Ischemic stroke and atherosclerosis at a young age

The Norwegian Stroke in the Young Study

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Contents

Scientific environment

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List of publications

This thesis is based on the following papers:

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II Fromm A, Thomassen L, Naess H, Meijer R, Eide GE, Kråkenes J, Vedeler CA, Gerdts E, Larsen TH, Kuiper KKJ, Laxdal E, Russell D, Tatlisumak T, Waje-Andreassen U.
The Norwegian Stroke in the Young Study (NOR-SYS): Rationale and design.
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III Fromm A, Haaland ØA, Naess H, Thomassen L, Waje-Andreassen U.
Risk factors and carotid Intima-media thickness in young ischemic stroke patients and controls. The Norwegian Stroke in the Young Study (NOR-SYS).
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IV Fromm A, Haaland ØA, Naess H, Thomassen L, Waje-Andreassen U. **Atherosclerosis in TOAST subtypes of young ischemic stroke. The Norwegian Stroke in the Young Study (NOR-SYS).**
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Abstract

Stroke risk and incidence increase exponentially with age. In Europe about one in three strokes occurs before the age of 65 years, with severe socioeconomic consequences on individual and society level. Despite improved diagnostic techniques and increasing knowledge, in 30-50% of young stroke patients the cause of stroke remains undetermined. However, due to the lack of one consistent definition for "young stroke", published data are inhomogeneous regarding patient age, stroke types included and variables studied. It has been claimed that young stroke patients have a favorable prognosis, including usually good recovery from neurological deficits, low recurrence risk, a fair social prognosis, and favorable long-term outcome. Newer follow-up investigations documented, however, high rates of stroke recurrence, vascular co-morbidity and mortality. Further, increased rates of psychological disorders, reduced health-related quality of life and impared social activity are frequent long-term consequences. The underlying cause of stroke is related to differing risk of recurrent cardiovascular events, being highest in patients with large-artery atherosclerosis and cardioembolism. Hence, there is a need for systematic investigation and data collection from well-defined young stroke populations aiming to reveal the true cause of stroke, leading to right treatment of underlying conditions, and to optimal and aggressive secondary prevention.

In the studies included in this thesis, we aimed to compare stroke characteristics in young ischemic stroke patients to older patient populations, and to controls free of cardiovascular disease. Data were obtained from 1217 ischemic stroke patients admitted to Haukeland University Hospital between 2006 and 2009 (the Bergen NORSTROKE study), and from 150 patients aged 15-60 years and 84 controls included in the Norwegian Stroke in the Young Study (NOR-SYS) between 2010 and 2012. Patients <50 years represented 8% of the NORSTROKE population. Undetermined cause of stroke was the most frequent subtype of stroke classified according to TOAST criteria in NORSTROKE and NOR-SYS. Of determined causes,

non-arrhythmic cardioembolism, small artery occlusion and cervical artery dissection were the most frequent diagnoses among younger patients, while cardioembolism due to arrhythmia was the most frequent cause of stroke among older patients. Risk factor burden increased with age, and patients of all investigated age and sex subgroups had a higher risk factor burden than controls. Of the risk factors investigated, hypertension, dyslipidemia, smoking, overweight and family history of cardiovascular disease were most frequent. Carotid Intima-media thickness (cIMT), a surrogate marker of atherosclerosis, was measured by ultrasonography in the NOR-SYS population, and performed in the common carotid artery (CCA), the bifurcation (BIF) and the internal carotid artery (ICA). Increased cIMT was associated with age and prevalent vascular risk factors. The overall cIMT difference between patients and controls was 12% in CCA, 17% in BIF and 29% in ICA. Mean cIMT was higher compared to controls in patients with large-artery atherosclerosis, small artery occlusion, and stroke of undetermined cause.

Our data add new information to our knowledge about young stroke patients concerning etiology, risk factors, and the prevalence of clinical and subclinical carotid artery disease. They support the conclusions drawn in previous studies regarding the varying impact of different risk factors on cIMT development dependent on age and sex. We find ICA to be the carotid segment distinctly associated with incident stroke, related to a family history of cardiovascular disease among young patients, and related to an increasing risk factor burden with increasing age. We further find that small artery occlusion and stroke of undetermined etiology represent stroke subtypes which hide a considerable number of patients suffering from atherosclerotic arterial disease in our cohort. Young stroke patients require extensive investigation with the purpose to detect and treat prevalent vascular disease and risk factors aggressively, to slow the progression of atherosclerotic disease, and to prevent future vascular events and subsequent disability, cognitive decline and death.

List of abbreviations

ABCD2	Age, Blood pressure, Clinical features, Duration, Diabetes (score)
ABI	Ankle-brachial index
AF	Annette Fromm
ARIC	Atherosclerosis Risk in Communities (study)
ASA	Acetylsalicylic acid
ASCO	Atherothrombosis, Small vessel disease, Cardiac causes, Other uncommon causes
BI	Barthel index
BIF	Bifurcation, carotid (standardized measurement site)
BMI	Body mass index
CAD	Carotid artery dissection
CAPRIE	Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (study)
CAPS	Carotid Atherosclerosis Progression Study (study)
CCA	Common carotid artery
CD	Cerebrovascular disease
CE	Cardioembolism
CE-MRA	Contrast-enhanced magnetic resonance angiography
CHD	Coronary heart disease
CHS	Cardiovascular Health Study
CI	Confidence interval
cIMT	Carotid Intima-media thickness
CT	Computed tomography
CTA	Computed tomography angiography
CVD	Cardiovascular disease
DSA	Digital subtraction angiography
DUS	Duplex ultrasound
DWI	Diffusion-weighted imaging
ECG	Electrocardiography
ECST	European Carotid Surgery Trial
e.g.	Exempli gratia, "for example"
ER-DP	Extended-release dipyridamole
HN	Halvor Næss
HR	Hazard ratio
ICA	Internal carotid artery
ICAS	Intracranial atherosclerosis
ICH	Intracerebral Hemorrhage
IMT	Intima-media thickness
LAA	Large-artery atherosclerosis
MCA	Middle cerebral artery
MI	Myocardial infarction
ML	Marianne Lundervik
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale

NOR-SYS	The Norwegian Stroke in the Young Study
OCSP	Oxfordshire Community Stroke Project
ØAH	Øystein Ariansen Haaland
PAD	Peripheral artery disease
PFO	Patent foramen ovale
PSV	Peak systolic velocity
RC	Relative change
REACH	Reduction of Atherothrombosis for Continued Health (study)
RF	Risk factors
SAH	Subarachnoidal hemorrhage
SAO	Small artery occlusion
SICH	Symptomatic intracranial hemorrhage
SOC	Stroke of other determined cause
SUC	Stroke of undetermined cause
TCCS	Transcranial color-coded sonography
TCD	Transcranial Doppler
TIA	Transient ischemic attack
TOAST	Trial of Org 10172 in Acute Stroke Treatment
TOF-MRA	Time-of-flight magnetic resonance angiography
UWA	Ulrike Waje-Andreassen
VA	Vertebral artery
VAD	Vertebral artery dissection
VB	Vertebrobasilar
WHO	World Health Organization

Introduction

Stroke

According to the World Health Organization (WHO), stroke is defined as "rapidly developing clinical signs of focal, at times global disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than of vascular origin"[1]. Ischemic stroke is a damage of brain tissue due to too low or interrupted blood flow and subsequent loss of oxygen and glucose supply. Stroke symptoms correlate with location and severity of the injury, and may vary from silent infarction with no clinical symptoms to slight confusion, numbness and/ or weakness of the limbs and/ or face, loss of speech, vision, balance or coordination, and even sudden unconsciousness and death. Stroke severity depends on the size of the affected area, time until reperfusion, and the presence and function of collateral arteries.

Epidemiology

Cardiovascular disease (CVD), including neurovascular disease, represents one of the 10 leading diseases worldwide, and is responsible for the majority of disability and deaths[2, 3]. Stroke incidence shows substantial variations over time[4] and in geographic distribution[3, 5]. The increased prevalence of modifiable cardiovascular risk factors (RF) and the aging of populations have resulted in stroke becoming a major health problem also in low-income and middle-income countries[3]. In Europe, average total stroke incidence rates are highest in eastern and lowest in southern European countries[6, 7]. In Norway, the annual incidence rate for first-ever and recurrent stroke has been estimated to 15,000 in 2007[8], including patients of all ages. The mean age for first-ever stroke in a Norwegian population-based study from 1994-1996 was 76 years[9]. Among young ischemic stroke patients aged 15-49 years, another population-

based study in Western-Norway found an annual incidence rate of 11.4 per 100,000 inhabitants[10]. Ischemic stroke is the most prevalent stroke subtype and represents approximately 85% of all stroke cases, while 15% are hemorrhagic strokes, mainly intracerebral hemorrhages (ICH) and subarachnoidal hemorrhages (SAH)[6]. Stroke risk and incidence increase exponentially with age[6, 11, 12]. About one third of ischemic strokes in Europe occur before the age of 65 years[6]. Regarding sex differences, females outnumber males below the age of 30 years, while males dominate at higher ages[10, 11, 13, 14].

Young Stroke

There is no specific definition of "young stroke". The young stroke population has been arbitrarily defined by several studies, e.g. with 45 years[15-19], 49 years[11, 20-22], 54 years[12, 23] and 55 years[14] as upper age limit. Additionally, previous population-based reports often included different stroke subtypes (ischemic stroke, transient ischemic attack (TIA), and hemorrhagic stroke), and referral bias need to be considered in hospital-based studies. Thus, reports vary strongly in terms of patient age and study methodology, and comparisons may be difficult.

Etiology of ischemic young stroke

The most commonly used tool for categorization of ischemic stroke etiology is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification[24]. In TOAST, causes of stroke are sorted into the five categories Large-artery atherosclerosis (LAA), Cardioembolism (CE), Small artery occlusion (SAO), Stroke of other determined cause (SOC) and Stroke of undetermined cause (SUC). However, the tool is controversial[25], and it does not give any guidelines concerning the extent and quality of investigation, which may vary between stroke centers.

Large-artery atherosclerosis

The LAA category requires >50% stenosis or occlusion of a major brain artery or branch cortical artery, presumably due to atherosclerosis, and takes a history of intermittent claudication, TIA in the same vascular territory, carotid bruit and diminished pulses in supportive consideration[24]. While LAA has increasing impact as cause of stroke from the age of 45 years[11, 14], only 4.9% of European young stroke patients aged 44 or younger had stenosis ≥50% or occlusion of at least one brain-supplying cervical artery in ultrasound examination, of which two thirds were

considered symptomatic[26]. LAA as overall cause of stroke was diagnosed in 4% of the population aged 18-44[14], while in Finland and Norway stroke in patients with the upper age limit of 49 years had previously been classified as caused by LAA in 7.5%[11] and 15%[20], respectively.

