage.[18] CMBs were rated in accordance with the Standards for Reporting Vascular changes on nEuroimaging (STRIVE) definitions and the Microbleed Anatomical Rating Scale,[21,22] and WMH by the Fazekas rating scale.[23] DWI lesions were defined as small, hyperintense lesions on DWI with low intensity in the corresponding region on ADC maps remote from ICH and the area of perihematomal edema.[24] EPVS were rated in basal ganglia and centrum semiovale regions on axial T_2-weighted sequences using a validated rating scale.[25,26]

Statistical analysis

We used the Student t-test, χ^2 test, Mann-Whitney U test and logistic regression, as appropriate, to analyze group differences in presence, frequency and distribution of baseline characteristics, CMBs (presence (yes/no) and number of CMBs), DWI lesions (yes/no), categories of EPVS in basal ganglia and centrum semiovale (0: no EPVS, 1: 1-10, 2: 11-20, 3:21-40, 4: >40 EPVS) and WMH (Fazekas ≥2 vs <2) between patients with and without contrast leakage on 7 T MRI.

RESULTS

We included 31 patients (mean age 60 years, standard deviation 12 years; 29% women). Baseline characteristics are listed in table 7.1. Median time interval between ICH and 7 T MRI was 20 days (IQR 9-67 days) and median time interval between 3 T and 7 T MRI was 7 days (IQR 1-44 days). Location of the ICH was supratentorial in 90% (lobar in eleven (36%), deep in seventeen (55%)) and infratentorial in three patients (10%). Five of the eleven patients with lobar ICH fulfilled the modified Boston Criteria for probable CAA and two patients for possible CAA.[19]

In seventeen patients (54%; seven with lobar, nine with deep, and one with infratentorial ICH) post-gadolinium FLAIR images showed contrast leakage distant from the hematoma. Contrast leakage most frequently occurred in a cortical location (sixteen patients, 94%), rarely deep (one patient had both cortical and deep contrast leakage, 6%), and never infratentorial. Nine patients (53%) had contrast enhancement in both hemispheres, two (12%) in the symptomatic hemisphere only, and six (35%) in the contralateral hemisphere only. One patient with a cerebellar ICH had supratentorial contrast enhancement in both hemispheres. Four patients (24%) had a

punctate lesion of contrast leakage (HARM 1), five patients (29%) multiple punctate lesions of contrast leakage (HARM 2), three patients (18%) showed focal sulcal contrast enhancement (HARM 3) and five patients (29%) bilateral and diffuse contrast leakage (HARM 4). Illustrative examples of patients with different HARM scale scores are provided in figure 7.1.

Among the patients with contrast leakage there was a relatively high proportion of women (0.41; risk ratio (RR) 2.5 (95% confidence interval (CI) 0.7-8.8)), a low proportion of patients with a history of atrial fibrillation (0; RR 0.4 (95%CI 0.2-0.6)) and a high mean diastolic blood pressure at admission (95 mm Hg; p=0.12), although these differences were not all statistically

Table 7.1 Baseline characteristics of patients with and without contrast leakage

Characteristics	All N = 31	Contrast leakage N = 17 (55%)	No contrast leakage N = 14 (45%)	Risk ratio (95% CI)	p-value
Patient characteristics					
Mean age, years (SD)	60 (12)	61 (13)	59 (12)		0.55*
Female sex, n (%)	9 (29)	7 (41)	2 (14)	2.5 (0.7-8.8)	0.10#
GCS at presentation, median (IQR)	15 (13-15)	15 (12-15)	14 (13-15)		0.53^
NIHSS at presentation, median (IQR)	5 (2-9)	6 (3-9)	4 (1-8)		0.31^
Mean systolic BP at admission, mm Hg (SD)	166 (33)	174 (36)	158 (28)		0.28*
Mean diastolic BP at admission, mm Hg (SD)	94 (21)	95 (24)	92 (16)		0.12*
History of hypertension, n (%)	19 (61)	9 (53)	10 (71)	0.7 (0.3-1.5)	0.38#
History of TIA, n (%)	4 (13)	3 (18)	1 (7)	1.9 (0.4-11.0)	0.39#
History of ischemic stroke, n (%)	3 (10)	2 (12)	1 (7)	1.4 (0.3-7.2)	0.67#
History of cardiac disease, n (%)	1 (3)	0 (0)	1 (7)	0.4 (0.3-0.7)	0.26#
Diabetes mellitus, n (%)	2 (7)	1 (6)	1 (7)	0.9 (0.2-3.8)	0.89#
Atrial fibrillation, n (%)	3 (10)	0 (0)	3 (21)	0.4 (0.2-0.6)	0.045#
Smoking (current or past), n (%)	13 (42)	5 (31)	8 (57)	0.6 (0.3-1.2)	0.15#
Anticoagulants, n (%)	5 (16)	2 (12)	3 (21)	0.7 (0.3-1.6)	0.47#
Antiplatelets, n (%)	6 (19)	4 (24)	2 (14)	1.4 (0.4-4.8)	0.52#
Hematoma characteristics					
Lobar ICH location, n (%)	11 (36)	7 (41)	4 (29)	1.4 (0.6-3.4)	0.47#
Median hematoma volume, mL (IQR)	14 (4-30)	14 (5-30)	11 (4-32)		0.74^
Subarachnoid extension, n (%)	5 (16)	3 (18)	2 (14)	1.2 (0.4-3.6)	0.80#
Intraventricular extension, n (%)	9 (29)	3 (18)	6 (43)	0.5 (0.3-1.1)	0.12#
Presence of CMBs					
CMBs (yes, %)	23 (74)	14 (82)	9 (64)	1.6 (0.8-3.3)	0.25#
0-1	12 (39)	6 (35)	6 (43)		ref
2-5	8 (26)	3 (18)	5 (36)		0.58@
6-10	5 (16)	2 (12)	3 (21)		0.0.71@
>10	6 (19)	6 (35)	0 (0)		0.99@
Any lobar CMBs (yes, %)	18 (58)	13 (77)	5 (36)	2.5 (1.1-5.7)	0.02#
Strictly lobar CMBs (yes, %)	5 (16)	4 (24)	1 (7)	2.5 (0.4-15.0)	0.22#

Table 7.1 Baseline characteristics of patients with and without contrast leakage (continued)

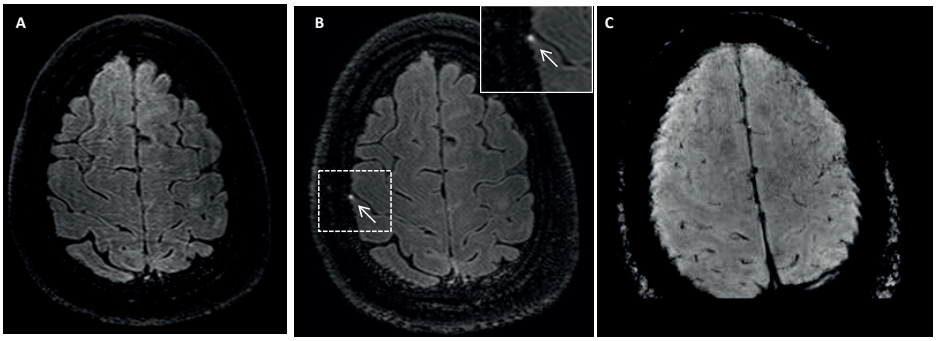
Characteristics	All N = 31	Contrast leakage N = 17 (55%)	No contrast leakage N = 14 (45%)	Risk ratio (95% CI)	p-value
Number of CMBs by location, median (IQR)					
Number of lobar CMBs	1 (0-4)	2 (1-8)	0 (0-2)		0.02^
Number of non-lobar CMBs	1 (0-4)	1 (0-10)	1 (0-1)		0.57^
Total number of CMBs	3 (0-9)	3 (1-23)	3 (0-5)		0.15^
White Matter Hyperintensities					
Fazekas score ≥2, n (%)	16 (52)	9 (53)	7 (50)	1.6 (0.7-3.4)	0.24#
DWI lesions					
Presence, n (%)$	4 (13)	3 (18)	1 (7)	2.0 (0.3-11.5)	0.36#
EPVS					
Basal ganglia$					
<10	12 (39)	6 (35)	6 (43)		ref
10-20	13 (42)	8 (47)	5 (36)		0.85
20-40	1 (3)	0 (0)	1 (7)		0.56
>40	0 (0)	0 (0)	0 (0)		1.00
Centrum semiovale$					
<10	3 (10)	0 (0)	3 (21)		ref
10-20	11 (36)	7 (41)	4 (29)		0.97
20-40	12 (39)	7 (41)	5 (36)		1.00
>40	0 (0)	0 (0)	0 (0)		1.00
Cortical superficial siderosis					
Presence, n (%)	3 (10)	3 (18)	0 (0)		0.20#
Modified Boston criteria					
Probable CAA, n (%)	5 (16)	4 (24)	1 (7)	2.5 (0.4-15.0)	0.17#
Probable CAA (including patients <55 years), n (%)	8 (26)	6 (35)	2 (14)	2.1 (0.6-7.4)	0.18 #

BP = blood pressure, CI= confidence interval, CMB = cerebral microbleed, DWI = diffusion weighted imaging, EPVS = enlarged perivascular spaces, GCS= Glasgow coma scale, ICH = intracerebral hemorrhage, IQR = interquartile range, NIHSS = national institutes of health stroke scale, SD = standard deviation, TIA = transient ischemic attack. *t-test, #chi square, ^Mann Whitney U, @logistic regression, $in 5 patients 3 T MRI was not available significant. All other patient characteristics were comparable in patients with and without contrast leakage (table 7.1). There was no difference in time interval between ICH and 7 T MRI in patients with (median 20 days; IQR 11-70 days) and without (median 22 days; IQR 5-67 days) contrast leakage (p=0.55).

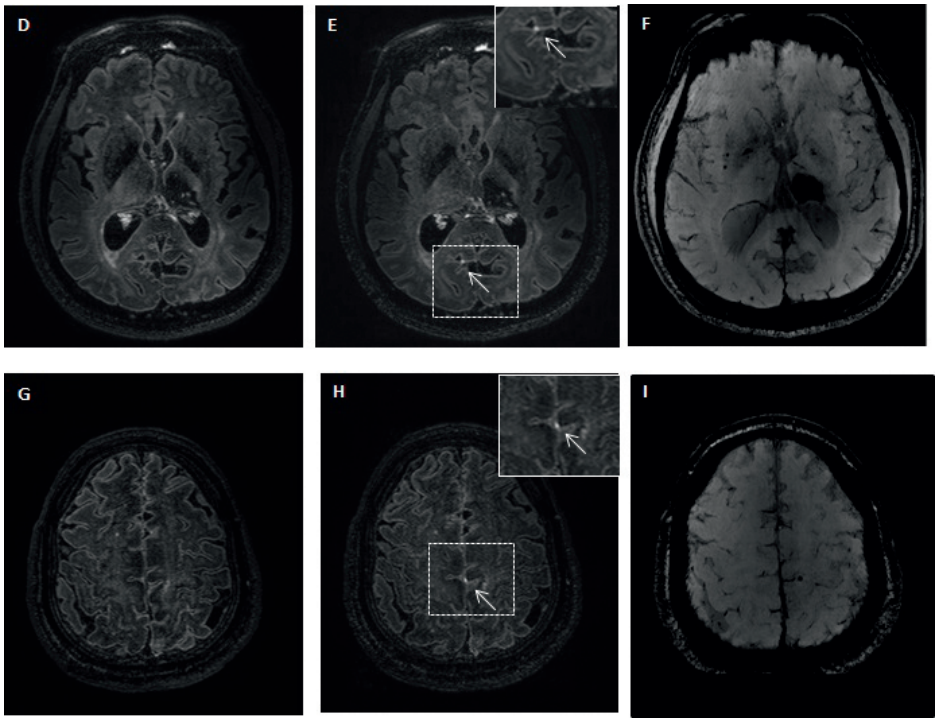
Patients with contrast leakage more often had lobar CMBs (77%) than those without contrast leakage (36%; RR 2.5 (95% CI 1.1-5.7), table 7.1) as well as a higher number of lobar CMBs (patients with contrast leakage: median 2, IQR 1-8 versus those without contrast leakage: median 0, IQR 0-2; p=0.02, table 7.1). Seven out of seventeen patients with contrast leakage had a lobar ICH of which five fulfilled the modified Boston criteria for probable (n=4) or possible CAA (n=1). The other two patients (44 and 51 years old) with lobar ICH had lobar CMBs or superficial siderosis but did not fulfill the modified Boston criteria for probable CAA, because they were younger than 55 years. We found no differences between patients with and without

contrast leakage in the proportion of patients with Fazekas score ≥2, categories of EPVS or the proportion of patients with DWI lesions (table 7.1). We found no spatial relationship between the location of the DWI lesions and the location of the contrast leakage.