Concerning intracranial arteries, the prevalence of large-artery stenoses differs widely across ethnicities[27]. In Europe, a large multicenter study has recently identified supratentorial intracranial stenosis or occlusion in 10.8% of ischemic stroke patients younger than 45 years compared to 15.5% of middle-aged patients (45-55 years), whereof 8.8 and 11.2% respectively were evaluated as symptomatic[26]. However, due to the limitations of the imaging methods, without advanced examinations e.g. with magnetic resonance imaging (MRI), or histopathologic (post mortem) examination, it is not possible to distinguish intracranial atherosclerotic stenoses from stenoses of other etiology as embolization or dissection, and early stages of atherosclerosis or signs of vulnerability of the atherosclerotic plaque may remain unidentified[28].

Small artery occlusion

Infarction due to SAO, or lacunar infarction, is categorized as a strictly subcortical lesion with a diameter of <1.5 cm, and with no signs of cortical dysfunction. The category is supported by the presence of hypertension or diabetes mellitus[24]. Lacunar infarcts stay frequently clinically silent[29], and coexist with ischemic white matter disease and microhemorrhages[30]. The frequency of ischemic young stroke due to SAO ranges between 13.5% in Europe[14], 13.8% in Finland[11] and 14.7% in Western Norway[20]. However, SAO as presumed clinical diagnosis is uncertain and may easily be confused by other etiologies, such as minor embolic infarctions[31].

Cardioembolism

The category of CE includes arterial occlusion due to a cardiac embolic source. CE is associated with several high-risk or medium-risk conditions, such as mechanical prosthetic valve, atrial fibrillation and left ventricular thrombus (high-risk), or patent foramen ovale (PFO), atrial septal aneurysm and recent myocardial infarction (MI)

(medium-risk)[24]. The frequency of ischemic young stroke due to CE has previously been reported to 16.7% in Europe[14], 19.6% in Finland[11] and 7.8% in Western Norway[20]. CE is further a significant source of multiple brain infarctions, both in one single or in multiple territories[32].

Stroke of other determined cause

Besides non-atherosclerotic vasculopathies, hypercoagulable states, hematologic disorders and other rare causes[24], the majority of ischemic strokes in young adults with "other determined etiology" arise from cervical artery dissection. Estimates range between 6% in Western-Norway[20], 9.7% in Europe[14] and 15% in Finland[11]. Dissections are more prevalent in the carotid arteries compared to the vertebral arteries (63% vs. 37%), and trauma, recent infection and genetic factors are assumed to play a role in pathogenesis[33]. The mean patient age differs between 45.7 years in carotid artery dissection (CAD) and 41.1 years in vertebral artery dissection (VAD), and males outnumber females in both types[34].

Stroke of undetermined cause

The category of SUC includes all ischemic stroke cases who´s cause cannot be determined despite extensive or due to cursory investigation, or where two or more potential causes of stroke have been identified[24]. Its rate has been estimated to 30-40% in recent series[11, 14, 20, 35]. Accordingly, SUC encompasses the majority of ischemic stroke patients, independent of age. Undetermined etiology is more common among females than males (38.0% vs. 30.5%)[14]. Since SUC includes not only negative or incomplete evaluation, but also multiple identified potential causes, SUC leads to large numbers of falsely "unsolved" stroke cases. Rare causes of stroke or diagnoses which require a challenging work-up or where diagnostic methods are limited are likely to be classified as SUC. Also, possibly symptomatic atherosclerosis with <50% stenosis is per TOAST definition assigned to SUC if no other determined cause was detected.

Risk factors

The concept of vascular RF goes back to the Framingham Heart Study, an epidemiological study which was started in 1948 as a result of increasing incidence of CVD in the United States, covering factors related to the development and clinical manifestations of coronary heart disease (CHD)[36]. Today, RF for CVD may be classified according to their potential for modification (non-modifiable, modifiable or potentially modifiable) and strength of evidence (well-documented or less well-documented)[37], as shown in Table 1.

Table 1. Risk factors for first-ever stroke

Non-modifiable Risk Factors	Well-Documented and modifiable Risk Factors	Less well-documented or potentially modifiable Risk Factors
Age	Cardiovascular Disease	Metabolic syndrome
Sex	- Prior TIA	Alcohol abuse
Race	- Coronary heart disease	Hyperhomocysteinemia
Low birth weight	- Heart failure	Drug abuse
Family history of stroke/ TIA	- Peripheral artery disease	Hypercoagulability
	Hypertension	Oral contraceptive use
	Cigarette smoking	Pregnancy/ Post-partum period
	Diabetes mellitus	Inflammatory processes
	Asymptomatic carotid stenosis	Migraine
	Atrial fibrillation, non-valvular	High Lipoprotein (a)
	Sickle cell disease	Sleep-disordered breathing
	Dyslipidemia	Malignancy
	Dietary factors	Impaired kidney function
	Obesity	Inherited thrombophilia
	Physical inactivity	Anti-phospholipid antibodies
	Postmenopausal hormone therapy	

(adapted from Goldstein et al.[38] and von Sarnowski et al.[39])

RF profiles change with increasing age, both in the general population[40] and in ischemic stroke patients[41]. The risk of fatal and nonfatal vascular events appears low in individuals with optimal RF profile at all ages, whereas the risk is increased once any RF level or status is considered non-optimal, and is highest among those with accumulation of RF[40, 42, 43]. Regarding gender, cardiovascular RF are more prevalent among male ischemic stroke patients[41]. Overall lifetime risk of stroke may not differ

substantially between men and women, but males may have about twice as high risk of CVD, CHD and MI, and traditional RF have a major influence on all mentioned diseases[40]. Regarding race, blacks show a higher RF burden than whites, despite gender[40].

However, vascular RF are also frequently present in young stroke populations[11, 19, 20, 22, 23, 39, 44, 45], particularly in males and in subjects ≥45 years[20, 22, 39]. A recent large multinational study of 4467 patients with cerebral infarction or TIA found that only 11.5% had none of eight well-documented and modifiable RF, and only 5.3% as well had none of further four less well-documented or potentially modifiable RF[39]. Ischemic stroke patients with no well-documented RF are usually younger, more likely females, the cause of stroke is predominantly of non-atherosclerotic origin, and overall vascular morbidity and long-term mortality are low in these patients[43].

Recurrence

There are limited data regarding recurrent ischemic events or recurrent stroke among young stroke populations. These populations have different RF profiles and etiologic profiles compared to older patient populations, and prognostic models derived from mostly elderly patients are not directly applicable. Recurrent arterial events in young patients have been observed in 10.9% 5 years after the index stroke in Finland[46], in 22.6% 9 years after the index stroke in The Netherlands[47], and in 37.5% 12 years after the index stroke in Western Norway[48]. Respective 8.9%[46], 14.3%[47] and 26.4%[48] had suffered recurrent ischemic stroke, and respective 2.2%[46], 10.5%[47] and 25.0%[48] had suffered other arterial events. The cumulative risk of recurrent stroke was 3.0% at 1 year, 6.6% at 3 years, and 9.4% at 5 years in Finland[46], and 19.4% at 20 years in The Netherlands[47]. The annual recurrence rate declined after the first year, and stabilized at ~1-2% after the first[46, 48] to fifth year[47]. Male gender, age, RF burden (in particular diabetes, dyslipidemia and smoking[47], and smoking and hypertension[46]),

cardiac disease and etiologic stroke subtype (in particular LAA) are factors associated with recurrent arterial events[46, 47].

Outcome

Regarding acute stroke treatment, 11.7% of all patients treated with intravenous thrombolysis (alteplase) within 4.5 hours after stroke onset between 2002 and 2010 were aged 18-50 years[49]. Compared to patients aged 51-80 years, younger patients showed a favorable outcome with functional independence (modified Rankin scale, mRS 0-2) in 72.1% vs. 54.5%, suffered symptomatic intracranial hemorrhage (SICH) within 24 hours in 0.6% vs. 1.9%, and had a mortality rate (mRS 6) at 3 months after thrombolysis of 4.9% vs. 14.4%[49]. SICH was associated solely with systolic blood pressure at baseline, while mortality and/ or poor functional outcome were associated with age, male gender, functional dependence (mRS 3-5) before the index stroke, prior stroke, stroke severity, baseline glucose and systolic blood pressure, signs of infarction in baseline CT imaging, and atrial fibrillation[49]. Regardless of acute treatment, functional outcome after ischemic stroke is dependent on age, being most favorable in the youngest (18-36 years) patients, and declining with increasing age[50]. At long-term follow-up of young stroke patients, a favorable functional outcome was reported for 86.7% after 3 years[16], and for 77.9% after 6 years[51]. Nevertheless, additional social, neuropsychological and neuropsychiatric consequences are high, as shown by the high frequencies of unemployment and divorce/ living alone[16, 52], and the high percentages of young stroke patients suffering from impaired memory/ cognitive performance, depression, fatigue, anxiety and sleep disturbances compared to controls[53, 54]. Major differences to control subjects and the general population regarding health-related quality of life in a Western Norwegian survey concerned physical and social functioning, and reduced health in general. The main associated factors were fatigue, depression and unemployment[55].

Mortality

Mortality after young ischemic stroke shows geographic differences, even within Europe[56]. Although a steady decline is registered in most European countries, mortality remains high in Eastern Europe. Russia reported 5 to 10-fold higher mortality rates among 35-44 year old patients than most western European countries[56]. Mortality rates one year after the index stroke are far higher in Estonia (21%)[57] than in Western Norway [51], Finland[58], Lille, France[16], and Nijmegen, The Netherlands[59] (all ~5-7%). Five-year mortality was 29% in Estonia[57] compared to about 11% in Western Norway, Finland and The Netherlands[51, 58, 59]. Beyond a 5-year follow-up period, the data are scarce. However, a further increase from ~12-17% after 10 years to ~27-31% after 18-20 years have been reported for Western Norway and The Netherlands[42, 53, 59]. Even though mortality in young ischemic stroke patients is much lower than in older patients[60], it is about tenfold compared to controls or the general population of similar age after 10 years[61, 62]. A notably higher risk of death in young stroke patients has been associated with increasing age, male gender, stroke severity, LAA, heart failure, vascular cardiac disease, hypertension, smoking, alcoholism, malignancies, seizures and the fact of living alone[51, 58, 61, 63, 64]. A vascular cause of death has been found in over 50% of the cases after 5 and 12 years[58, 61].

Predictors

Risk factor burden

The number of present RF is associated with recurrent arterial events and mortality in young ischemic stroke populations[42, 43]. This association was clearest for well-documented RF (table 1): The corresponding event rates for patients with ≥1 well-documented RF compared to patients with none were 13.6% vs. 4.7% for recurrent ischemic stroke, 6.1% vs. 0.0% for MI and other arterial events, and 14.3% vs. 3.4% for long-term mortality. Further, the event rates increased proportionally to increasing RF counts[43]. The presence of less well-documented RF increased the cumulative risk of death to 20.6% compared to 8.7% in patients with no less well-documented RF, while differences regarding recurrent ischemic stroke or non-cerebrovascular arterial events could not be shown[43]. At long-term follow-up 18 years after the index stroke in a Western Norwegian population, the mortality rate was 12.5% in patients with no RF, 18.5% in patients with 1 RF, 25.4% in patients with 2 RF, and 53.1% in patients with ≥3 RF[42].

Stroke subtype

Clinical outcome is related to the subtype of stroke[58, 65-68]. In a recent Swedish young stroke study, patients with LAA had the highest risk of recurrent stroke (19.2%) and death (9.6%) 2 years after the index stroke[66]. In Finland, young stroke patients with LAA had the highest mortality rate of 21% compared with other TOAST subtypes 5 years after first-ever stroke [58]. Mortality was lowest among patients with small-vessel disease in both studies (1.6% in Sweden, 5% in Finland). The subtype of stroke has further been associated with imaging-based findings in young stroke patients. Multiple cerebral infarctions in a single territory are associated with cervical, mainly VA dissections , as well as with high-risk cardioembolic sources and LAA. Multiple cerebral infarctions in multiple territories are associated with high-risk cardioembolic sources[32]. Silent cerebral infarctions and leukoaraiosis are associated with small-vessel disease[69].

Multiple cerebral infarcts

Multiple acute cerebral infarcts among young stroke patients were independently associated with an unfavorable 3-month outcome, and also with an increased cumulative risk of death at 8-year follow-up compared to patients with single lesions[32]. Death rates were highest for multiple lesions in multiple territories, when the lesions were distributed bilaterally, or when both the anterior and the posterior circulation were involved. However, an influence on recurrent ischemic stroke has not been shown.[32]

Silent infarcts and leukoaraiosis

Silent infarcts and leukoaraiosis overall are rare findings in young individuals and their prevalence increases with age[29]. Nevertheless, a recent study found silent infarcts in 13% of young ischemic stroke patients, whereof 54% had 1, 20% had 2, and 27% had ≥3 lesions[69]. Leukoaraiosis was prevalent in 7% of all patients, mainly to a mild (42%) or moderate degree (54%)[69]. Silent infarcts and leukoaraiosis were prevalent in 3% of all patients[69]. Multiple silent infarcts increase the risk of recurrent ischemic stroke (Hazard ratio (HR) 2.48, 95% confidence interval (CI) 1.24-4.94, p=0.010), and moderate to severe leukoaraiosis increases independently the risk of death (HR 3.43, 95% CI 1.58-7.42, p=0.002)[70].

Atherosclerosis

Pathophysiology

Atherosclerosis is a multifactorial, slowly progressing, chronic inflammatory disease, characterized by the development of atherosclerotic lesions (atheromata, plaques) mainly from the innermost layer of the arterial wall, the intima[71], but secondarily involving also the media and adventitia layers[72]. The disease begins in infancy[73] and progresses with individual variations in time and location throughout lifetime, dependent on cardiovascular RF[74-77]. Atherosclerosis is further associated with genetic factors[77]. It affects the entire artery tree, but involves mostly systemic large- and medium- sized arteries, most commonly the aorta, carotid, coronary and peripheral arteries[78]. The involvement of lipids, immune cells, smooth muscle cells, connective tissue, extracellular matrix, and thrombotic material indicates the disease´s complexity, and varying proportions of these components explain the heterogeneity of lesions[79, 80]. Endothelial cells are considered the key feature in vascular homeostasis, and their dysfunction and injury are assumed first steps in atherogenesis[81], mediated by accumulation of lipids and inflammatory mediators[71, 82]. Endothelial dysfunction may progress to the stage of a fatty streak, which still may go into regression, or develop further into atheromata[71]. Regression and progression are mediated by the balance of anti-inflammatory and pro-inflammatory mechanisms[83, 84]. Inflammatory processes in the atherosclerotic artery may lead to increased blood levels of activated T-cells, cytokines, C-reactive protein, fibrinogen, interleukines, and other markers of immune activation, which have been found elevated in acute coronary syndromes[85-89]. Atherosclerosis has further been associated with infections in several studies. E.g. chlamydia pneumonia may stimulate disease progression and plaque activation[90], but plays most likely no predominant role[91]. Cytomegalovirus has been found in atherosclerotic lesions and can increase atherosclerosis in experiments[92]. Clinical data imply further the virus´ importance in transplantation-related atherosclerosis causing

graft rejection[93]. Chronic inflammation leads to multifocal plaque development, predominantly at bifurcations, branch points and curvatures of the arterial tree, whereas straight artery segments often remain spared[94]. Inflammation-mediated neovascularization and intra-plaque hemorrhage, along with lipid core necrosis and fibrous cap thinning, differentiate the stable from the unstable, vulnerable plaque prone to rupture[95]. Plaque rupture preferentially occurs in areas of fibrous cap thinning secondary to inflammatory processes[96].

Extracerebral atherosclerosis

Atherosclerosis in extracranial cerebral arteries is the origin of several mechanisms behind ischemic cerebrovascular events. These include atheroembolism, thromboembolism from atherosclerotic plaque surface, occlusion due to plaque rupture and thrombosis, reduced perfusion due to stenotic or occlusive plaques and dissection or subintimal hematoma[97].

Carotid artery disease

The bifurcation, sinus portion and siphon are the carotid artery segments most prone to atherosclerosis, regardless of ethnicity, gender and age[98]. The European prevalence of asymptomatic moderate (≥50%) and severe (≥70%) atherosclerotic carotid stenosis has recently been estimated to 0.2% and 0.1% in males and to 0% for both degrees of stenosis in females ≤ 50 years in the general population. The prevalence increased with age and the number of RF to 7.5% and 3.1% in males and to 5.0% and 0.9% in females ≥80 years[99]. Overall, severe asymptomatic carotid artery stenosis has been associated with an annual stroke risk of 2-5%[100, 101]. Most clinical decision making is based on the degree of stenosis. However, the correlation between the degree of stenosis and ischemic events is not consistent. Other lesion characteristics, such as molecular and cellular processes responsible for plaque composition, have therefore

been suggested as potential markers of plaque vulnerability with impact on the stroke risk[102-104].

Angiographic abnormalities are common in young ischemic stroke patients[13, 45]. A large multinational European study based on ultrasound imaging recently reported an overall prevalence of atherosclerotic carotid artery stenosis ≥50% and occlusion in 4.9% of stroke patients aged 18-44 years (3.4% symptomatic), and 11.0% in those aged 45-55 years (9.2% symptomatic)[26]. Overall, extracranial carotid artery disease was detected in 9.5% of patients aged 18-44, and in 27.6% of those aged 45-55, reflecting the contributing role of premature atherosclerosis to early-onset stroke[105]. Non-stenotic plaques were observed more frequently among males and middle-aged patients[26]. Besides the risk of death due to stroke, patients with asymptomatic carotid stenosis have an even greater risk of death due to MI[101, 106].

Vertebral artery and vertebrobasilar disease

Symptomatic obstructive vertebral artery (VA) disease is less common than the carotid artery equivalent, and the details of the disease are less well understood[97]. The origin of the VA and the proximal, extravertebral segment are most prone to atherosclerotic lesions[98]. VA atherosclerosis is estimated to account for approximately 20% of all posterior circulation strokes[107, 108]. However, in a population-based study of apparently symptomatic stenosis in patients with first-ever posterior circulation TIA or minor stroke, the prevalence of ≥50% vertebrobasilar (VB) stenosis was significantly higher than the frequency of ≥50% carotid stenosis in patients with an anterior circulation event. Furthermore, VB stenosis was more often associated with multiple ischemic episodes and a higher risk of early recurrent stroke[109]. In European young ischemic stroke patients, the frequency of extra- and intracranial VB flow abnormalities was approximately similar to the prevalence of extracranial carotid artery stenosis and occlusion (10.3% vs. 9.5%)[26]. Comparable to the observations regarding carotid artery disease, middle-aged patients more frequently showed VB flow abnormalities compared to the younger age group (12.1% vs. 6.9%)[26].

Intracranial atherosclerosis

Intracranial LAA is a major cause of TIA and ischemic stroke throughout the world, but shows ethnological and geographical differences regarding prevalence[27, 110]. Occlusive intracranial disease most often affects medium sized arteries and their proximal branches: the anterior, middle and posterior cerebral arteries, the posterior and anterior inferior cerebellar arteries, and the distal basilar artery[110].

Among Caucasians, approximately 1% of ischemic strokes were associated with intracranial atherosclerosis (ICAS) in US Americans[111], and 2.2-6.5% of ischemic strokes have been associated with ICAS in Germany[112, 113]. In Norway, the prevalence of ICAS in the anterior circulation among 607 ischemic stroke and TIA patients of all ages was 7.7%, whereof 4.9% were symptomatic lesions significantly associated with ≥50% degree of stenosis[114]. The expected prevalence of symptomatic stenoses in both the anterior and posterior circulation was calculated to 6.4%[114]. In a multinational European young stroke study, 24.1% of patients with extracranial internal carotid artery (ICA) stenosis or occlusion additionally had intracranial stenosis or occlusion[26]. Stenosis or occlusion in the intracranial arteries (11.2% and 3.2%, respectively) were overall more common than in the extracranial ICA (4.2% and 5.2%, respectively). They were mostly related to the middle cerebral artery (MCA, 12.2%), and symptomatic in 10.4%. The prevalence of intracranial stenosis increased with age[26]. However, to what extent atherosclerosis was the process behind the referred stenoses and occlusions remains uncertain. A substantial proportion of the revealed lesions may have occurred due to e.g. cardiac or arterio-arterial embolism, and the application of high-technological methods would be necessary to distinguish atherosclerotic from thromboembolic causes[115].

Coronary atherosclerosis

CHD is the leading cause of death in the US. In 2009, almost 390.000 US Americans died of CHD, consistent with one in six of all deaths[116]. Data from the WHO mortality database indicate a decrease of mortality rates due to CHD among young Europeans aged 35-44 over the period 1980-2007 for most countries except for Russia, with the lowest rates registered for France, Italy and Sweden[56].