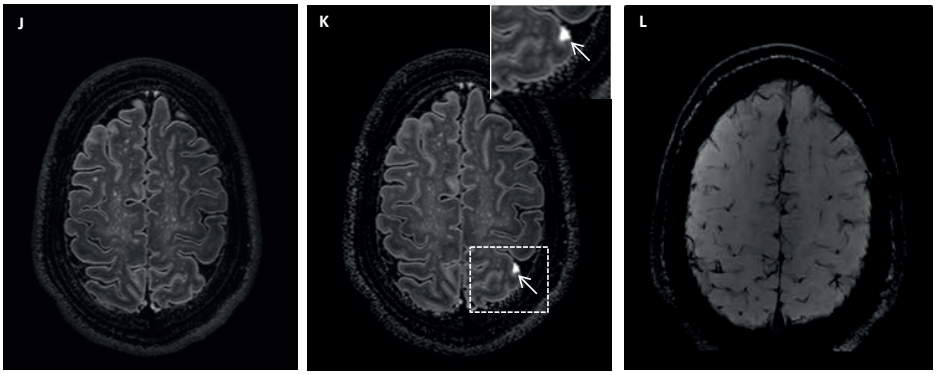
Figure 7.1 Examples of patients with different HARM scale scores



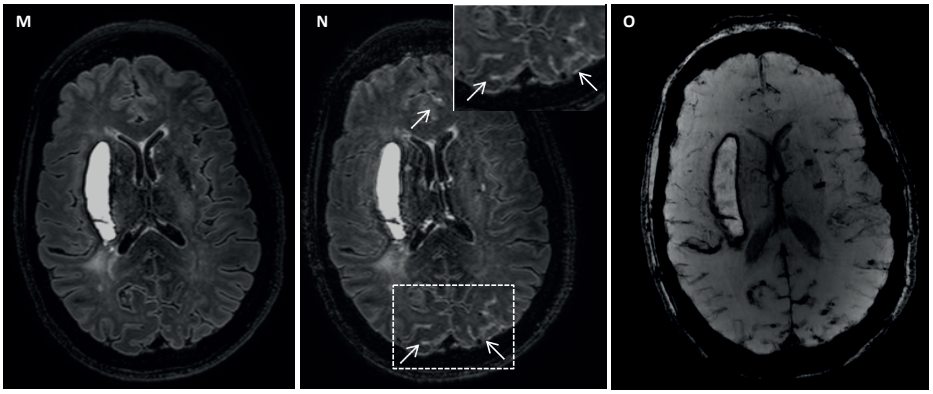
HARM 1: Punctate lesion of contrast leakage. Pre– (panel A) and post-gadolinium (panel B) FLAIR images of a 41 years old man with an infratentorial hemorrhage, a right frontal punctate lesions (white arrow) of contrast leakage and no abnormalities at that site on SWI (panel C).



HARM 2: Multiple punctate lesions of contrast leakage. Pre– (panels D and E) and post-gadolinium (panels G and H) FLAIR images of a 54 years old man with left deep ICH, right occipital (white arrow) and left frontal lesions (white open arrow) of contrast leakage and no abnormalities at those sites on SWI (panel F and I).



HARM 3: Focal sulcal contrast leakage. Pre– (panel J) and post-gadolinium (panel K) FLAIR images of a 45 years old man with a left deep ICH, left parietal focal sulcal contrast leakage (white arrow) and no abnormalities at that site on SWI (panel L).



HARM 4: Bilateral and diffuse contrast leakage. Pre– (panel M) and post-gadolinium (panel N) FLAIR images of a 67 years old woman with right deep ICH, extensive bilateral and generalized occipital contrast enhancement (white arrows) and multiple cerebral microbleeds at that site on SWI (panel O).

DISCUSSION

This study shows that over 50% of the patients with spontaneous ICH have contrast leakage distant from the hematoma as evidenced by high field 7 T MRI in the subacute phase, up to 70 days after ICH. It occurs both in patients with lobar and in those with deep and infratentorial ICH, with a predominantly cortical location. In half of the patients contrast leakage is moderate or severe (HARM 3 and 4) and presence of contrast leakage is associated with presence and number of lobar CMBs.

The proportion of patients with contrast leakage in our cohort (54%) is higher than that in a recent study using 3 T MRI delayed post-gadolinium FLAIR images in healthy elderly subjects (mean age 73 years; 19% with contrast leakage), in patients with mild cognitive impairment (mean age 69 years; 30%) and in patients with Alzheimer's disease (mean age 72 years; 40%).[14] In a retrospective cohort study of 46 spontaneous ICH patients, contrast leakage was found distant from the hematoma in 85% of patients with conventional 1.5 T and 3 T MRI performed in the acute phase (median 11 hours) after hemorrhage onset.[18] In that study, a possible but not significant association was found with a higher national institutes of health stroke scale score at baseline and shorter time to MRI. Location of the hematoma, however, was not reported in that study. Our patients were scanned in the subacute phase (median 20 days), which may explain the smaller proportion of patients with contrast enhancement, despite higher field MRI. The smaller proportion in our study might also be explained by another potential mechanism of the BBB disruption. In the acute study, the BBB disruption may be due to direct injury of the hematoma.[18] The persisting contrast leakage that we found might still be related to the direct effect of the hematoma but could also be an indicator of BBB disruption as a result of cSVD.[27] A previous study described contrast leakage in two of nineteen patients with probable CAA, presenting with either atypical or multiple hemorrhages or superficial siderosis, on post-gadolinium T_1-weighted sequences on 1.5 T MRI.[28] That study did not report the time interval between symptom onset and MRI. In 51 patients with lacunar stroke who underwent MRI in the subacute phase (mean 64 days, SD±12), BBB permeability was present more generalized in most of the sulci and EPVS, in contrast to the areas of focal enhancement in our study of ICH patients. In that study BBB permeability was visualized using pre- and post-gadolinium T_1-weighted MR sequences and two image processing methods.[9]

We found no association of contrast leakage with DWI lesions on 3 T MRI. A possible explanation might be the variable time interval between 3 T and 7 T MRI with a median time interval of 7 days (IQR 1-56 days), and DWI lesions may start to disappear after approximately 7 to 10 days.[29]

The results of our study suggest that increased permeability of the BBB might play a role in cSVD related ICH, in particular in the presence of lobar CMBs. A potential mechanism explaining contrast leakage is, the cascade of events that follow the direct injury and mass effect of ICH, consisting of a release of clot-derived factors, cortical spreading depression and an inflammatory response, including leukocyte infiltration, microglia activation, cytokine and chemokine elevation and glutamate neurotoxicity. These lead to additional brain injury and BBB disruption, supporting the hypothesis that contrast leakage may be a consequence of ICH.[1,30-36] This might also explain why contrast leakage can be found in a different lobe than the hematoma or the contralateral hemisphere.

Another hypothesis based on animal studies is that BBB disruption may in fact precede ICH.[37,38] This hypothesis is supported by our previous observation of a patient with multiple lobar ICHs who showed focal contrast enhancement before appearance of a CMB exactly at the site of that contrast enhancement.[39] As we assessed patients only after they had experienced the ICH, our study cannot draw conclusions on causal inference. MRI contrast leakage indicative of BBB disruption may be another marker of the underlying cSVD pathology next to the ICH and classic MRI markers of cSVD, including WMH, lacunes, EPVS, CMBs, recent small subcortical infarcts and brain atrophy.[21] To date, it remains unclear why in some patients cSVD manifests as ischemia whereas in others cSVD causes hemorrhage. Even in the cSVD that is related to hypertension and not CAA, some patients appear to be more prone to hemorrhage than others as suggested by the fact that new CMBs are found more frequently in those who already had CMBs before, than in those who did not.[40-42]

We found contrast leakage predominantly cortical, even in patients with deep ICH. A potential explanation is that hypertensive vasculopathy not only affects the deep penetrating vessels but also the superficial cortical vessels or that it affects deep penetrating vessels in a different way than superficial cortical vessels.[43] Also, it could be that patients with non-lobar ICH have CAA in addition to hypertensive vasculopathy (for example HARM score 4 in figure 7.1).[44] Other studies describe that contrast leakage or BBB disruption might be caused by cortical spreading depression.[31,45-48] The association of contrast leakage with lobar CMBs and that four of the five patients in our study with probable CAA according to the modified Boston criteria had contrast leakage, tentatively supports a relation with CAA. The predilection of contrast leakage in cortical rather than deep areas was also found by others in cognitive healthy elderly subjects, and in patients with mild cognitive impairment or Alzheimer's disease and ischemic stroke.[14-16,18,49,50]

We found no association with other cSVD markers than CMBs (i.e. DWI lesions, EPVS and WMH). In a previous cohort of ICH patients there was also no association of contrast leakage with WMH.[18] In the study in cognitively healthy elderly patients, patients with mild cognitive impairment and Alzheimer's disease, there was also no association of contrast leakage with lacunes or WMH.[14] This might indicate that contrast leakage is a marker independent of other cSVD features, and possibly be particularly associated with CAA.

Strengths of our study are that we were able to prospectively collect patients with spontaneous ICH from multiple centers and assess these patients in the 7 T MRI scanner in the subacute stage of ICH. Another strength is that 7 T MRI has a high signal-to-noise ratio with also better contrast-to-noise in FLAIR images than conventional MRI scanners. This allows the use of voxel sizes less than a millimeter and consequently the possibility of a better yield by being able to detect small punctate lesions of contrast leakage, including in deeper areas.[51] We used FLAIR

images instead of T_1-weighted images, because this MR sequence has a better sensitivity in detecting low concentrations of Gadolinium.[14,52,53] Furthermore, FLAIR images are less sensitive to the effects of gadolinium contrast in the blood vessels, because of a stronger signal decay due to the effects of blood flow in combination with shortening of the T_2 relaxation time at higher concentrations of gadolinium.[14,54] Post-gadolinium FLAIR enhancement on 3 T MRI is a novel technique which has also been used in cranial nerve imaging for identifying both normal and abnormal cranial nerves.[55-57]

Our study also has limitations. First, the sample size was small. Second, including severely affected ICH patient for 7 T MR imaging is challenging and this may have resulted in a selection bias in that we included less affected patients with smaller hematomas and a relatively lower NIHSS scores. Third, assessing contrast leakage on post-gadolinium FLAIR images may be more rater dependent than techniques such as DCE-MRI and cannot be used to quantify the amount of leakage. Fourth, raters were not blinded for ICH location which might have included detection bias. Finally, there was variation in time interval between ICH and MRI between patients. As the median time interval was similar between patients with and without contrast leakage this will not have affected the analyses of different cSVD markers in these groups.