Atherosclerosis is the main cause of CHD[71]. Thrombotic coronary occlusion subsequent to rupture of a thin-cap fibroatheroma is the most common mechanism behind coronary ischemia, MI and cardiac death[117-120]. However, there are indications that histopathological characteristics do not depend on the angiographic degree of stenosis at the culprit site[121-124]. Despite successful percutaneous coronary intervention and medical treatment of acute coronary syndromes, recurrent major adverse cardiovascular events are frequent (~20%) within few years, mostly presenting as unstable or progressive angina[123]. US American intracoronary ultrasound studies demonstrated the presence of asymptomatic coronary artery plaques in 17% of donor hearts in 13-19 year old teenagers, and in 60% in 30-39 year old adults[125].

In young ischemic stroke and TIA, CHD has been found in 4.2% and established MI in 3.1% at the time of the index event in a large multinational European study population aged 18-55 years[39]. The corresponding proportions in northern European young ischemic stroke populations aged 15-49 years were 5.9% and 10.2% in Western Norway[20], and 4.9% and 3.7% in Finland[11]. A composite prevalence of CHD and MI among young stroke patients has been reported for 6.0% in data derived from 15 European stroke centers[22]. Further, acute coronary disease is associated with recurrent arterial events and vascular death after ischemic stroke[63, 126]. In the Finnish study population[46], 1.2% suffered fatal or nonfatal MI within 5 years after the index stroke. The cumulative risk for MI or other non-cerebrovascular arterial events was 0.5% at 1 year, 1.2% at 3 years, and 2.4% at 5 years[46]. Composite cardio-aortic causes

of death were reported for 31% among Finnish 30-Day survivors at 5-year follow-up[58], albeit including embolic sources. In Western Norway 4.3% developed first-ever MI and 2.6% had died due to MI (1.3%) and sudden death (1.3%)[51] during a mean follow-up period of 5.7 years. A repeated follow up after 11.8 years revealed CHD including MI in 13.2% of young ischemic stroke patients, compared to 5.4% among matched controls from the general population[48]. At a median observation time of 11.1 years, 4.0% had died due to MI (3.0%) and sudden death (1.0%)[61].

Lower extremity peripheral atherosclerosis

The prevalence of peripheral artery disease (PAD) is dependent on age, atherosclerosis RF profile, and concomitant manifestations of atherosclerotic disease at other sites[127, 128]. In well-defined epidemiological study populations, the prevalence of PAD varied from 3.7% in low-risk individuals[129] to 29% in older or high risk patients[130]. However, the prevalence of asymptomatic PAD has been estimated to 20% in the general adult population[131], and females are more likely to present without symptoms with the consequence of underdiagnosis and undertreatment[132]. Clinical symptoms of PAD vary, and the disease is frequently underdiagnosed. PAD guidelines suggest PAD to be asymptomatic in 20-50%, or presenting with atypical leg pain in 30-40%, with typical claudication in 10-35%, and with critical ischemia in1-3%[133]. In a recent study, investigations for PAD were performed in patients admitted for coronary angiography and/or coronary intervention[134]. PAD had been diagnosed before admission in 17% and unrecognized PAD was revealed in further 15%. Classic intermittent claudication was uncommon (11%), 23.1% described atypical leg pain, and the majority (59.8%) of newly diagnosed PAD patients did not have any form of leg pain[134]. However, PAD is frequent in patients with diabetes mellitus[135], and presence of concomitant sensitive neuropathy may contribute to the underestimation of PAD[136].

In young ischemic stroke, PAD was prevalent in 2.2% of a multinational European study population[39], and in 1.8% in Finland[11]. In Western Norway, intermittent claudication was reported for 4.3% of patients[20]. Further, the presence of PAD was identified as one of the strongest predictors of 5-year mortality[58].

Co-prevalence of carotid, coronary and peripheral disease

Cerebrovascular disease (CD), CHD and PAD together account for 4.35 million deaths, 49% of all deaths in Europe each year[137]. All three conditions are manifestations of atherothrombotic disease, all have serious implications for morbidity and mortality, but the impact of PAD is the most often underestimated one[130]. The international REduction of Atherothrombosis for Continued Health (REACH) registry includes data on cardiovascular events (cardiovascular death, MI, stroke, hospitalization for atherothrombotic events) for about 68.000 outpatients with established CVD, or at least 3 vascular RF[138]. One-year results show a high incidence of cardiovascular events in individuals with established arterial disease, most particularly in PAD patients[139] (Figure 1), which is consistent with other studies[140, 141]. Among patients with symptomatic arterial disease, 15.9% had symptomatic disease in multiple vascular beds[142].

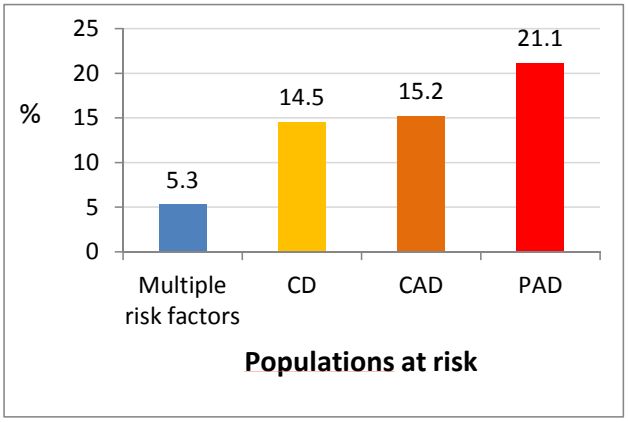

Figure 1: Cardiovascular events within 1-year of diagnosis in patients with multiple risk factors, cerebrovascular disease (CD), coronary heart disease (CHD), and peripheral arterial disease (PAD) (adapted from Steg et al.[139])

The prevalence of vascular disease in all three vascular beds was 3.3% in CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events)[143] and 1.2% in REACH[142]. Combined cerebrovascular disease and PAD was prevalent in 3.8% in CAPRIE and in 1.2% in REACH. Further, one-year event rates increased with the number of symptomatic arterial disease sites[139]. The rate of cardiovascular events was 5.3% for patients with RF only, 12.6% for patients with one, 21.1% for patients with two, and 26.3% for patients with three symptomatic sites of arterial disease[139].

PAD has recently been associated with the prevalence of more severe CHD, such as left main or multivessel CHD, but is nevertheless frequently overlooked[134]. Patients with multivessel CHD have shown a 2-fold higher risk of being diagnosed with PAD compared to single vessel CHD patients. When PAD was revealed in multivessel CHD, more frequently a moderate to severe degree of PAD was confirmed than in single vessel CHD patients[134].

In stroke and TIA populations, abnormal Ankle-brachial index (ABI) as marker of PAD has been reported for 26-31%[144-146] and the risk of subsequent arterial events and mortality was increased in these patients[145, 146]. Patients with severe ABI lowering (<0.8) had, compared to those with normal ABI (0.9-1.3), a higher prevalence of severe extracranial carotid disease (15% vs. 5%) and intracranial large-artery disease (72% vs. 48%)[147]. The corresponding incidence rates at one-year follow up were 19% vs. 11% for composite arterial events, 15% vs. 10% for stroke, and 4% vs. 2% for MI. However, no associations were found for mild ABI lowering (0.80-0.89)[147]. As early as 30 days after the index stroke, the cumulative stroke recurrence rate was higher in patients with ABI ≤0.9 (19.2%) compared to those with ABI > 0.9 (3.3%), and both atherothrombotic cause of stroke and previously asymptomatic PAD were independently associated with stroke recurrence risk[148].

Imaging of atherosclerosis

The choice of method for arterial imaging is based on a benefit-risk evaluation. Invasive imaging includes the risks inherent with arterial access[149]. Methods based on the utilization of ionizing radiation are limited by restrictions for repeated use[150]. Magnetic resonance angiography (MRA) is independent from ionizing radiation, but its use is limited by claustrophobia, extreme obesity, or incompatible implanted devices[97]. Duplex ultrasound (DUS) is safe and inexpensive, and the examinations may be repeated almost unrestrictedly, with few exceptions (e.g. transorbital). Iodinated contrast media are restricted to patients with adequate renal function, and gadolinium-based contrast agents have been associated with the development of nephrogenic systemic fibrosis[151-154]. Summarizing, DUS and time-of-flight MRA (TOF-MRA) are the only methods with no or low proven side effects in vascular evaluation. However, both methods have limitations.

Extracranial arteries

Conventional digital subtraction angiography (DSA) has been established as the standard of reference against which other imaging methods are compared[97]. Among several alternative methods for carotid stenosis measurement, the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[155] method and the European Carotid Surgery Trial (ECST)[156] method are the most used ones in clinical trials. There are feared complications of DSA. The risk of morbidity and mortality are estimated to be 1-4% in patients with atherosclerosis[157]. Also asymptomatic cerebral infarctions due to microembolization have been reported[158]. However, when performed by experienced physicians, the incidence of stroke associated with the procedure is <1%[149].

Computed tomography angiography (CTA) compares favorably with DSA for evaluation of precerebral stenoses through direct imaging of the arterial lumen. Besides the ability to evaluate the degree of stenosis, although impaired in heavily calcified plaques[159], CTA is further capable of the assessment of surface irregularities, ulceration, and plaque composition[160-162]. Positron emission tomography (PET)-CT is an emerging technique intending to image and quantify plaque inflammation through absorption of a radionuclide tracer by lymphocytes and macrophages[163].

Magnet resonance angiography (MRA) provides imaging of >70% stenosis and occlusion with a high sensitivity and specificity, although dependent on the equipment used[164]. MRA is able to provide imaging of the arteries with insensitivity for calcifications, but stenoses may be overestimated and there are difficulties in distinguishing subtotal from total occlusions[97]. However, MRI has a great potential for plaque characterization, and can provide information on plaque volume and composition[165], including fibrous cap integrity, necrotic core, and intraplaque hemorrhage[166]. Dynamic contrast enhancement is used to evaluate plaque vascularity and inflammation[167, 168]. The development of targeted contrast agents may in the future allow plaque characterization on molecular and cellular level[169].

DUS is globally accepted as first-line diagnostic tool in the evaluation of carotid stenosis. Quantification of the degree of stenosis can be done by ratio-percent methods based on morphological analysis, or on analysis of peak systolic velocity (PSV) in the ICA and common carotid artery (CCA)[170, 171]. PSV in the ICA correlates with angiographically determined degree of stenosis[171], and DUS has high sensitivity and specificity in the detection of >70% stenosis (respective 86% and 87%) and occlusion (respective 98% and 100%)[164].

Morphological evaluations are provided by B-mode ultrasonography. Plaque surface is commonly described as regular, irregular or ulcerous, and surface irregularities have been correlated to plaque vulnerability and risk of cardiovascular events[172]. Echolucent plaques represent lesions with a high content of soft tissue, such as lipids or hemorrhage, while echogenic plaques represent primarily fibrous tissue content[173,

[174]. The correlation of hypoechoic plaques and subsequent ipsilateral cerebrovascular events has repeatedly been shown[175, 176]. Plaque classifications further describe homogeneity or heterogeneity in plaque composition[177, 178]. Intravenous microbubbles-based contrast agents are used to increase the visibility of the arterial blood flow. They are further used for evaluation of plaque neovascularization and perfusion[179]. Late onset of plaque enhancement is increased in symptomatic lesions compared to asymptomatic ones[180], most likely due to increased neovascularization[181]. In addition, advanced targeted contrast agents may in future allow characterization of atherosclerotic lesions on a molecular level[182, 183]. Intima-media thickness (IMT) of the carotid artery is a marker of systemic atherosclerosis and its measurement is widely used in prediction of risk for ischemic arterial events[184, 185].

Intracranial arteries

DSA currently is the gold standard for imaging of intracranial artery pathology[186].

CTA may, due to broad availability, short performance time and minimal invasiveness, be the modality of choice in most cases. Its sensitivity is 98%[187].

TOF-MRA is frequently used in diagnostics of the intracranial arteries. However, its sensitivity is only 70%[187]. The method tends to overestimate the degree of stenosis[188] and intracranial hemodynamic low-flow conditions may result in an additional overestimation of the degree of stenosis, or even in false diagnosis of arterial occlusion[187]. Gadolinium-based contrast agents increase accuracy[188].

Transcranial ultrasonographic methods include transcranial Doppler (TCD) and transcranial color-coded sonography (TCCS), most commonly through the transtemporal, transnuchal or orbital bone window, providing insonation of the circle of Willis and the branches of the intracranial arteries. The sensitivity of TCCS may be improved by use of ultrasound contrast agents, gas filled “microbubbles” which scatter ultrasound signals and increase the magnitude of the received echo[189]. The

detection of intracranial stenoses is provided by measurement of blood flow velocities[190, 191].

Coronary arteries

Conventional coronary x-ray arteriography depicts the arterial lumen only, is unable to reveal compensatory enlargement of arteries through positive remodeling and may thus give the false impression of a normal artery when the lesion does not impinge on the lumen[192].

Computed tomography (CT) is able to identify calcium, and the amount detected by coronary CT correlates well with histologically identified coronary atherosclerosis[193]. The evaluation of coronary artery calcification adds to clinical risk scoring providing predictive information[194], and has been the predominant method for risk assessment by CT so far. Predominantly lipid-rich plaques may be differentiated from predominantly fibrous plaques by CT, but there is a large degree of overlap regarding attenuation on CT, decreasing the method´s specificity[195]. CTA is capable of excluding the presence of significant stenoses[196], but is limited when high signals from calcified plaques obscure the arterial lumen.

Ultrasound examinations for evaluation of coronary atherosclerosis have so far mainly been limited to the measurement of carotid Intima-media thickness (cIMT). It is evident that increased cIMT is associated with subsequent coronary events[197, 198]. However, the imaging of coronary atherosclerosis has recently been improved by the addition of catheter-based gray-scale and radiofrequency intravascular ultrasonographic imaging to three-vessel coronary angiography[123]. This combination showed that lesions which were nonculprit at the time of baseline acute coronary syndrome, but had led to major adverse cardiovascular events at follow-up, frequently gave only mild stenosis, but were characterized by a large plaque burden, a small luminal area, or both[123]. Contrarily, nonculprit lesions with a low plaque burden and

<40% loss of cross-sectional luminal area did not lead to major adverse events. Further, thin-cap fibroatheromas, identified by radiofrequency intravascular ultrasonography, represented the highest-risk phenotype[123].

Peripheral arteries

DSA has, as a robust technique for diagnosing arterial stenoses or occlusion, been the standard for evaluation of lower extremity atherosclerosis. The method is limited by 2-dimensional view and the complications mentioned above[152, 153], and has been widely replaced by non-invasive techniques.

CTA provides high scanning speed, a great longitudinal field of view and high imaging quality to minimum voxel dimensions of 0.4 to 0.6 mm, which is optimal for the standard performance from the aortic diaphragmatic hiatus through the toes, including visualization of smaller, distal arteries[199]. Dense vessel calcification or metallic devices may lead to blooming artifacts and result in overestimation of stenosis. However, PAD patients commonly undergo multiple imaging studies during their lifetime, and reduction in radiation exposition is mandatory[199].

Contrast-enhanced MRA (CE-MRA) is a robust technique, and can be sequentially performed to image the aortoiliac, femoropopliteal, and tibial regions through the peripheral runoff arteries[199]. There is excellent agreement between 3 Tesla MRA and conventional DSA regarding the degree of stenosis[200]. The use of TOF-MRA, based on flow-dependent enhancement, is challenging due to inadequate signal for deep intraabdominal vessels, flow-related artifacts at the site of stenosis, and scanning is time-consuming[201]. However, TOF-MRA is as adequate as DSA for evaluation of tibial and pedal arterial stenosis or occlusion[202]. MRI is further able to show calcification and lipid-rich necrotic plaque core, and to measure total plaque volume[203, 204]. Non-CE-MRA gains renewed interest due to concerns regarding complications after use of contrast agents, and new techniques are emerging[199].

DUS combines broad functional and morphological information by gray scale and color pulsed Doppler techniques. However, complete examination from the aorta and through the peripheral vessels is time consuming and technically challenging, and may not be feasible in many patients due to abdominal girth or intestinal gas. Further, when sequential stenosis is present, DUS is less sensitive for detection of additional lesions further downstream[199]. The quality is dependent on the examiner's experience, and inter-observer agreement is only moderate for evaluation of aortoiliac and femoropopliteal vessels[205].

Neurosonology, carotid intima-media thickness and atherosclerotic plaques

Neurosonology is a noninvasive, readily available, inexpensive imaging technique, which is independent from application of ionizing radiation or iodinated contrast agents, and may be repeated almost unrestrictedly for diagnostic investigation of the extracranial arteries, when reasonable duration of examination is achieved. High-resolution pulse-echo (B-mode) sonography is based on grey-scale characteristics of the tissue examined. This method is able to visualize arterial wall structures including IMT and plaques[206, 207]. It further allows for evaluation of plaque characteristics, such as volume, surface, composition and morphology, which define plaque vulnerability and may be used in vascular risk stratification[176, 208-211]. The evaluation of plaque vulnerability may be improved further by use of microbubbles contrast agents, which are able to depict plaque neovascularization and intralesional hemorrhage[212-216]. Color duplex flow imaging and power duplex imaging may be applied to detect artery caliber variations and to grade arterial stenosis and local hemodynamic alterations.

Carotid intima-media thickness

Atherosclerosis is a process directly affecting the arterial wall. Thus, it has been suggested that measurements of IMT may be more accurate than measurements of

luminal diameter, not at least due to frequent compensating luminal expansion at sites of stenosis[217]. In B-mode sonography of IMT, the varying echogenicity of different tissues is used to detect the transition from the hypoechogenic lumen of the artery into the hyperechogenic intimal layer (lumen-intima boundary) as internal border of the intima-media complex, and the transition from the hypoechogenic medial layer into the hyperechogenic adventitia (media-adventitia boundary) as its external border[217] (marked in Figure 2). These observations have been verified by histology[218]. The differentiation between the intimal and the medial layer is not possible by ultrasonography. IMT measurements at the near wall (marked in Figure 2) do not correlate with measurements at the far wall due to inverted order of the tissue boundaries, resulting in transition from the surrounding tissue into the adventitial layer as external boundary, and the transition from the medial into the intimal layer as internal boundary. Adventitia-media and intima-lumen boundary cannot be depicted[219]. Thus, IMT measurement is recommended at the far wall[220]. The measurements should be obtained at end-diastole to avoid stretching effects during systole resulting in IMT reduction[221]. Further, the angle between ultrasound beam and tissue should be 90° to achieve optimal imaging quality[220]. CIMT measurements can be done in the CCA, the carotid bifurcation (BIF), and the ICA. However, it has repeatedly been demonstrated that measurements from BIF and ICA compared to CCA measurements result in higher proportions of missing values and value variability due to anatomical obscurity and subsequent imaging difficulties[222]. Current guidelines suggest obtainment from CCA, which is easier accessible and results in higher reliability, as does measurements in areas free of plaque[220]. However, after recent analyses one has concluded that CCA-IMT does not improve long-term risk prediction regarding first-ever stroke or MI[223, 224], while ICA-IMT does[224]. Another practical concern is whether the use of mean or rather maximum values is favorable. Both have been reported in several key studies, what makes comparison difficult[206] (Table 2).

Table 2. Major epidemiological studies on cardiovascular disease prediction by carotid Intima-media thickness

Study	*Publication*	*n*	*Outcome*	*Segments assessed*	*IMT Summary*
ARIC[225]	1997	12,841	MI	CCA, BIF, ICA, combined	Mean IMT
ARIC[185]	2000	14,214	Stroke	CCA, BIF, ICA, combined	Mean IMT
CHS[226]	1999	4,476	MI / Stroke	CCA, ICA, combined	Maximum IMT
Rotterdam Study[227]	2002	5,851	MI	CCA, BIF, ICA, combined	Maximum IMT
Rotterdam Study[228]	2003	5,479	Stroke	CCA	Mean IMT
CAPS[229]	2006	6,962	MI / Stroke	CCA, BIF, ICA	Mean IMT
Framingham Offspring Study[224]	2011	2,965	All CVD	CCA, ICA	Mean CCA-IMT / maximum ICA-IMT

(adapted from Robertson et al.[230])

Finally, IMT is prone to variability due to the type of ultrasound equipment, and to the experience of the sonographer and the IMT-reader. Former can be overcome by stringent research settings (though not applicable in clinical routine settings), and the latter may benefit from use of automated edge detection systems[220, 231].

Increased IMT correlates well with vascular RF, but has also been associated with an increased risk of CVD independent of cardiovascular RF[206]. IMT has become a surrogate marker of atherosclerosis from early to late stages[232, 233]. It is frequently used as predictor of CVD risk[206], and as marker of efficacy in interventional studies including both asymptomatic, at-risk, and CVD populations[234-238]. It has been hypothesized that increased IMT represents subclinical vascular disease, which was supported by graded associations between IMT and concurrent atherosclerotic change in coronary angiography studies[239, 240]. In the Atherosclerosis Risk in Communities (ARIC) study, assessment of middle-aged, CHD-free participants led to the conclusion that IMT $\geq$ 1 mm was associated with an increased incidence of CHD at 4- to 7-years follow-up[225]. Also the risk of stroke increased with IMT, although the

relationship was not linear[185]. Similar findings were made in the Cardiovascular Health Study (CHS)[226] and in the Rotterdam Study[227] in older populations. Further, in the Rotterdam Study, cIMT > 0.84 mm was predictive of incident stroke[228]. The Carotid Atherosclerosis Progression Study (CAPS) found IMT to be predictive of MI, stroke and death at 4.2-year follow-up in a wide range of age[229]. Recent data from the Framingham Offspring Study supported that both mean CCA-IMT and maximum ICA-IMT were predictive of future vascular events, but, remarkably, only ICA-IMT improved clinical risk classification[224].