Our findings need confirmation, preferably in a larger cohort of ICH patients with and without CAA. Furthermore, it would be of interest to study the relation between contrast leakage and outcome. Also, sequential scanning of patients with contrast leakage to follow changes in contrast enhancement over time would be of interest. Further work is needed to determine whether FLAIR contrast enhancement, as a biomarker of BBB disruption, is able to predict recurrent ICH or cognitive deterioration.[21]

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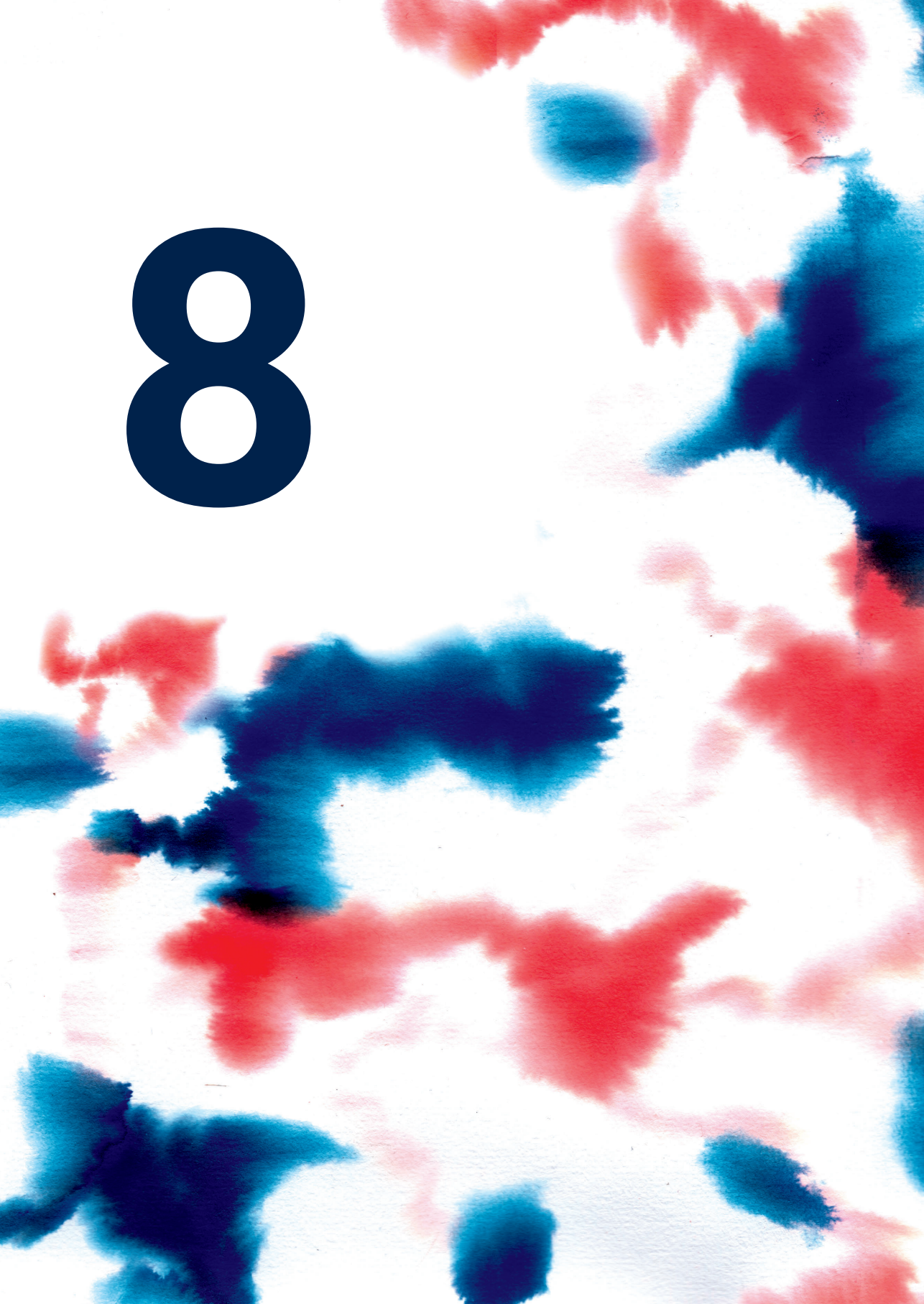
8

GENERAL DISCUSSION

GENERAL DISCUSSION

The overall aim of studies described in this thesis was to explore the etiology in spontaneous intracerebral hemorrhage (ICH; non-traumatic intracerebral hemorrhage without a secondary cause). We identified time trends and risk factors in different subgroups of ICH and assessed presence, severity and distribution of markers of small vessel disease (SVD), cerebral microinfarcts (CMIs) and blood-brain barrier (BBB) disruption on MRI in patients with ICH. In this chapter, I discuss the main findings of this thesis, methodological considerations, implications for clinical practice and future directions for research.

Main findings of this thesis

- In the Netherlands, between 1998 and 2010, ICH incidence, and 30-day and 1-year case fatality declined in men and women <75 years, but not in persons ≥75 years. ICH mortality also declined between 1980 and 2010, in men and in women <75 years, and remained stable in patients ≥75 years. The most prominent decline in mortality was observed in patients aged 55 to 74 years, with a steeper decline in men than in women (chapter 2).
- Hypertension is a risk factor for both lobar and non-lobar ICH, whereas diabetes, male sex, alcohol overuse, underweight, and being black or Hispanic are risk factors for non-lobar ICH only (chapter 3).
- Cerebral microinfarcts are common in patients with ICH and related to presence and number of cerebral microbleeds, but not related to vascular risk factors and other MRI markers of small vessel disease (chapters 4 and 6). On histopathology, cortical cerebral microinfarcts and cerebral microbleeds were more often found in the superficial than in the deep layers of the cortex in patients with lobar ICH, whereas in non-lobar ICH these lesions were more often found in the deeper than in the superficial layers of the cortex (chapter 5). In a 7 tesla MRI study we confirmed the finding of the more frequent superficial than deep localization in the cortex of cerebral microinfarcts in lobar ICH patients in vivo (chapter 6).
- Post-gadolinium enhancement on fluid attenuated recovery (FLAIR) images distant from the hematoma is common in days to weeks after ICH. This measure of blood-brain barrier disruption is located predominantly in cortical areas and related to lobar cerebral microbleeds; therefore it is possibly related to cerebral amyloid angiopathy (chapter 7).

Epidemiology and risk factors of intracerebral hemorrhage

For a long time, overall ICH incidence, case fatality and mortality have remained stable.[1] The results of this thesis show that in the Netherlands, between 1998 and 2010, ICH incidence, 30-day and 1-year case fatality declined in men and woman younger than 75 years, but not in those 75 years and older. Between 1980 and 2010, ICH mortality decreased in patients younger than 75 years, with the largest decline in the age group 55 to 74 years old and more in men than in women. In contrast, mortality remained stable in patients of 75 years and older. These results underline the importance not to combine epidemiological data in both sexes and for all age groups, because both changes over time, as well as the identification of groups in which interventions were more or less effective, may be missed.

The decline in ICH incidence in in patients younger than 75 years may be explained by better primary prevention and secondary prevention (after other vascular events) in the last years. Guidelines were updated with more stringent control of hypertension, diabetes and hypercholesterolemia and programs to stop smoking and prevent and treat alcohol overuse.[2–6] The decrease in case fatality can be attributed to improvements in treatment in the acute phase, particularly the introduction of organized stroke unit care.[7] In recent years early intensive blood pressure lowering has been implemented. A pooled analysis of individual patient-level data including 3,809 participants of the INTERACT2 and ATACH-II trials showed a modest effect of lower blood pressures on good functional outcome (modified Rankin scale score ≤3 at 90 days; [adjusted OR 0.90, 95% CI 0.87-0.94 per 10 mm Hg higher systolic blood pressure] i.e. per 10 mm Hg lower systolic blood pressure a 10% increase in the odds of a better functional outcome).[8–10] These trials were published in 2013 and 2016 and our analysis included data until 2010. A future study is needed to look for an association between implementation of early intensive blood pressure lowering and case fatality. Case fatality was not the primary outcome in the INTERACT 2 and ATACH-II trials, but the individual patient data meta-analysis of these studies showed a significant association between a higher (achieved) mean systolic blood pressure and death within 90 days.[10] The implementation of early intensive blood pressure lowering might therefore have a beneficial effect on case fatality.

The lack of a decline in incidence in patients of 75 years and older might be the result of increasing use of antithrombotic medication in elderly patients,[11] which may supersede the effect of improvements in primary and secondary prevention (i.e. control of hypertension, diabetes and hyperlipidemia and programs to stop smoking and prevent alcohol overuse) in this age group. Patients using non-vitamin K antagonist oral anticoagulants (NOACs), for the prevention of ischemic stroke in non-valvular atrial fibrillation, have a lower risk of ICH compared to patients using warfarin (HR 0.48, 95% CI 0.34-0.67).[12] NOACs were introduced at the end of (and after) our study period (1998-2010), so we were not able to assess the effect of the introduction of NOACs.[13–16] Considering the lower risk of ICH, the introduction of these alternative anticoagulants might result in a lower incidence of ICH in elderly patients.

In our registry in the Netherlands, we were not able to identify time trends of incidence by location (lobar or non-lobar) of the ICH. An earlier study based on a population-based registry in Dijon, France, described a decrease in incidence of both lobar and deep ICH in persons younger than 60 years between 1985-1992 and 2001-2008.[17] In persons of 75 years and older the incidence of lobar, but not deep ICH, more than doubled between 1985-1992 and 2001-2008.[17] This might be explained by a higher incidence in elderly persons of cerebral amyloid angiopathy (CAA),[18,19] for which control of vascular risk factors is less effective and the risk of anticoagulant-related ICH even higher.[17,20] The absence of decline in case fatality in elderly

patients may also be explained by the increasing use of anticoagulants, because the use of anticoagulants is associated with larger hematoma volume and more hematoma expansion and both volume and expansion are associated with in-hospital mortality.[21] Additionally, early care-limiting decisions in elderly patients, because of a presumed lower chance to survive with good functional outcome, might also play a role. Various studies showed that early care limitations are independent predictors of mortality in patients with ICH.[22,23]

Identification of risk factors might help in understanding time trends of incidence and the etiology of subgroups of ICH. The results of this thesis show that hypertension is a risk factor for both lobar and non-lobar ICH, albeit with a more than two times smaller effect in lobar ICH. Longstanding hypertension is considered to affect small, deep, perforating intracranial arteries and arterioles, causing lipohyalinosis, microatheromas, fibrinoid necrosis and microaneurysms.[24] These changes increase the probability of vessel rupture in deep and infratentorial locations. In leptomeningeal vessels, similar changes have been described, particularly fibrinoid necrosis and microaneurysms, in patients with and without coexisting CAA.[18,25–27] This suggests that partly similar pathophysiological mechanisms play a role in lobar ICH as in non-lobar ICH. Leptomeningeal vessels appear to be affected to a different degree by hypertension than deep perforating arteries.

Diabetes, male sex, alcohol overuse, underweight, and being black or Hispanic compared to being white are risk factors for non-lobar ICH only. No differences were found between deep and infratentorial ICH. Most of these risk factors also affect the deep perforating intracranial, cerebellar and pontine arteries and arterioles, directly or indirectly through hypertension, and subsequently increase the likelihood of non-lobar ICH.[24,28] Abnormal glucose metabolism causes endothelial dysfunction and arteriolosclerosis.[24,29] Alcohol overuse is a risk factor for hypertension, so hypertension may act as a mediator between alcohol overuse and non-lobar ICH.[30] Alcohol can also impair platelet function and lower coagulation factors in the context of liver cirrhosis.[31,32] Underweight is associated with low serum cholesterol, which may cause necrosis of arterial smooth muscle cells, enhancing vulnerability of the endothelium to formation of microaneurysms.[33] The higher risk of non-lobar ICH in men than women may in part be explained by the higher prevalence of hypertension, diabetes and alcohol overuse in men,[34–36] but also by male-specific risk factors of stroke, such as decreasing testosterone levels in older men.[37,38] The increased risk of non-lobar ICH of blacks and Hispanics compared with whites might be explained by the higher prevalence of hypertension, diabetes and alcohol overuse in these populations.[39,40] It is unclear if and to what extent the abovementioned mechanisms affect leptomeningeal vessels, but apparently to a lesser degree, because diabetes, male sex, alcohol overuse underweight and being black or Hispanic are not associated with lobar ICH.