However, atherosclerosis only partly explains intima-medial thickening. Several other mechanisms are subject of discussion, such as hypertrophy due to shear stress to the artery wall, or hypertension[241, 242]. A recent systematic review on the improvement in CVD risk stratification by additional imaging of subclinical atherosclerosis concluded with a reasonable benefit in individuals at intermediate risk[243]. The combination of cIMT and plaque assessment has shown stronger improvement of CVD prediction than one of these methods alone[244, 245], and may further increase the potential to identify subclinical vascular disease[231].

Therapeutic opportunities

Life-long administration of antiplatelet drugs represents the prevention treatment of choice in atherothrombotic diseases[246]. Both clopidogrel monotherapy and combined acetylsalicylic acid (ASA) plus extended-release dipyridamole (ER-DP) treatment are superior to ASA monotherapy in secondary prevention[247, 248]. There is also a benefit from dual antiplatelet therapy with clopidogrel and ASA in secondary prevention compared to ASA alone, although at the cost of an increased rate of bleedings[249]. The comparison of treatment with combined ASA/ER-DP vs. clopidogrel monotherapy did not reveal superiority of one of the alternatives[250] and the choice between the two is still a matter of debate.

A recent study confirmed fatty streaks as precursors of plaques[251], and their prevention will prevent or slow the development of clinically significant lesions[76]. Concepts about the timing and aggressiveness of lifestyles changes and RF treatment, both in ostensibly "healthy" individuals and in those with established CVD, have gotten increasingly into focus[252-256]. Atherosclerosis as an inflammatory disease offers several opportunities for treatment, such as immunosuppressant and anti-inflammatory agents, or vaccination. Cyclosporine inhibits T-cell activation and smooth muscle cell proliferation, and inhibita intimal thickening[257, 258]. Antiinflammatory agents include statins with lipid-lowering properties and also pleiotropic effects, which are not directly dependent on the reduction of the cholesterol level[259, 260]. Statins have beneficial effects on clinical outcome in CHD[261] and ischemic stroke[262], and the need to begin treatment according to the pathogenesis of atherosclerosis rather than at the time of initial clinical manifestation has been emphasized[263]. Finally, vaccination with disease-related antigens and the purpose to induce protective immunity is an attractive approach under experimental investigation[264, 265].

Aims of the thesis

1. To compare and evaluate differences and similarities regarding demographics, stroke characteristics, and clinical performance among patients aged 15-49 years and among patients aged ≥ 50 years. We hypothesized that young stroke patients would differ in etiology, co-morbidity, stroke severity and complications, and have a more favorable outcome than older patients.
   This hypothesis is discussed in paper I.

2. To describe the comprehensive diagnostic methods applied in the Norwegian Stroke in the Young Study (NOR-SYS).
   Rationale, methods and aims of the study are discussed in paper II.

3. To evaluate the burden of vascular RF among young ischemic stroke patients, and to investigate their impact on cIMT compared to controls free of CVD. We hypothesized a higher prevalence of RF and increased cIMT among young stroke patients. This hypothesis is discussed in paper III.

4. To evaluate and compare the prevalence of carotid atherosclerosis in all TOAST subtypes of young ischemic stroke by cIMT measurement. We hypothesized atherosclerotic carotid artery disease to be prevalent among patients with undetermined cause of stroke. This hypothesis is discussed in paper IV.

Subjects and methods

The data for paper I were obtained from a cohort of the Bergen NORSTROKE registry, and gathered between February 2006 and March 2009. All registered patients with established and documented ischemic stroke were included.

Paper II represents a description of the comprehensive NOR-SYS research protocol, and does not contain patient data.

The data studied in papers III and IV were obtained from a first cohort of NOR-SYS patients and their partners, enrolled in the study between September 2010 and June 2012. All data on TOAST classification in paper IV were obtained from the Bergen NORSTROKE registry.

The Bergen NORSTROKE study

In the Bergen NORSTROKE study, all patients admitted to the Centre for Neurovascular Diseases at the Department of Neurology, Haukeland University Hospital, and diagnosed with TIA, ischemic stroke, or ICH, have been registered consecutively since 2006. Registration is based on written informed consent by the patient or a legal representative, and contains demographic, clinical, radiological and laboratory data, including short-term outcome. The study has been approved by the local ethics committee. The Department of Neurology serves a well-defined population of approximately 240,000 inhabitants[266].

The Norwegian Stroke in the Young Study (NOR-SYS)

NOR-SYS is a prospective 3-generations study, gathering anamnestic, clinical, radiological and laboratory data. The study has been approved by the local ethics committee. Patients are included consecutively within the inclusion period September 2010 to August 2015, based on written informed consent by the patient or a legal representative during hospitalization. Inclusion criteria are age 15-60 years, and documented acute ischemic stroke. Patients with non-arterial cerebral infarction, such as caused by SAH, sinus venous thrombosis, trauma or cerebral tumor, are excluded from participation. Due to ethnic variations regarding the development of atherosclerosis, patients of non-Caucasian ethnicity are investigated according to the research protocol, but are not included and registered in NOR-SYS from June 2012. Non-Caucasian patients included before that date are excluded from all statistical analyses (concerns papers III and IV). Partners were chosen as healthy controls in NOR-SYS due to their role as reference persons to joint offspring in future analyses. Controls with established CVD were excluded from statistical analysis.

Participation in NOR-SYS

Of 162 patients consecutively admitted to the Neurovascular Center between September 2010 and June 2012, 2 patients (1.2%) refused study participation. Seven patients were excluded from statistical analysis: four non-Caucasian patients due to ethnic variations regarding the prevalence of atherosclerosis, and three patients with incomplete data set due to hardware errors. Further three patients were excluded due to protocol violation.

Of 161 potential controls (patient´s partner and/ or other parent to joint offspring), five were deceased, four were non-Caucasians, 17 refused participation, in 12 the investigators were denied to offer inclusion by the patient, and 39 did not respond to

the invitation to participate. Thus, of 123 potentially available controls, 63 (70.8%) females and 21 (61.8%) males finally participated. Seven controls with prior cardiovascular events were excluded from statistical analysis in this study.

Diagnostic work-up

In paper I, the studied population (cohort I) represents ischemic stroke patients of all ages, admitted to the Neurovascular Center, and registered in the Bergen NORSTROKE study. Cohort I has been dichotomized into a group of patients of young and early mid-age (15-49 years), and a group of middle-aged and old patients (≥50 years). Stroke investigation included CT or MRI scan of the cerebrum, electrocardiography (ECG), Holter monitoring, echocardiography, and Duplex sonography of the precerebral arteries. RF ascertainment included MI, angina pectoris, PAD, atrial fibrillation, hypertension, diabetes mellitus, and smoking. Stroke severity was assessed by use of the National Institute of Health Stroke Scale (NIHSS) on admittance, and on day 7 or at discharge. The degree of physical disability was described by mRS score, and performance in activities of daily living was classified by Barthel Index (BI). Stroke subtypes were classified according to TOAST criteria[24]. Clinical stroke classification was performed by use of the Oxfordshire Community Stroke Project (OCSP) scale[267]. ICA stenosis was classified as 30-49%, 50-69%, 70-99% or occlusion by neurosonology. Clinical complications were documented.

All patients included in NOR-SYS (cohort II, analyzed in papers III and IV,) were additionally investigated according to the NOR-SYS research protocol, which has been described separately in paper II. The population was dichotomized into a young (15-44 years) and a middle-aged (45-60 years) group. Anamnestic data collection was performed by standardized questionnaires concerning socio-demographics, life styles and nutrition, history of vascular RF and prior vascular or general disease, current medication, and circumstances around stroke onset. Ten RF were evaluated in Paper

III: Prior stroke, CHD, PAD, family history of CVD (stroke, CHD, PAD), diabetes mellitus, hypertension, dyslipidemia, smoking, high alcohol consumption, and Body mass index (BMI) > 25. Family history concerned parents and siblings. RF burden was defined as the composite number of RF diagnosed before admission or revealed during hospitalization.

TOAST classification

Causative subtyping of cohort I and II has been performed by one experienced vascular neurologist (HN), who was blinded for ultrasonographic NOR-SYS results.

cIMT measurements

cIMT measurements were performed in supine position by insonation of the bilateral carotid arteries with a 9-3 MHz linear array transducer (iU22 Philips Medical Systems, Bothell, WA, UWA), or a 12-3 MHz linear array transducer (CX50 Philips Medical Systems, Bothell, WA, UWA). Preset vertical markers in a horizontal distance of 10 mm each were used to define the distal CCA segment (20-10 mm proximally to the tip of the flow divider, the BIF segment 10-0 mm proximally to the tip of the flow divider, and the proximal ICA segment 0-10 mm distally to the tip of the flow divider in longitudinal view (Figure 2). To ensure center position of the artery scan plane, IMT was sought visualized on the far and, if possible, on the near wall. Overall, a complete carotid scan required 12 multi-angle measurements obtained from the far wall of all three segments in the enddiastolic phase of the cardiac cycle. Meijer´s carotid arc was used to standardize the scan angles to 180◦, 150◦, 120◦ and 90◦ in the right CCA segment, and to 180◦, 210◦, 240◦ and 270◦ in the left CCA segment. IMT measurements in BIF and ICA were bilaterally obtained from one respective angle representing the most pathological finding. Data were stored as frozen images, and analyzed at an Xcelera® work station by automated Philips QLAB® quantification (Figure 2) software after completed examination according to the NOR-SYS ultrasonographic research protocol.

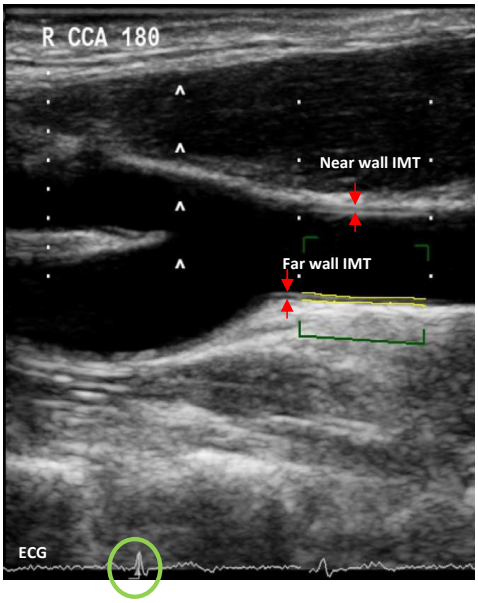

Figure 2:

Demonstration of right 180° CCA-IMT (red arrows), and measurement at end-diastole (green circle) by automated edge detection using Philips QLAB® (yellow edges)

^ Tip of the flow divider

All IMT analyses were performed by the respective sonographer herself. IMT analysis was sought performed over a standardized 10 mm distance for each measured angle at each measured segment, and stored as a mean value. Satisfactory analysis required continuous visualization over a distance of at least 7 mm (70%). In case of artery wall irregularities or plaques, additional maximum measurements were performed at the site of the largest distance between media-adventitia boundary and the lumen-intima boundary of the lesion.