FETCH study

To further explore the etiology in ICH we conducted the Finding the ETiology in Cerebral Hemorrhage (FETCH) study. This is a multicenter, observational cohort study, with prospective patient enrollment in the University Medical Centers of Utrecht, Leiden and Nijmegen between October 2013 and December 2018. In FETCH we included a total of 221 adult patients with ICH confirmed by computed tomography (CT). We excluded patients with a known cause (tumor, cavernoma, coagulation disorder) or macrovascular cause on CT angiography (CTA). If a brain CTA with CT Perfusion was performed at admission, we performed a control brain CT after 24 to 48 hours. We performed 3 tesla (T) and 7 T MRI preferably within 10 days, but if not possible up till 3 months after the ICH. We performed telephone follow up at 3, 12, 24 and 48 months, in which we asked for new vascular events, determined the modified Rankin scale score and assessed cognitive status by 'the modified Telephone Interview for Cognitive Status (TICS-M).[41] In patients who died as a consequence of ICH we asked families for consent for routine pathological examination. After autopsy, we could request the residual material for research purposes.

Not all included patients underwent all imaging modalities, so at the end of the study period we were able to acquire 3 T MRI in 166 patients and 7 T MRI in 51 patients.

Cerebral microinfarcts in intracerebral hemorrhage

CMIs are, as the name implies, small lesions of presumed ischemic origin.[42] They are a frequent finding on brain autopsy in different disease settings particularly in patients with dementia, but also in patients with cerebrovascular disease.[43,44] Until 2012, CMIs were called the 'invisible lesion', because up till then structural imaging could not visualize CMIs. High resolution structural MRI has enabled visualization of the largest CMIs (1-2 mm) in vivo.[45] They can be detected in the acute stage (<2 weeks) with diffusion-weighted imaging (DWI) and in the acute (within hours on T_2-weighted images, within days on T_1-weighted images) and chronic stage with high-field MRI (hypointense on T_1-weighted images, hyperintense, isointense or cavitated on fluid attenuated inversion recovery (FLAIR) images, strictly intracortical and ≤ 3 mm).[42] The main presumed causes of CMIs are 1) cerebral SVD, 2) microemboli and 3) hypoperfusion.[42] Especially the presence of CMIs in the context of cerebral SVD makes CMIs interesting lesions to investigate in patients with ICH.

Neuropathological studies have shown that CMIs are prevalent in patients with SVD.[44] An autopsy study in more than 1,000 community dwelling-persons found an association of cortical CMIs with CAA and an association of subcortical (i.e. not cortical, but location not further specified in that study) CMIs with atherosclerosis and arteriolosclerosis.[46] Multiple other autopsy

studies in patients with Alzheimer's disease and CAA also found an association between more severe CAA and the occurrence and higher number of CMIs.[47–49]

In vivo studies found that CMIs are often found in patients with CAA with and without lobar ICH, on 1.5 and 3 T MRI, with proportions ranging from 40 to 60%.[50,51] These studies also found associations with the presence of cortical superficial siderosis, but not with any or lobar microbleeds (CMBs).[50,51] In patients with Dutch type hereditary CAA (also known as Hereditary Cerebral Hemorrhage with Amyloidosis – Dutch type, a hereditary form of CAA) CMIs demonstrated on 7 T MRI, can be, along with white matter hyperintensities (WMH), one of the earliest markers of the disease, compared with other MRI markers and clinical symptoms.[52] A meta-analysis of studies investigating presence of DWI lesions (i.e. acute CMIs <5 mm) in ICH patients, showed associations of DWI lesions with WMH and presence and number of CMBs.[53] In patients attending a memory clinic and population based cohorts, CMIs were found to be associated with other MRI markers of SVD, including lacunar infarcts, a higher volume of WMH and cerebral atrophy.[54–56] Based on these findings, the questions arose whether (acute and/or chronic) CMIs are present in patients with lobar and non-lobar ICH, whether they are associated with other MRI markers of SVD in ICH patients, and whether CMIs are associated with CAA-related ICH or also with arteriolosclerotic vasculopathy-related ICH.

In this thesis we showed that CMIs are found in two-thirds of patients with lobar and non-lobar ICH on 7 T MRI. In ICH patients, CMIs are related to presence and number of CMBs, but not to other MRI markers of SVD. In patients with ICH, we found no associations of presence of CMIs with vascular risk factors, such as hypertension, diabetes or current or past smoking. Previously reported associations of presence of CMIs with vascular risk factors have shown conflicting results. An earlier 7 T MRI study found no differences in presence and number of CMIs in a group of 48 patients with type 2 diabetes mellitus compared with controls.[57] In a 3 T MRI study in 238 patients attending a memory clinic no associations were found for presence of CMIs with diabetes or hypertension, but that study did find an association with hyperlipidemia.[54] In a cohort of 231 patients with TIA and ischemic stroke, investigated with 3 T MRI, no associations were found for smoking, alcohol use, diabetes, hypertension or hyperlipidemia with presence of CMIs.[58] In a population based study including 861 participants that underwent 3 T MRI, CMIs were associated with hypertension.[56] It seems that the disease setting is important in interpreting the results of associations of vascular risk factors with CMIs.[42] Because of our selection of patients with ICH, risk factor patterns are obviously different than in the general population or a memory clinic setting and therefore influence the association with CMIs. The impact of vascular risk factors on the development of CMIs remains unclear.

This thesis showed that CMIs were more often located in the superficial than in the deep part of the cortex in patients with lobar ICH compared to non-lobar ICH patients, in both histopathological examination and in vivo examination on 7 T MRI. This is in line with a recent post-mortem study, which found half of CMIs in the more superficial layers of the cortex in 12 cases with definite CAA with and without lobar ICH.[59] CAA might specifically cause damage to superficial branches of leptomeningeal or cortical arteries. Indeed, a neuropathological study including 113 consecutive brain autopsies, showed that CAA mainly affected the superficial cortical branches of pial arteries.[60] The majority of lobar ICH patients in our histopathology study (75%) had definite CAA and half of the lobar ICH patients that underwent 7 T MRI in the FETCH study fulfilled the modified Boston criteria for probable CAA.[61] This suggests that CAA might specifically affect superficial branches of cortical arteries with superficial cortical CMIs as a consequence. Superficial cortical CMIs might therefore be a specific marker for CAA.

The abundance of ischemic lesions in ICH is intriguing. The underlying SVD in ICH apparently causes both hemorrhagic lesions (macrobleeds, CMBs, cortical superficial siderosis) and ischemic injury in the appearance of CMIs and WMH. CMIs might be caused by the acute event of the ICH. The mass effect of the hematoma and subsequently increase in intracranial pressure could cause hypoperfusion. CMIs can also be the effect of treatment (rapid blood pressure lowering). The presence of DWI lesions (i.e. acute CMIs) in the first days after ICH suggest an association with the either the acute event or the effect of treatment or a combination of both. A meta-analysis investigating associations between DWI lesions and ICH found a significant association between mean arterial pressure reduction and DWI lesions.[53] The concurrent presence of chronic, cavitated CMIs however indicates an ongoing process. SVD could cause impaired cerebral blood flow and loss of autoregulation resulting in chronic subclinical ischemia.[24] Other mechanisms underlying the ongoing process in SVD, might be apoptosis of oligodendrocytes (leading to WMH), local subclinical inflammation and damage to the BBB.[24]

Blood-brain barrier disruption in intracerebral hemorrhage

The BBB is a network of microvasculature between the systemic blood circulation and the central nervous system, formed by vascular endothelium connected by tight-junctions, astrocytes and pericytes.[62] The BBB regulates the transport of proteins and inflammatory cells to control the immune system, maintain ion homeostasis and restrict the entrance of toxins.[62,63] One of the hypotheses of the mechanism underlying SVD, is that the BBB becomes permeable to substances that would normally remain within the blood and outside the central nervous system.[24,63] The BBB permeability presumably is the consequence of increased leakiness of endothelial walls of small vessels, resulting in thickening and breakdown of the vessel walls and perivascular edema.[63] Multiple studies have shown presence of increased BBB permeability in patients with vascular dementia or Alzheimer's disease and more BBB

permeability with increasing severity of WMH. In these studies BBB permeability was measured by either biochemical tests (e.g. cerebrospinal fluid (CSF) / serum albumin ratio) or imaging (e.g. contrast CT or MRI, PET).[64]

One of the techniques that can be used to visualize BBB disruption is post-gadolinium enhancement of the CSF on FLAIR images. Gadolinium contrast agents contain larger molecules that normally do not cross an intact BBB and can therefore be used as a marker of BBB disruption.[65] Post-gadolinium FLAIR images are used instead of post-contrast T_1-weighted images, because FLAIR images have a better sensitivity in detecting low concentrations of gadolinium.[66–68] FLAIR images are also less sensitive to the effects of gadolinium in the vessels, because of a stronger signal decay due to the shortening of the T_2 relaxation time at higher concentrations of gadolinium in combination with the effects of blood flow.[66,69] This technique has shown presence of BBB disruption on 3 T MRI in healthy individuals (19%), in patients with mild cognitive impairment (30%), in patients with Alzheimer's disease (40%),[66] and in patients with ischemic (all subtypes) stroke (33%).[70] A previous study in patients with ICH, demonstrated BBB leakage distant from the hematoma in 85% of 46 ICH patients on 1.5 and 3 T MRI performed in the acute phase after the onset of ICH (median 11 hours).[71] In this thesis we provide further evidence that BBB disruption is present in patients with ICH remote in time and place from the hemorrhage itself. We used 7 T MRI with delayed (at least 10 minutes after contrast injection) FLAIR images with long (>8 minutes) acquisition time and found BBB leakage in 54% of patients with lobar and non-lobar ICH, distant from the hematoma, and in the subacute phase after ICH onset (median 20 days).

All we have demonstrated so far is evidence of BBB disruption in the presence of ICH. It remains unclear if BBB disruption is a cause or the consequence of the ICH. BBB disruption can be caused by the direct injury of the ICH. The hematoma causes mass effect with increasing intracranial pressure and reducing local cerebral blood flow with ischemia as potential result. Both the mass effect and the ischemia can disrupt the cellular architecture of the BBB.[72] Secondary injury of the ICH can be caused by release of clot-derived factors, cortical spreading depression and inflammatory responses.[72–78] The combination of primary and secondary injury after ICH explains both peri- or intrahematomal BBB leakage and BBB leakage distant from the hematoma.

If BBB disruption was causative in SVD, one would expect more progression of SVD markers on follow up imaging in patients with BBB leakage compared to those without.[63] A study in 70 patients with lacunar and cortical stroke showed more progression of WMH at 3-years follow up in patients with BBB permeability (in enlarged perivascular spaces (EPVS)) in the basal ganglia at baseline compared to patients without BBB permeability.[79] A study supporting the causative hypothesis of BBB disruption in ICH, is a case report of a patient with multiple lobar

ICHs and BBB leakage at a site where a CMB was found at follow up imaging 20 months later.[80] Our finding that BBB leakage is still present in the subacute phase after ICH, could suggest that BBB disruption is not just a consequence of the ICH. As we only assessed BBB disruption in patients after their ICH, our study cannot draw conclusions on causal inference. More imaging follow-up studies are needed to prove a possible causative role of BBB disruption underlying the SVD in ICH.

This thesis showed that the BBB leakage in patients with lobar and non-lobar ICH is predominantly located in the cortical areas and in association with lobar CMBs. We did not find the earlier described BBB permeability in the (EPVS in) basal ganglia in lacunar and cortical stroke.[79] This might be explained by the differences in patient groups. We investigated patients with both CAA-related and arteriolosclerotic vasculopathy-related ICH while the other study included lacunar strokes associated with arteriolosclerosis and cortical (large-artery) strokes. Furthermore, the other study used post-contrast T_1-weighted images and not post-gadolinium FLAIR, which might also be the reason that they did not find more cortical BBB permeability.[79] We found BBB leakage in four of five patients with probable CAA. This suggests that cortical BBB disruption might be a marker of CAA. This is supported by a post-mortem study that showed more plasma protein extravasation (as marker of BBB disruption) in CAA patients compared to controls (without neurological disease or brain lesions) and an association of plasma protein extravasation with more severe CAA and number of CMBs.[81]

Methodological considerations

The various studies described in this thesis had different designs, each with their own strengths and limitations. The internal validity of the studies in this thesis could have been affected by different sources of bias and confounding.