As described previously, several large studies found increasing cIMT to be related to prevalent and incident CVD. We defined cIMT ≥ 1.0 mm as cut-off point for arterial disease[225, 268], and we defined cIMT 0.8-0.99 mm as gray-zone values suspect for arterial disease[228, 269]. cIMT ≥ 1.5 mm was defined as plaque[220]. cIMT was categorized accordingly into < 0.8 mm/ 0.8-0.99 mm/ 1.0-1.49 mm/ ≥ 1.5 mm in figures and statistical analyses of IMT distribution (papers III and IV).

Additional ultrasonographic and anthropometric examinations

In addition to anamnestic data collection and cIMT measurements as described above, the carotid artery and MCA were bilaterally examined for stenoses by neurosonography. Ultrasonographic examination of the infrarenal abdominal aorta and the femoral arteries including femoral artery IMT, and of epicardial, visceral abdominal and subcutaneous abdominal adipose tissue were performed. ABI was obtained bilaterally. BMI and Waist-Hip ratio were calculated.

Radiological and cardiac examinations

In addition to standard radiological and cardiac examinations in stroke investigation, NOR-SYS patients were referred to MRI scan including axial FLAIR, MRA, diffusion-weighted (DWI) and susceptibility weighted imaging (SWI) within 24 hours after admission, to arterial stiffness measurements by aplanation tonometry, to coronary CTA including CT of the thoracic aorta in case of plaques revealed by femoral artery sonography, and to ambulatory 24 hour blood pressure registration within 3 months after hospitalization.

All examinations included in the NOR-SYS research protocol are comprehensively described in Paper II, but data obtained from these have not been published yet.

Neurosonographic settings, training and data reliability tests

The NOR-SYS participants enrolled in statistical analyses for paper III and IV have been examined by 2 sonographers (AF, UWA). Both sonographers have been trained and certified for the NOR-SYS ultrasonographic research protocol in collaboration with the University Medical Centre of Utrecht, The Netherlands, prior to study inclusions. All neurosonological studies were performed using iU22 or, when performed at the intensive care unit (concerns 2 patients included in the studies

discussed in papers III and IV), using CX50 (both Philips Medical systems, Bothell, WA, USA). The NOR-SYS protocol settings in iU22 and CX50 have been prepared and stored in cooperation with Vingmed AS Norway.

Data reproducibility regarding cIMT, studied in Papers III and IV, was evaluated by intra-observer, inter-observer and inter-equipment tests within the research group (AF, ML, UWA). Intra-observer examinations were performed in 10 volunteers of varying age and with varying degree of pathological findings by complete NOR-SYS ultrasonographic examination at baseline, and repeated after 10-14 days. Intra-observer correlation for cIMT measurements (AF) was found substantial, calculated to 0.78, with a mean absolute cIMT difference of 0.08 mm. Inter-observer correlation of sonographers (AF, UWA) was performed in 12 random patients included in NOR-SYS during initial hospitalization and found substantial, calculated to 0.83, with a mean absolute cIMT difference of 0.11 mm. The inter-equipment variability was tested in 5 volunteers by one sonographer (AF) applying both ultrasound systems, and correlation for iU22/CX50 was excellent, calculated to 0.94, with a mean absolute cIMT difference of 0.04 mm.

Statistical analyses

For paper I, Chi-square test, Fisher´s exact test, student´s t-test and logistic regression were performed by use of STATA 11.0.

For statistical analysis of cIMT values (papers III and IV), maximum IMT values were achieved for each carotid segment: the highest of four mean CCA-IMT values (obtained from four fixed angles), mean BIF-IMT and mean ICA-IMT (both obtained from the site representing most pathology) were chosen for left and right carotid artery separately. When performed, maximum IMT values obtained from sites of irregularities or plaques were chosen for analysis instead of mean values.

For paper III, data were formatted in STATA 12.1, and statistical analyses were performed by R 3.0.0 by a statistician (ØAH). T-test, univariate linear regression, multivariate logistic regression and Fisher´s exact test were applied, and simulations were used when appropriate.

For paper IV, data were formatted in STATA 12.1, and statistical analyses were performed by R 3.0.1 by a statistician (ØAH). A standard t-test and Fisher´s exact test were applied.

Results

Comparison between ischemic stroke patients < 50 years and ≥ 50 years (Paper I)

Of 1217 consecutive acute ischemic stroke patients enrolled in the Bergen NORSTROKE study, 100 patients (8.2%) were < 50 years ("young", mean age 40.8 ± 7.6), and 1117 patients were ≥ 50 years ("old", mean age 73.4 ± 11.8) at the time of the index stroke. Males represented 72% of young patients, and 55.8% of old patients. Current smoking (44.1% vs. 23.6%, p<0.001) and mechanic aortic valve (5.0% vs. 1.9%, p=0.05) were more frequent among young patients, while prior cerebral infarction (4.0% vs. 16.2%, p<0.001), MI (4.0% vs. 13.9%, p=0.003), pectoral angina (4.0% vs. 14.4%, p=0.002), hypertension (27.0% vs. 53.8%, p<0.001), paroxysmal atrial fibrillation (2.0% vs. 9.4%, p=0.009), and chronic atrial fibrillation (0.0% vs. 9.46%, p<0.001) were more frequent among old patients.

Undetermined cause of stroke was the most frequent subtype of stroke among young patients (39.0%) and among old patients (41.9%). The most frequent determined causes were cervical artery dissection and non-arrhythmic CE among young patients (both 18%), and CE due to arrhythmia (23.9%) among old patients.

Stroke severity on admission measured by NIHSS (score 5.7 vs. 6.3, p=0.45) and the classification of stroke syndromes based on OCSP (p=0.33) did not differ. Neither were there differences in functional short-term outcome based on mRS (p=0.11).

The Norwegian Stroke in the Young Study (NOR-SYS) (Paper II)

Paper II represents a comprehensive description of design, rationale and methods included in the NOR-SYS research protocol. Paper II does not contain any results.

Risk factors and carotid Intima-media thickness in young ischemic stroke patients and controls (Paper III)

In total, 150 patients aged 15-60 years, and 84 CVD-free controls were studied.

Of patients, 30.0% were aged 15-44 at study inclusion, and 32.7% were female. Of 84 controls, 25.0% were aged 15-44 at study inclusion , and 75.0% were female.

Risk factor burden

Patients of all age and sex subgroups had a higher RF burden than controls (age 15-44: $p=0.027$; age 45-60: $p<0.001$; females: $p<0.001$; males: $p=0.021$; total: $p<0.001$). The RF burden was highest among middle-aged patients with 7 out of 10 investigated RF present.

Carotid Intima-media thickness

The overall cIMT difference between patients and controls was 12% for CCA, 17% for BIF and 29% for ICA. Associations between RF and IMT increase varied in the different carotid segments. In multivariate analyses of all 234 participants, IMT increase was associated with age ($p<0.001$). Further, CCA-IMT was associated with male sex ($p=0.023$) and hypertension ($p<0.001$). Increased BIF-IMT was associated with dyslipidemia ($p=0.018$) , coronary heart disease ($p=0.017$) and smoking ($p=0.012$).

Atherosclerosis in TOAST subtypes of young ischemic stroke (Paper IV)

In total, 150 patients aged 15-60 years, and 84 CVD-free controls were studied.

TOAST subtypes of stroke

The causes of stroke were 5.3% LAA, 26.7% CE, 21.3% SAO, 10.0% SOC (including 8.0% dissections), and 36.7% SUC. CE ($p=0.008$), SOC ($p<0.001$), dissections ($p=0.01$) and SUC ($p<0.001$) were more frequent among patients aged 15-44 years compared to those aged 45-60 years. CE ($p=0.017$) and dissections ($p=0.047$) were predominant in males.

Carotid Intima-media thickness in TOAST subtypes

Compared to mean cIMT among controls (0.86 mm), mean cIMT was increased among patients with LAA (1.56 mm, $p=0.002$), SAO (1.11 mm, $p=0.006$), and SUC (1.10 mm, $p=0.004$). Mean cIMT did not differ from controls among patients with CE (0.89 mm, $p=0.775$), SOC (0.86 mm, $p=0.974$), and, among SOC, in patients with dissection (0.95 mm, $p=0.476$).

A similar pattern was found for middle-aged patients (LAA: 1.57 mm, $p=0.011$; SAO: 1.23 mm, $p=0.005$; and SUC: 1.20 mm, $p=0.008$; CE: 1.06 mm, $p=0.396$, SOC: 1.15 mm, $p=0.399$; dissection: 1.15 mm, $p=0.399$; controls: 0.95 mm).

Solely LAA differed from controls among young patients (LAA: 1.44 mm, $p<0.001$; SAO: 0.68 mm, $p=0.29$; and SUC: 0.66 mm, $p=0.403$; CE: 0.66 mm, $p=0.359$, SOC: 0.72 mm, $p=0.219$; dissection: 0.81 mm, $p=0.124$; controls: 0.59 mm).

Segmental IMT distribution differed across TOAST subtypes, age groups and sexes.

Discussion

Age-dependent variations of stroke

Age

In NOR-SYS, the patients´ upper age limit has been set to 60 years due to high life expectancy in Norway. One assumed a potential benefit of study inclusion also for middle-aged patients prior to regular retirement at age 67. This creates a hangover of 10-15 years compared to previous young stroke studies, and a 10 years overlap with the NORSTROKE population described in Paper I (cohort I). Changes in stroke etiology have been recently suggested by the approximate age of 45 years[11], and this assumption has been addressed by division of our NOR-SYS population (cohort II) into age groups, as previously practiced by other studies[14, 39]. Our two studied cohorts cannot be compared directly due to the overlap regarding age, the missing old patient population in NOR-SYS, and a 16 months discrepancy with respect to study duration. However, in the respective study periods, 8% of 1217 patients in cohort I were aged 15-49 years, and 92% were aged ≥ 50 years. Of 150 patients in cohort II, 30% were aged 15-44 years, and 70% were aged 45-60 years. This confirms the increasing incidence of stroke with age for our population.