Information bias is any systematic difference from the truth that arises in the collection, recall, recording and handling of information in a study.[82] In our epidemiological study analyzing time trends in ICH incidence, case fatality and mortality, we used the national hospital discharge register (HDR) and the Dutch population register. With this method we were able use the nationwide data of more than 40,000 persons with a broad range of age groups for both sexes. The HDR and Dutch population register are based on International Classification of Diseases (ICD) codes, so misclassification of an admission as ICH might have occurred. We therefore assessed accuracy of coding ICH diagnosis by checking medical records of a random sample of >1000 patients in a university hospital and a large teaching regional hospital coded as ICH. We showed correct coding in >91% of patients. The less than 100% accuracy of coding could have resulted in an overestimation of the incidence on the one hand. On the other hand, an ICH could have been miscoded as well and the percentage of misclassification is small, so it is not

likely that misclassification has influenced the observed trends. By using the HDR, we might have missed patients that had an ICH but were not admitted to a hospital, for example patients that had an ICH in a nursing home. This could cause an underestimation of the incidence.

In our systematic review and meta-analysis we included data from cohort, case-control and case-crossover studies. Information bias probably played a role because of 1) different and changing risk factor definitions; 2) changing methods and extent to which patients were investigated for the presence of risk factors between patients with lobar ICH and non-lobar ICH; 3) misclassification of hematomas, if investigators were not blinded for risk factors. The change of the definition of a risk factor may have caused an underestimation of the association of a risk factor with lobar or non-lobar ICH. For example, for the risk factor hypertension the limit for the systolic blood pressure became more strict over time, so patients that in the past would not have been classified as hypertensive, would be according to current standards. A hematoma is possibly more likely to be classified as non-lobar if investigators were not blinded for hypertension-status. This might have might have resulted in overestimation of the risk factor hypertension. In our meta-analysis we used the information of 42 studies with more than 26,000 patients, so the influence of the above-mentioned sources of bias on the results is probably small, because each study used different methods, taking into account various factors.

In the FETCH study information bias may also have been at play, because 1) in assessing the MRI markers of SVD, raters were not blinded to location of the ICH, which could cause both an under- and overestimation of the presence and number of some lesions; 2) not all lesions were rated by the same reader, but each lesion type was rated by two readers and a random sample of at least 20% was rated by both readers independently. Discrepancies were discussed in a consensus meeting and if necessary, discussed with an experienced neuroradiologist or stroke neurologist. This could result in, if at all, both an under- and overestimation of presence and number of a lesion; 3) MRI scanners in three different centers were used, which could cause differences in detection of MRI markers because of variation in image quality. By using the same scan protocols across centers possible systematic errors in MRI measures were avoided.

Selection bias, occurring when individuals or groups in a study differ from the population of interest leading to a systematic error in an association,[83] has played a role in the FETCH study. Including severely affected ICH patients is challenging. In a large group of these patients, often with a reduced level of consciousness, family members have to give consent and are often reluctant, because they do not want their family member to undergo more investigations. If patients are able to give consent, they are sometimes equally reluctant, because they also do not want more investigations due to the severity of their symptoms. Moreover, not all ICH patients are able to undergo MRI, because they should be able to lie still for at least 30 minutes

and to follow instructions. This might have resulted in the inclusion of younger, less affected patients with relatively mild symptoms. Elderly patients with larger hematomas and a depressed level of consciousness on admission have a high risk of death,[84] and are consequently not included in our imaging studies. This might have resulted in, for example, an underestimation of the presence and severity of cortical superficial siderosis in ICH patients, because cortical superficial siderosis is associated with a larger lobar ICH volume.[85] It is unclear what this means for the severity of other SVD markers. In our 7 T studies, we included relatively young patients (mean age 60 years), which could result in lower proportions of patient with CMIs and BBB disruption, because both markers are related to older age.[42,63] Furthermore, we were not always able to perform all planned imaging (3 T and 7 T MRI), because of clinical deterioration or death before the planned follow up imaging. Also, logistic reasons, as availability of scan slots and a need for transfer to a regional hospital or a rehabilitation center before all procedures had been performed, may have played a role. This might have resulted in the absence of data on both ends of the spectrum of ICH patients. Nevertheless, we were fortunate to include and perform, with the help of the neurology and radiology departments in the Leiden University Medical Center, Radboud university medical center Nijmegen and University Medical Center Utrecht, 3 T MRI in more than 150 ICH patients, and 7 T MRI in >50 ICH patients, making this a unique cohort.

Confounding is a distortion that modifies an association between an exposure and an outcome, because a factor is independently associated with the exposure and the outcome.[86] In our systematic review and meta-analysis we cannot rule out confounding in the included studies. Some, but not all, adjusted for other risk factors. In the studies based on the FETCH cohort, we used multivariable regression analysis, to minimize the influence of confounding.

An important question in interpreting the data from the studies in this thesis is to what extent the conclusions are generalizable to all patients with ICH. In our systematic review and meta-analysis we included studies from all over the world. However, studies from countries in Africa, South America and South-East Asia were underrepresented, so extrapolating the conclusions to patients in these regions should be done with caution. Some risk factors might be more prevalent in Hispanics, blacks and Asians.[39] The study of trends in time of ICH incidence, case fatality and mortality and the studies from the FETCH cohort were performed in the Netherlands. The results can probably be extrapolated to other countries in Northern and Western Europe, but not to other regions because of differences in ethnic composition of the populations and health care systems. The patients in the FETCH study were included in three tertiary referral, university hospitals. However, each university hospital in the Netherlands also has a regional function in addition to its tertiary referral position. Most patients are referred by their general practitioner or directly brought in by the ambulance services (>95% in the FETCH study). Some of the patients are referred by colleagues in a general hospital, for example for the possibility

of neurosurgical intervention. The proportion of cerebellar ICH (13% in the FETCH study) is therefore presumably higher in university hospitals compared to general hospitals. Younger patients are more frequently referred to university hospitals directly. An underlying cause of the ICH is more likely to be found in younger patients,[87] but these patients were excluded in our study.

Implications for clinical practice and future research

For a long time, ICH has been considered one entity that should be diagnosed and treated in the same way. ICH incidence and case fatality seemed to remain stable despite improvements in diagnostic techniques and many clinical trials in this field. In this thesis, we have shown that in the Netherlands incidence, case fatality and mortality in fact are declining since the eighties and nineties in men and women younger than 75 years. In patients of 75 years and older incidence, case fatality and mortality remained stable. One of the reasons for the lack of decrease in incidence in elderly patients might be the increasing use of anticoagulant medication in this group. NOACs were mostly introduced after our study period. It would be of interest to study whether the increased use of NOACs resulted in a decrease in incidence, case fatality and mortality in the last decade. Secondly, early intensive blood pressure lowering in the acute phase after ICH has also been implemented after our study period. The question is if early intensive blood pressure lowering resulted in a (further) decline in case fatality in all age groups. Third, it would be interesting to investigate if the growing knowledge regarding the importance of controlling risk factors, such as hypertension, led to a decline in incidence in patients of 75 years and older.

In our systematic review and meta-analysis we found that hypertension is a risk factor for both lobar and non-lobar ICH and that hypertension is not the only risk factor for non-lobar ICH. The term "hypertensive ICH" to distinguish deep or non-lobar ICH from lobar ICH seems inappropriate and should, in my opinion, no longer be used. Furthermore, the differences in risk factor profiles of lobar and non-lobar ICH would merit further research into distinct secondary prevention strategies to prevent recurrent events in lobar and non-lobar ICH. One of the challenges in the period after an ICH is, if and when to restart antiplatelet or anticoagulant medication if a patient was already using this as secondary prevention after an earlier vascular event. A recent study showed that ICH survivors are at a high risk of vascular events in the first five years after their ICH.[88] More interestingly, that study showed that non-lobar ICH was associated with ischemic events (HR 1.85, 95% CI 1.01-3.40), whereas lobar ICH was related to hemorrhagic events (HR 2.38, 95% CI 1.17-4.86).[88]A recent population-based study from China demonstrated that 41% of recurrent strokes in the 9 years after an ICH were ischemic strokes.[89] The results of the RESTART trial showed a lower recurrence of ICH in patients that restarted antiplatelet therapy (after a median of 76 days, IQR 29-146) after ICH while on antithrombotic therapy, compared to those that did not (HR 0.51, 95% CI 0.25-1.03).[90] This trial has taught us

that restarting antiplatelet therapy weeks after ICH is safe and might be beneficial in some subgroups. Considering our finding that the risk factor profile of non-lobar ICH is more similar to ischemic (large-vessel) stroke than to lobar ICH, it may be interesting to investigate if it is beneficial to start antiplatelets in patients with non-lobar ICH that did not use antiplatelets before the ICH.

CMIs and contrast leakage on 7 T MRI are novel markers of the SVD underlying ICH. CMIs in the superficial layers of the cortex and contrast leakage are associated with lobar ICH and possibly with CAA. Because 7 T MRI is not routinely used in clinical practice, the next question would be if both markers and the same associations can also be found on 3 T MRI in patients with ICH. Another factor limiting use of detection of CMIs in clinical practice is it is time consuming and difficult. It takes approximately 20 to 30 minutes to assess the MRI scan of one patient and sensitivity is relatively low (compared to neuropathological examination).[42] Further research and clinical use could benefit from a (semi-)automated detection tool. To further elucidate the meaning of presence of a CMI in ICH, it would be interesting to investigate if functional outcome, cognitive decline and recurrent ICH in patients with lobar and non-lobar ICH are associated with presence and number of CMIs.

Most studies investigating BBB disruption are cross-sectional. The question remains if it is a temporary (indicating a process triggered by ICH) or persisting phenomenon (suggesting an ongoing process of SVD). In future studies, repeat and/or serial imaging could be used to investigate the evolution of BBB disruption over time and to look for possible associations with SVD progression and recurrent ICH in patients with and without BBB leakage. Furthermore, if the role of BBB disruption in the pathogenesis is more clear, it could be a potential target for future treatments to stop or delay progression of SVD.

In conclusion, the studies in this thesis have shown that we should stop regarding and treating ICH as one single disease. Future research must be focused on tailoring treatment and secondary prevention to ICH in different age groups, sexes, location of ICH and differences in risk factor profiles. The novel markers CMIs and BBB leakage can be used to gain more insight in the SVD underlying ICH.

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SUMMARY

Intracerebral hemorrhage (ICH) is caused by rupture of a small artery or arteriole into the brain parenchyma. Spontaneous ICH is ICH not caused by a trauma or a secondary cause, such as a vascular malformation, coagulation disorder or tumor. Classically, deep (in basal ganglia or thalamus) ICH is associated with hypertension and lobar (in cortical areas of the frontal, parietal, temporal or occipital lobe) ICH in elderly patients with cerebral amyloid angiopathy (CAA). The subdivision of ICH in deep and lobar ICH however, is an oversimplification. Not all patients with deep ICH have hypertension and CAA is the cause of lobar ICH in only one-third of elderly patients.

ICH is a devastating disease. Worldwide more than three million people are affected by ICH each year. Around 40% of ICH patients die within 30 days. More importantly, in the last decades, incidence, case fatality and functional outcome remained stable. Changes over time might vary according to sex and in different age groups. Identifying risk factors could help in understanding the etiology of ICH. As pathogenesis of ICH may vary according to the location of the ICH, risk factor profiles may be different by location as well.

Advances in imaging and histopathologic assessment of imaging biomarkers have shown that ICH is predominantly caused by small vessel disease (SVD). SVD is an umbrella term for a group of pathologic processes with various etiologies that affect the small arteries, arterioles, venules and capillaries of the brain. Difference in patterns of presence, severity and distribution of SVD markers on MRI might indicate distinct etiologies of ICH. Cerebral microinfarcts (CMIs) and blood-brain barrier (BBB) disruption, as measured by leakage of gadolinium contrast on MRI, may be additional markers of SVD in ICH.

The overall aim of this thesis was to explore the etiology in spontaneous ICH by identifying time trends for and risk factors in different subgroups of ICH and assessing presence, severity and distribution of CMIs and BBB disruption on MRI, as novel marker of SVD in patients with ICH.

Part I: Epidemiology and risk factors of intracerebral hemorrhage

In **chapter 2,** we assessed time trends in incidence, 30-day and 1-year case fatality of ICH in the Netherlands between 1998 and 2010 and mortality between 1980 and 2010, stratified by age-groups and sex. We selected patients hospitalized for first ICH through linkage of the national hospital discharge register and Dutch population register. Out-of-hospital deaths were identified in the national cause of death register. Between 1998 and 2010, 41,068 ICH cases (49% women) were identified, of which 6% were out-of-hospital deaths. In men and women younger than 75 years incidence declined (P<0.01), but not significantly in men between 35

and 54 years old. Thirty-day and 1-year case fatality declined in patients younger than 75 years ($p<0.01$), but not significantly in women between 35 and 54 years old. In patients of 75 years and older ICH incidence and 30-day and 1-year case fatality remained stable. Between 1998 and 2010 mortality declined in men and women younger than 75 years with the most prominent decline in mortality in patients between 55 and 74 years and a steeper decline in men than in women. Mmortality also remained stable in patients of 75 years and older. The observed time trends may be explained by increasing control of vascular risk factors, improved treatment in the acute phase and better secondary prevention during the previous two decades of which the elderly do not seem to benefit. Specific efforts targeting the causes and consequences of ICH in the elderly may lead to lessening of the burden of ICH.

In **chapter 3** we described a systematic review and meta-analysis of studies reporting on risk factors according to the location of the ICH. We selected 42 studies including a total of 26,174 patients with ICH (9,141 lobar and 17,033 non-lobar). Hypertension was a risk factor for lobar ICH (risk ratio (RR) 1.83, 95% confidence interval (CI) 1.39-2.42). Risk factors for non-lobar ICH were hypertension (RR 4.25, 95% CI 3.05-5.91), diabetes (RR 1.35, 95% CI 1.11-1.64), male sex (RR 1.63, 95% CI 1.25-2.14), alcohol overuse (RR 1.48, 95% CI 1.21-1.81), underweight (RR 2.12, 95% CI 1.12-4.01), and being black (RR 2.19, 95% CI 1.21-3.96) or Hispanic (RR 2.13,95% CI 0.94-4.81) in comparison with being white. Since hypertension is a risk factor for both lobar and non-lobar ICH and hypertension is not the only risk factor for non-lobar ICH, we consider the term "hypertensive ICH" to distinguish deep or non-lobar ICH from lobar ICH inappropriate. Moreover, the differences in risk factor profiles between lobar and non-lobar ICH suggest that with regard to secondary prevention ICH should not be treated as one disease.

Part II: Characterization of small vessel disease in spontaneous intracerebral hemorrhage

To further explore the etiology in ICH we conducted the Finding the ETiology in Cerebral Hemorrhage (FETCH) study. This multicenter, observational cohort study enrolled patients in the University Medical Centers of Leiden, Nijmegen and Utrecht between October 2013 and December 2018. We included a total of 221 adult patients with ICH without a known cause and performed 3 tesla (T) MRI in 166 patients with 3 T MRI and 7 T MRI in 51 patients.

Cerebral microinfarcts

CMIs are small lesions in the brain of presumed ischemic origin. They are commonly found on brain autopsy in patients with dementia, but also cerebrovascular disease. On high field MRI (3 T or 7 T) CMIs are defined as a lesion hypointense on T_1-weighted images, hyperintense, isointense or cavitated on fluid attenuated inversion recovery (FLAIR) images, strictly intracortical and smaller than 3 mm. In **chapters 4 and 6** we investigated the presence of CMIs on 7 T MRI in patients with ICH.

Chapter 4 describes an explorative study of 12 patients with ICH and 15 controls (healthy controls without a history of neurologic or psychiatric disorder) who underwent 7 T MRI. We found CMIs in nine of 12 patients with ICH and in five of 15 controls. CMIs occurred in patients with and without cerebral microbleeds (CMBs). In **chapter 6** we further explored this finding. In 40 patients of the FETCH study with 7 T MRI we found CMIs in around two-thirds of patients with both lobar and non-lobar ICH. Presence of CMIs was related to presence and number of CMBs, but not to vascular risk factors (hypertension, diabetes, current or past smoking, history of TIA or stroke) or other markers of SVD on MRI (white matter hyperintensities, lacunes, cortical superficial siderosis or enlarged perivascular spaces). These results support the common finding of concurrent ischemic lesions in patients with both lobar and non-lobar ICH, which may be the result of the acute event of the ICH or reflect the ongoing process of SVD underlying ICH.

In **chapter 5** we studied the substrate of CMIs and CMBs in patients with lobar and non-lobar ICH with a combination of post-mortem 7 T MRI and histopathological examination. We selected 12 case from the database of autopsy case of the UMC Utrecht and eight additional case from the Netherlands Brain Bank, who died from ICH (9 lobar and 11 non-lobar). From each case we scanned three formalin-fixed coronal brain slabs with 7 T MRI (14 hours scan protocol). On the images we identified 132 CMIs and 204 CMBs, with a higher number of CMIs in lobar ICH case (median 6, IQR 1-33) compared to non-lobar ICH case (median 1 IQR 0-2, p=0.03). We examined 62 CMIs and CMBs in detail on histopathology. In lobar ICH, CMIs and CMBs were more often located in the superficial than in the deeper layers of the cortex. In non-lobar ICH we found CMIs and CMBs more often in the deeper than superficial layers of the cortex. We also assessed severity of CAA, vessel wall splitting, loss of smooth muscle cells and fibrin leakage associated with CMIs and CMBs and found similar abnormalities in patients with lobar and non-lobar ICH. The results of this study showed us that although CMIs and CMBs were found in different segments of the cortex in lobar ICH compared to non-lobar ICH patients, otherwise similar histopathological features suggest shared pathophysiological mechanisms in arteriolosclerotic vasculopathy-related ICH and CAA-related ICH.

In **chapter 6** we confirmed the finding of the more superficial localization of CMIs in the cortex in lobar ICH patients compared to non-lobar ICH patients in vivo on 7 T MRI. The majority of the lobar ICH patients in this study and in the histopathological study had definite or probable CAA according to the modified Boston criteria. Superficial cortical CMIs might therefore be a specific marker for CAA.

Blood-brain barrier disruption

The BBB is the boundary between the systemic blood circulation and the central nervous system, consisting of endothelial cells connected by tight-junctions, astrocytes and pericytes.

The BBB regulates transport of proteins, controls the immune system and limits the entrance of toxins to the brain. Increase in BBB permeability might be one of the mechanisms underlying SVD. Disruption of the BBB can be visualized by demonstrating leakage of gadolinium contrast agent in the cerebral spinal fluid distant from the hematoma on FLAIR MR images. In **chapter 7** we assessed presence and severity of BBB disruption with 7 T MRI in 31 ICH patients included in the FETCH study. Median time between ICH and 7 T MRI was 20 days (IQR 9-67 days). Seventeen patients (54%, 7 lobar and 10 non-lobar ICH) had gadolinium contrast leakage distant from the hematoma as a marker of BBB disruption. Contrast leakage was located predominantly in the cortical areas and associated with presence and number of lobar CMBs and is therefore possibly related to CAA.

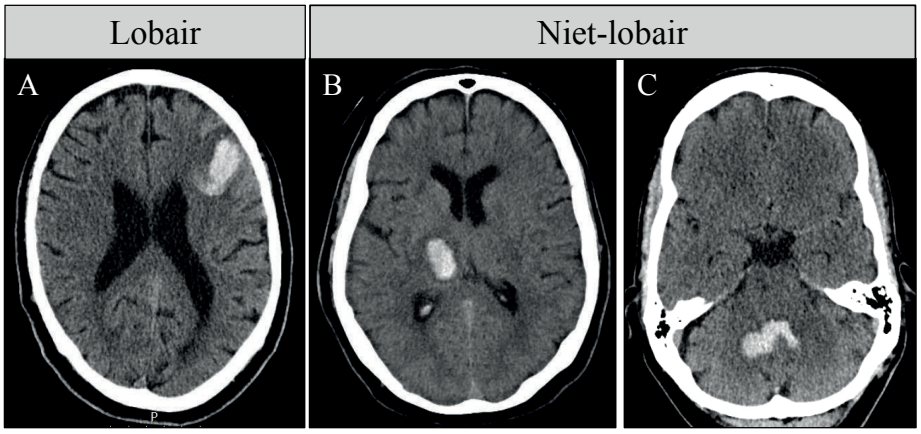
Conclusion

In this thesis, we have shown that time trends in incidence, case fatality and mortality of ICH vary in different age groups and sexes and that risk factor profiles are different for lobar and non-lobar ICH. We should stop regarding and treating ICH as one single disease. The novel markers CMIs and BBB disruption can be used in understanding the underlying SVD.

NEDERLANDSE SAMENVATTING

Een intracerebrale bloeding (ICB) is een bloeding in de hersenen veroorzaakt door het scheuren van een kleine arterie of arteriole. Een spontane (of primaire) ICB is een bloeding niet veroorzaakt door een ongeval (trauma) of door een andere oorzaak, zoals een afwijking van de bloedvaten, een stollingsstoornis of een onderliggende hersentumor. In het vervolg van de samenvatting wordt met ICB een spontane ICB bedoeld. Een ICB in de diepe structuren van de hersenen (i.e. basale kernen en thalamus) wordt in het algemeen geassocieerd met een hoge bloeddruk. Een oppervlakkige of lobaire ICB (i.e. in de buurt van de schors van de frontale, pariëtale, temporale of occipitale kwab) bij oudere patiënten wordt vaak gerelateerd aan cerebrale amyloïd angiopathy (CAA). Dit is een aandoening veroorzaakt door de ophoping van het eiwit amyloïd-bèta in de wanden van kleine en middelgrote cerebrale bloedvaten, waardoor ze broos worden en makkelijk kunnen scheuren. De tweedeling van ICBs in diepe ICBs met een hoge bloeddruk als oorzaak en lobaire ICBs met CAA als oorzaak is echter een oversimplificatie, want niet alle patiënten met een diepe ICB hebben hypertensie en bij slechts een derde van de lobaire ICBs bij oudere patiënten wordt CAA als oorzaak gevonden. Bovendien omvat deze tweedeling niet de ICBs in de hersenstam en kleine hersenen en de lobaire ICBs bij jongere (<50 jaar) patiënten. Er zijn dus meerdere oorzaken voor een ICB en de oorzaken kunnen mogelijk verschillen per locatie van de ICB. Tot op heden worden alle patiënten met een ICB onafhankelijk van de locatie grotendeels op dezelfde manier behandeld. Zie figuur 1 voor voorbeelden van ICBs in verschillende locaties.

Figuur 1. Voorbeelden van een lobaire intracerebrale bloeding (A) en niet-lobaire bloedingen (B: diepe bloeding, C: bloeding in de kleine hersenen)



Een ICB is een ernstige aandoening. Wereldwijd worden jaarlijks meer dan 4 miljoen mensen getroffen door een ICB. Ongeveer 40% van de patiënten met een ICB overlijdt binnen een maand.

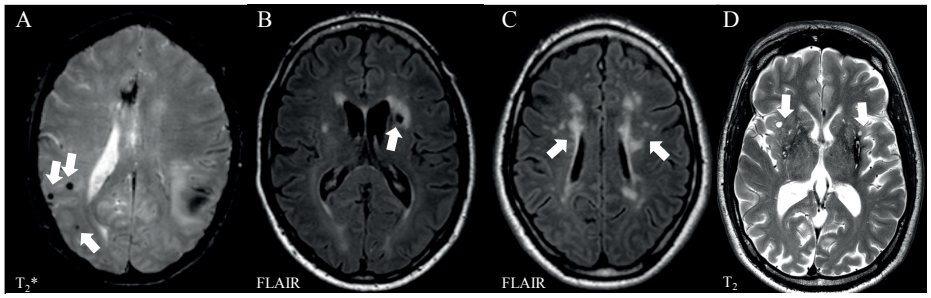
In de laatste decennia is de incidentie (zie onderstaand kader voor een verklarende woordenlijst van verschillende termen gebruikt in deze samenvatting) en uitkomst na een ICB niet verbeterd. Het is de vraag of dit voor mannen en vrouwen en voor alle leeftijdsgroepen heftzelfde is. Het identificeren van risicofactoren voor het ontstaan van een ICB kan helpen bij het ontrafelen van de oorzaken. Omdat de oorzaken lijken te verschillen voor ICBs op verschillende locaties (lobair, diep, hersenstam of kleine hersenen), kunnen risicofactor-profielen voor verschillende locaties ook anders zijn.

Verklarende woordenlijst:

- 30-dagen sterfte: sterfte in de eerste 30 dagen na en ten gevolge van een ziekte
- 1-jaars sterfte: sterfte in het eerste jaar na en ten gevolge van een ziekte
- Cerebrale amyloïd angiopathie: aandoening veroorzaakt door de ophoping van het eiwit amyloïd-bèta in de wanden van kleine en middelgrote cerebrale bloedvaten, waardoor ze makkelijker kunnen scheuren
- Cortex: hersenschors (buitenste laag van de grote hersenen)
- Hematoom: bloeding
- Incidentie: aantal nieuwe gevallen van een ziekte per aantal personen per tijdseenheid
- Interkwartielafstand: het verschil tussen eerste en het derde kwartiel in een verdeling
- Mediaan: de middelste waarde in een getallenreeks die gerangschikt is naar grootte
- Meta-analyse: onderzoek waarbij de resultaten van eerder uitgevoerde onderzoeken worden gecombineerd om één secuurdere uitkomst te verkrijgen.
- Mortaliteit: sterfte, nadat een ziekte is doorgemaakt
- Relatief risico: de verhouding tussen 2 absolute risico's. Het absolute risico is de verhouding tussen het aantal keren dat iets voorvalt in een groep en het totale aantal van die groep.
- Small vessel disease: ziekte van de kleine vaten in de hersenen

Door vooruitgang in beeldvorming en microscopisch onderzoek van op beeldvorming gevonden afwijkingen, is aangetoond dat ICBs grotendeels veroorzaakt worden door ziekten van de kleine bloedvaatjes, in het Engels *small vessel disease* (SVD) genoemd. SVD is een overkoepelende term voor aantasting van kleine arteriën, arteriolen, capillairen en venules van de hersenen door verschillende oorzaken. Verschillen in patronen van aanwezigheid, ernst en verdeling van SVD kernmerken op MRI scans kunnen wijzen op uiteenlopende oorzaken van ICBs. Voorbeelden van deze kenmerken of markers zijn microbloedingen (MBs), wittestofafwijkingen (WSA), lacunes en verwijde perivasculaire ruimtes. In figuur 2 kunt u voorbeelden zien van hoe deze markers op MRI scans zichtbaar zijn. De relatief nieuwe markers cerebrale microinfarcten (CMIs) en verstoring van de bloed-hersenbarrière (BHB), aangetoond door middel van lekkage van gadolinium contrast op MRI scans, zijn niet eerder onderzocht bij patiënten met een ICB.

Figuur 2. Voorbeelden van MRI markers van SVD bij patiënten met een ICB op 3 tesla MRI. A: Cerebrale microbloedingen, B: lacune, C: wittestofafwijkingen en D: verwijde perivasculaire ruimtes; aangegeven met de witte pijlen



Het overkoepelende doel van dit proefschrift is om meer te weten te komen over de oorzaken van ICBs. Dit hebben we gedaan door het identificeren van trends in de tijd van en risicofactoren voor verschillende subgroepen van ICBs. Daarnaast hebben we ernst en verdeling van CMIs en BHB lekkage, als nieuwe markers van SVD, beoordeeld bij patiënten met een ICB.

Deel 1: Epidemiologie en risicofactoren voor intracerebrale bloedingen

In **hoofdstuk 2** hebben we leeftijds- en geslachts-specifieke trends van incidentie, 30-dagen en 1-jaars sterfte in Nederland tussen 1998 en 2010 en mortaliteit tussen 1980 en 2010 bepaald. Hiervoor hebben we patiënten geselecteerd die opgenomen waren in het ziekenhuis voor een eerste ICB door het koppelen van de landelijke medische registratie, de gemeentelijke basisregistratie en het doodsoorzaken register van het centraal bureau voor statistiek. Tussen 1998 en 2010 waren er 41.068 patiënten (49% vrouwen) opgenomen met een ICB, waarvan 6% buiten het ziekenhuis is overleden. De incidentie van ICBs bij mannen en vrouwen jonger dan 75 jaar nam af ($p<0,01$), maar niet significant voor mannen tussen 35 en 54 jaar. Dertig-dagen en 1-jaars sterfte namen ook af voor patiënten jonger dan 75 jaar ($p<0,01$) maar niet significant voor vrouwen tussen 35 en 54 jaar. Van patiënten van 75 jaar en ouder bleven de incidentie, 30-dagen en 1-jaars sterfte stabiel. Tussen 1980 en 2010 nam de mortaliteit van mannen en vrouwen jonger dan 75 jaar af met de meest prominente daling voor patiënten tussen 55 en 74 jaar en meer voor mannen dan voor vrouwen. Ook de mortaliteit bleef voor patiënten van 75 jaar en ouder stabiel. De geobserveerde trends in de tijd kunnen verklaard worden door een betere controle en behandeling van vasculaire risicofactoren (zoals hypertensie, diabetes, roken en overmatig alcohol gebruik), een verbeterde behandeling in de acute fase van een ICB en toename van secundaire preventie in de laatste decennia. Oudere patiënten lijken hier echter geen profijt van te hebben. In de toekomst kan het specifiek richten van onderzoek en behandeling op oorzaken en gevolgen van ICBs bij oudere patiënten, leiden tot een afname in ziektelast van ICBs.

In **hoofdstuk 3** beschrijven we een systematisch literatuuroverzicht en meta-analyse van studies over risicofactoren voor lobaire en niet-lobaire (i.e. diep, hersenstam en kleine hersenen) ICBs. De 42 geselecteerde onderzoeken includeerden in totaal 26.174 patiënten met een ICB (9.141 lobair en 17.033 niet-lobair). Hypertensie was een risicofactor voor zowel lobaire (relatief risico (RR) 1,83; 95% betrouwbaarheidsinterval (BI) 1,39-2,42) als niet-lobaire (RR 4,25; 95% BI 3,05-5,91) ICBs. Verder vonden we voor niet-lobaire ICBs ook nog de risicofactoren diabetes (RR 1,35; 95% BI 1,11-1,64), mannelijk geslacht (RR 1,63; 95% BI 1,25-2,14), overmatig alcoholgebruik (RR 1,48; 95% BI 1,21-1,81) en ondergewicht (RR 2,12; 95% BI 1,12-4,01). Daarnaast waren er etnische verschillen, waarbij zwarte mensen (RR 2,19; 95% BI 1,21-3,96) en latino's (RR 2,13; 95% BI 0,94-4,81) een hoger risico hadden op een niet-lobaire bloeding in vergelijking met witte mensen. Omdat hypertensie een risicofactor is voor zowel lobaire als niet-lobaire ICBs en hypertensie niet de enige risicofactor is voor niet-lobaire ICBs, beschouwen we de term hypertensieve ICB voor onderscheid tussen lobaire en niet-lobaire bloedingen als obsoleet. Bovendien suggereren de verschillende risicofactor-profielen voor lobaire en niet-lobaire ICBs dat ICBs wat betreft secundaire preventie niet als één ziekte moet worden beschouwd.

Deel 2: Karakteriseren van small vessel disease bij spontane intracerebrale bloedingen

Voor het verder ontrafelen van de oorzaken van ICBs hebben we de 'Finding the ETiology in Cerebral Hemorrhage' (FETCH) studie uitgevoerd. Dit is onderzoek, waarin we patiënten met een recent doorgemaakte ICB hebben geïncludeerd in de universitaire medische centra van Leiden, Nijmegen en Utrecht tussen oktober 2013 en december 2018. In de FETCH studie hebben we in totaal 221 volwassen patiënten onderzocht met een ICB zonder secundaire oorzaak en hebben bij in totaal 166 patiënten een 3 tesla (T) MRI scan en bij 51 patiënten een 7 T MRI scan verkregen.

Cerebrale microinfarcten

CMIs zijn hele kleine herseninfarcten die ontstaan doordat een klein deel van het hersenweefsel te weinig bloed en/of zuurstof heeft gekregen. In het verleden zijn CMIs veelvuldig beschreven bij autopsiestudies, waarbij het hersenweefsel onder de microscoop onderzocht wordt. Ze zijn gevonden bij bijvoorbeeld patiënten met dementie, maar ook bij patiënten met een beroerte. De laatste jaren is het ook mogelijk om CMIs al tijdens het leven zichtbaar te maken op MRI scans met hoge veldsterke (3 T of 7 T). CMIs worden gedefinieerd als afwijkingen in de cortex, kleiner dan 3 mm, hypointens op T_1-gewogen opnames en hyperintense, isointens of met een holte op fluid attenuated inversion recovery (FLAIR) opnames. In **hoofdstuk 4 en 6** hebben we de aanwezigheid van CMIs op 7 T MRI scans bij patiënten met een ICB onderzocht.

Hoofdstuk 4 beschrijft een eerste onderzoek naar de aanwezigheid van CMIs bij patiënten met een ICB met behulp van 7 T MRI scans. We onderzochten 12 patiënten met een ICB en vergeleken deze met 15 gezonde controle patiënten zonder een neurologische of psychiatrische voorgeschiedenis. We vonden CMIs bij 9 van de 12 patiënten met een ICB, tegenover 5 van de 15 gezonde controle patiënten. CMIs kwamen voor bij patiënten met en zonder de gelijktijdige aanwezigheid van MBs. In **hoofdstuk 6** hebben we deze bevinding verder uitgezocht. Bij twee derde van 40 patiënten met een lobaire of niet-lobaire bloeding met een 7 T MRI scan vonden we één of meer CMIs. De aanwezigheid van CMIs was gerelateerd aan aanwezigheid en aantal MBs. We vonden geen relatie met de vasculaire risicofactoren hypertensie, diabetes, roken of een eerder doorgemaakte TIA of herseninfarct. Er was ook geen associatie met andere op MRI gevonden afwijkingen (WSA, lacunes, corticale superficiële siderose of verwijde perivasculaire ruimtes). Onze resultaten ondersteunen de eerder beschreven bevinding van het tegelijkertijd vinden van ischemische afwijkingen bij patiënten met een recente lobaire of niet-lobaire ICB. De CMIs kunnen het gevolg zijn van het acute ontstaan van de bloeding (bijvoorbeeld door massaeffect en verhoging van de intracraniële druk), maar ook het gevolg van een doorgaand proces van SVD bij de ICB.

In **hoofdstuk 5** hebben we op MRI scans gevonden CMIs en MBs nader onderzocht met een combinatie van 7 T MRI scans en microscopisch onderzoek van hersenweefsel van patiënten die aan een ICB zijn overleden. Hiervoor hebben we hersenweefsel gebruikt van 12 ICB patiënten gevonden in de database met obducties verricht in het UMC Utrecht en hersenweefsel van 8 ICB patiënten opgevraagd bij de Nederlandse Hersenbank in Amsterdam (totaal 9 lobair en 11 niet-lobair). Van iedere patiënt hebben we 3 hersenplakken gescand in de 7 T MRI scanner. Op de verkregen opnames hebben we in totaal 132 CMIs en 204 MBs gevonden. We vonden meer CMIs bij patiënten met een lobaire ICB (mediaan 6, interkwartielafstand (IQR) 1-33) dan bij patiënten met een niet-lobaire ICB (median 1, IQR 0-2, p=0,03). Vervolgens hebben we 62 CMIs en MBs in meer detail microscopisch. Bij lobaire ICBs vonden we CMIs en MBs vaker in de oppervlakkige lagen van de cortex dan in de diepere lagen van de cortex. Bij niet-lobaire ICBs vonden we CMIs en MBs juist meer in de diepe lagen van de cortex dan in de oppervlakkige lagen. We hebben verder gekeken naar andere kenmerken van de CMIs en MBs en het omliggende weefsel, maar vonden geen verschillen in ernst van CAA, splitsing van de vaatwand, verlies van gladde spiercellen in de vaatwand en lekkage van fibrine tussen lobaire en niet-lobaire ICBs. De resultaten van dit onderzoek laten ons zien dat, ondanks een verschil in locatie in de cortex van CMIs en MBs, de verder vergelijkbare histopathologische kenmerken kunnen wijzen op overeenkomstige pathofysiologische mechanismen bij ICBs gerelateerd aan verschillende locaties en onderliggende oorzaken.

In **hoofdstuk 6** hebben we deze bevinding van de meer oppervlakkige lokalisatie van CMIs bij patiënten met lobaire ICBs kunnen bevestigen op 7 T MRI scans bij levende patiënten na een ICB. De meerderheid van de patiënten in deze studie en onze microscopie studie (**hoofdstuk 5**) voldeden aan de modified Boston criteria voor 'waarschijnlijke' of 'zekere' CAA. Oppervlakkige corticale CMIs zouden derhalve een specifieke marker voor CAA kunnen zijn.

Bloed-hersenbarrière

De BHB is de grens tussen de systemische bloedsomloop en het centraal zenuwstelsel. De BHB zorgt ervoor dat schadelijke stoffen de hersenen niet kunnen bereiken, reguleert het transport van eiwitten en controleert het immuunsysteem. Een van de mechanismen die ten grondslag ligt aan SVD is het meer doordringbaar worden van de BHB. Verstoring van de BHB kan aangetoond worden door middel van lekkage van gadolinium-houdend contrast in het hersenvocht op afstand van het hematoom op FLAIR opnames. In **hoofdstuk 7** hebben we dit onderzocht bij 31 patiënten uit de FETCH studie met een 7 T MRI scan. De mediane tijd tussen de ICB en de 7 T MRI was 20 dagen (IQR 9-67 dagen). Bij 17 van de 31 patiënten (54%, 7 lobaire en 10 niet-lobaire ICB) met een ICB vonden we contrast lekkage op afstand van het hematoom als marker van BHB verstoring. Contrast lekkage werd hoofdzakelijk corticaal gevonden en was geassocieerd met aanwezigheid en aantal lobaire MBs. Ook contrast lekkage is daarom mogelijk gerelateerd aan CAA.

Conclusie

In dit proefschrift heb ik aangetoond dat trends in de tijd van incidentie en mortaliteit uiteenlopen in verschillende leeftijdsgroepen en tussen mannen en vrouwen. Daarnaast zijn risicofactorprofielen anders in lobaire ICBs in vergelijking met niet-lobaire ICBs. Mijns inziens moeten we stoppen om ICBs te zien en behandelen als één ziekte. De nieuwe markers CMIs en BHB lekkage kunnen gebruikt worden om de onderliggende SVD meer te doorgronden.

ABBREVIATIONS

3D = three dimensional
3 T = 3 tesla
7 T = 7 tesla
ADC = apparent diffusion coefficient
ALT = alanine aminotransferase
APC = annual percentages change
APOE = apolipoprotein E
AST = aspartate aminotransferase
BIC = Bayesian information criterion
BP = blood pressure
BBB = blood-brain barrier
BMI = body mass index
CAA = cerebral amyloid angiopathy
CAD = coronary artery disease
CDR = cause of death register
CI = confidence interval
CMI = cerebral microinfarct
CMB = cerebral microbleed
CSF = cerebrospinal fluid
cSS = cortical superficial siderosis
cSVD = cerebral small vessel disease
CT = computed tomography
CTA = computed tomography angiography
DBP = diastolic blood pressure
D-CAA = Dutch-type hereditary cerebral amyloid angiopathy
DCE-MRI = dynamic contrast enhanced magnetic resonance imaging
DM = diabetes mellitus
DWI = diffusion weighted imaging
ECG = electrocardiogram
EPVS = enlarged perivascular spaces
F = female
FETCH study = Finding the ETiology in spontaneous Cerebral Hemorrhage study
FLAIR = fluid attenuated inversion recovery
GCS = Glasgow coma scale
H&E = hematoxylin and eosin
HARM = hyperintense acute reperfusion marker

HDL = high-density lipoprotein
HDR = hospital discharge register
HR = hazard ratio
ICD = international classification of diseases
ICH = intracerebral hemorrhage
IQR = interquartile range
IV = inverse variance
LOSC = loss of smooth muscle cells
LVH = left ventricular hypertrophy
M = male
MRA = magnetic resonance angiography
MRI = magnetic resonance imaging
NA = not applicable
NIHSS = national institutes of health stroke scale
NOAC = non-vitamin antagonist oral anticoagulant
NOS = Newcastle-Ottawa scale
OR = odds ratio
PET = positron emission tomography
PR = population register
RR = risk ratio
SBP = systolic blood pressure
sCAA = sporadic cerebral amyloid angiopathy
SD = standard deviation
SE = standard error
SMC = smooth muscle cells
SWI = susceptibility weighted imaging
STRIVE = STandards for Reporting Vascular changes on nEuroimaging
SVD = small vessel disease
TIA = transient ischemic attack
TICS-M = modified telephone interview for cognitive status
TR = repetition time
TE = echo time
TE = treatment effect
TSE = turbo spin echo
VWS = vessel wall splitting
WMH = white matter hyperintensities

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Wilhelmus Martinus (Wilmar) Tim Jolink was born on February 1st 1988 in Gouda, the Netherlands. In 2005 he graduated with honors from his high school, the Coornhert Gymnasium, in Gouda. The same year, Wilmar started his medical training at Utrecht University. During medical school, he was an active member of the Faculty of Medicine students association (MSFU "Sams) and he was treasurer of the board in 2008-2009. In the fourth year of medical school he did a 10-week research internship on symptomatic carotid occlusion at the University Medical Center Utrecht, under supervision of prof. dr. C.J.M. Klijn, which formed the basis of his interest in neurology and academic research. In the final year of his medical training Wilmar did a research internship on outcome after intracranial hemorrhage due to dural arteriovenous fistulae under supervision of prof. dr. C.J.M. Klijn. After obtaining his medical degree in 2012, he started his PhD project studying the etiology of spontaneous intracerebral hemorrhage under supervision of prof. dr. C.J.M. Klijn and prof. dr. G.J.E. Rinkel. In 2014 started as a resident neurology in the University Medical Center Utrecht under supervision of prof. dr. J.H.J. Wokke, dr. T. Seute and prof. dr. G.J. Biessels. As part of his PhD project, Wilmar had the opportunity to do a research internship at the J. Philip Kistler Stroke Research Center at Massachusetts General Hospital, Harvard Medical School, in Boston, United States of America, where he was supervised by dr. S.J. van Veluw and prof. dr. S.M. Greenberg. After defending his thesis in October 2020, Wilmar will continue his residency training, which he plans to complete in 2021. Wilmar is married to Natanja Oosterom and they live together in Utrecht.

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This thesis

Wilmar MT Jolink, Catharina JM Klijn, Paul JAM Brouwers, L Jaap Kappelle, I Vaartjes. Time trends in incidence, case fatality, and mortality of intracerebral hemorrhage. Neurology. 2015 Oct 13;85(15):1318-1324.

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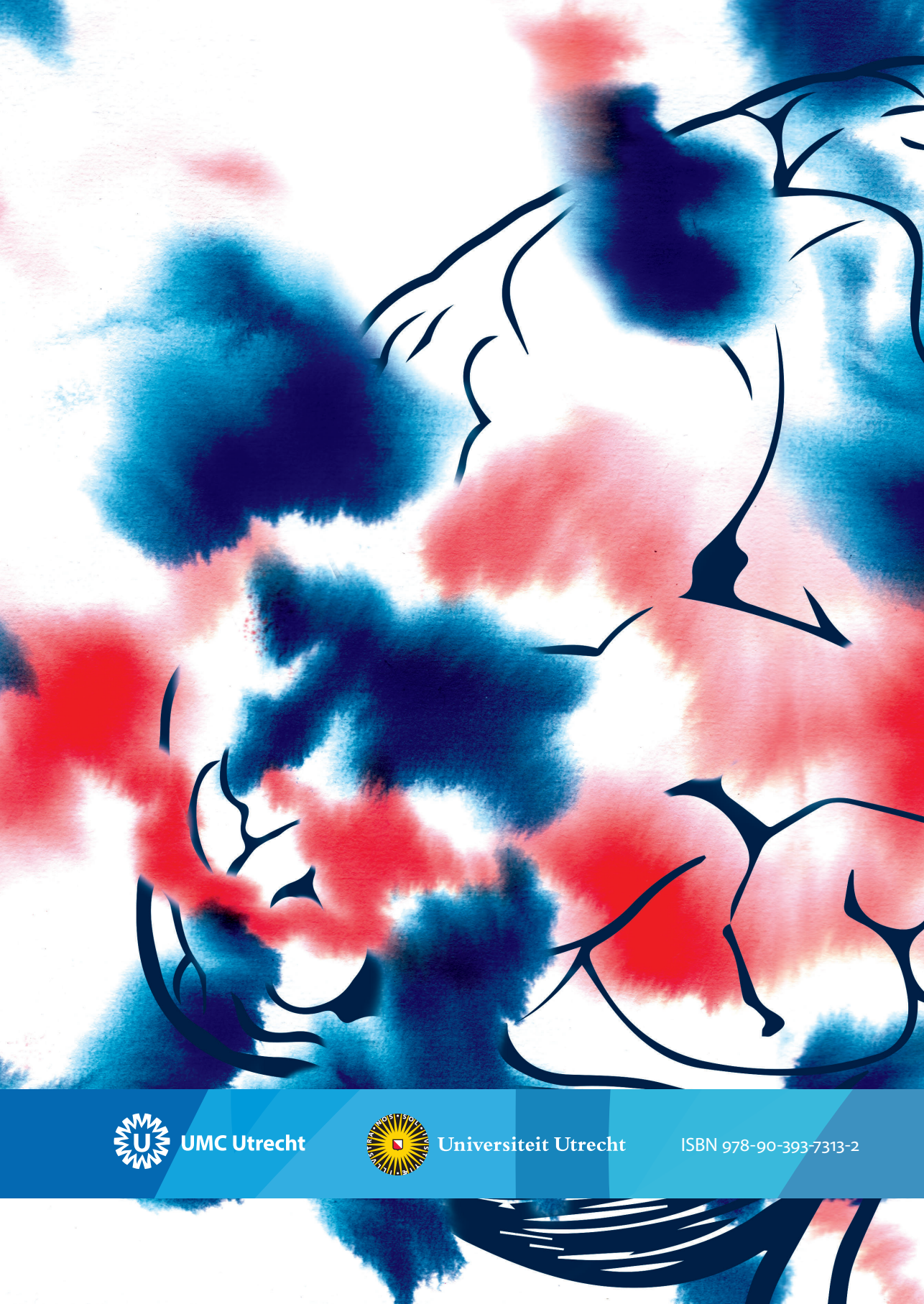

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