Sex

Males were outnumbering females in both cohorts, including both age groups in NOR-SYS. We observed a decrease of the female population from young age (42%) to mid-age (29%) in cohort II, but there was a relative increase from young age/ mid-age (28%) to mid-age/ old age (44%) in cohort I. Both observations are in line with other studies. There is evidence for females being at a higher risk of stroke before age 30, e.g. related to prothrombotic state, pregnancy, and the perinatal period[11, 21]. Female risk declines thereafter, only to relatively increase again at older age, possibly

due to later accumulation of RF, hormonal changes leading to sudden increase of atherosclerotic disease[270], and a higher life expectancy compared to males.

Risk factors

RF were frequently prevalent in both cohorts, including the CVD-free controls enrolled in NOR-SYS. Among patients of all investigated age groups, current or previous smoking (51% -77%) and hypertension (27% -74%) were the most frequent RF. Additionally, but only evaluated in young and middle-aged patients of cohort II, BMI > 25 (62% and 68%), dyslipidemia (60% and 83%) and family history of CVD (20% and 66%) were frequently present. All RF were increasing with age, except smoking which showed an inverse u-shaped course, peaking at mid-age. NOR-SYS controls were comparably healthier than patients with only smoking (57% and 71%), BMI > 25 (52% and 67%), and a family history of CVD (43% and 64%) as most frequent RF in both age groups.

In cohort II, the overall RF burden was lowest among young controls followed by middle-aged controls, and RF burden was highest among middle-aged patients followed by young patients. RF burden increasing with age has been reported for other young stroke populations before[22, 39]. Even though clinically healthy controls had the lowest RF burden among the entire NOR-SYS population, RF were frequently present. Of the 10 RF investigated, we found an accumulation of up to four RF among young and up to six RF among middle-aged controls, compared to corresponding five and seven maximum accumulated RF among patients. RF burden was not evaluated for cohort I.

Etiology of stroke and TOAST classification

Observational studies performed during the last decades have shown that the causes of ischemic stroke vary substantially throughout lifetime[271], and that the prognosis after ischemic stroke is associated with the stroke subtype[65].

Both cohorts had a large proportion of SUC in common (42% of cohort I, 37% of cohort II), which is in line with other studies[11, 14, 15, 17, 18, 20, 21, 271, 272], and has been described as a limitation of the TOAST classification[273-275]. The SUC category does not only include patients with true unknown cause of stroke, but also patients with several potential causes, and patients with incomplete diagnostic work-up, resulting in oversizing of the category. We further found agreement between both cohorts concerning the most frequent determined causes: In the younger age groups of cohort I and II, SOC (respective 23% and 22%) and CE (respective 21% and 38%) were most frequent, while CE (respective 29% and 22%) and SAO (respective 15% and 24%) were most frequent in the older age groups.

Our results suggest the following trends for the different determined subtypes of stroke in our study populations:

1. CE, as the largest determined category, is strongly associated with young stroke. Its importance may decrease during mid-age, but finally increases further at older ages. Incident cardioembolic stroke is known to increase with age[271]. However, CE was one of the major subtypes of determined cause also in several young stroke studies[11, 15, 17, 21, 272], and a slight decrease at mid-age has been observed by others, too[14, 272]. This may be explained by the underlying sources of CE, which differ in different age groups. Conditions assumed to be of low or uncertain risk, such as PFO, are a frequently found cause of stroke among young patients, but decrease over time and are rarely documented among the older ones[272, 276]. Inversely, high-risk disorders, such as atrial fibrillation, are rarely documented among the young, but dominate at older ages[276]. Our data support these observations. However, our results may be biased by differing target diagnostics with increased utilization of bubble-test and transesophageal echocardiography in young patients, and increased focus on the detection of cardiac arrhythmias in older patients.

2. SOC, the second large determined category among young patients in our population and other studies[11, 15, 17, 18, 20, 21, 272], contains to a high degree patients with arterial dissection as underlying cause of stroke. In our cohorts, dissections are highly

prevalent before the age of 45 years, decline steeply at mid-age, and are an almost non-existent cause of stroke at older ages. A multinational study recently estimated the mean age to 45.7 years for CAD, and to 41.1 years for VAD[34]. However, other young stroke data support a decline of SOC towards mid-age, although the rates of dissection declined more gently[14, 272].

3. SAO is quite constantly represented at all ages in our population, with a slight temporal increase at mid-age, which may be associated with the observed enlargement of the RF burden with age, particularly of hypertension[277]. This observation is partly supported by recent young stroke data[14], while other investigators have described a constant increase towards mid-age[272], and a further increase at older age[271]. Particularly hypertension is known to be associated with lacunar stroke[277], and microatheroma in intracerebral small arteries, lipohyalinosis and fibrinoid necrosis have been identified as causes of lacunar infarction[278]. However, besides SUC, SAO is the second-most uncertain TOAST category, as neuropathological examinations are required to state SAO with certainty. SAO shares the RF and frequently coexists with LAA[279], which supports its partly atherosclerotic origin. However, it has been discussed that an uncertain proportion of SAO patients in fact may have suffered embolic stroke, such as CE or arterio-arterial embolism, appearing as a single lesion of lacunar size[31, 280-282]. Hence, the SAO category most likely represents a variety of causes.

4. LAA is rare among young stroke patients (3% in cohort I, 2% in cohort II), but increases proportionally with age to 7% at mid-age, and 12% at older ages. This corresponds with other studies[11, 15, 17, 271, 272], while higher proportions already at young age were reported elsewhere[15, 18, 20, 21]. However, TOAST criteria are rigid, and the LAA category is defined arbitrary, requiring the demonstration of an occlusion or stenosis $\geq$ 50% in the artery related to the infarction. In clinical practice, the estimation of severity is mostly based on the evaluation of the degree of stenosis by angiographic methods or by peak velocities in color-coded ultrasonography, and does not include criteria pointing out plaque instability, such as hypoechogenicity[283],

ulceration, thinning of the fibrine capsule or intralesional haemorrhage[284, 285] and neovascularization[286]. Hence, emboligenic unstable atherosclerotic plaques giving a low-grade or moderate degree of stenosis may be frequently overlooked, although there is evidence addressing stroke caused by their kind[176, 213, 287, 288]. True LAA may thus be underestimated in a large number of studies due to arbitrary definition.

Classification systems have important limitations. Phenotypic systems, such as ASCO (Atherothrombosis, Small vessel disease, Cardiac causes and Other uncommon causes), are based on organization of abnormal test findings into several etiological groups without a loss of information. They are valuable, but limited by the fact to assign stroke patients to a vast number of categories, a disadvantage when used in clinical research[273]. On the other hand, causative systems, such as TOAST, aim to sort out the causative mechanism of stroke during a decision-making process by integration of clinical information, test results and RF. This method is limited by important investigator bias regarding the decision-making process and the diagnostic technologies applied[273], and its reliability is only moderate (kappa 0.42-0.54)[289-292]. Still, due to its applicability, TOAST has been used widely in epidemiological and clinical studies without defined requirements regarding the diagnostic extent. TOAST facilitates the investigation of certain populations (e.g. young stroke), the impact of RF or genetic markers, brain imaging and prognosis[11, 14, 65, 271, 291, 293], and its use affects decisions for future treatment.

Short-term outcome

Analysis of cohort I showed that neither stroke severity and characteristics at admittance, nor short-term outcome at day 7 (or at discharge) differed in comparison between young/middle-aged and middle-aged/ old patients, with BI as the only exception. Even though we do not have long-term follow-up data on cohort I, our results indicate that ischemic stroke is a severe event independently from age. Several other studies have led to this conclusion, not only addressing physical functioning, but also evaluating social and psychological effects.

Carotid intima-media thickness

By integration of three statistical methods to compare segmental cIMT (Relative change (RC), mean values, distribution), we found increased values for each carotid segment in the total patient population, compared to controls. This result is to a high degree explained by the similar pattern found among middle-aged patients. It is well-known that cIMT increases with age[294], justifying the difference between young and middle-aged patients, particularly when their respective RF burden is taken in consideration[295]. Further, it is well-known that cIMT increase is associated with incident stroke[185, 198], justifying the differences between middle-aged patients and controls. Additionally, middle-aged patients represent 70% of our patient population, and dominate our total results.

ICA-IMT has previously been found to have a distinct impact on CVD risk prediction, stronger than IMT obtained from BIF or, most commonly performed, from CCA[224]. Although our cohort is relatively small, this assumption may be supported by our data. In our population, ICA was the one segment with reasonable indications for IMT increase among patients compared to controls, not only observed in the total and middle-aged population, but also among female and young participants, and was further associated with prior stroke. We did not find ICA-IMT differences between male patients and controls by any of the three statistical tests, but the unbalanced case-control relation for males (1 : 0.2) does not allow consistent conclusions.

The RC of cIMT between patients and controls was highest among the middle-aged population, again with strong impact on the results of the total population. The carotid segment most prone to IMT increase was the ICA. Although we only found a statistical trend among young patients ($p=0.079$), the approximately 20% ICA-IMT increase compared to controls is assumed clinically relevant, and statistically supported by testing for differences in mean ICA-IMT and ICA-IMT distribution. When the impact of RF on ICA-IMT increase among young participants was analyzed, we found a family history of CVD to be the only associated factor. Our

results may be affected by the relatively small sample size of the young population (n=66). Nevertheless, this may indicate a genetic predisposition, although we did not yet investigate genetic markers and cannot pull further conclusions on this issue. Our results may be most reliable for the well-controlled female population, where we found a 28% RC of ICA-IMT. This was unexpectedly high both compared to a 4% RC in CCA-IMT, and a 9% RC in BIF-IMT, but also compared to rather low and constant segmental RC of 1-8% in all three segments among males, although the male group was least representative. Age was found to be the only factor associated with ICA-IMT increase in females. We did not include hormonal issues or age at menopause in this analysis, but our results may be related to hormonal influences on the development of atherosclerosis[270, 296, 297]. Furthermore, we evaluated the impact of being a patient in analysis of RF-IMT associations. ICA in males was found the only segment in any subgroup, where being a patient mattered. In all other constellations, patients and controls contributed equally. But, again, male controls were insufficiently represented, implying limitations for interpretation.

Mean IMT values were in all patient and control subgroups lowest in CCA and highest in BIF, which correlates with previous ultrasonographic and histological observations[98, 298]. Further, our data met the knowledge of cIMT increase with age and cardiovascular risk[74, 206]. Our values were lower in young than in middle-aged participants. Our control population and the young population had the lowest RF burden, and normal mean cIMT values (< 0.8 mm) were predominantly found in these groups. On the other hand, pathological mean cIMT values (≥ 1.0 mm) were found in BIF in all subgroups but the young. ICA values were pathological in middle-aged patients and males, which we also have identified as the subgroups with the highest RF burden. Mean plaque values (≥ 1.5 mm) were solely found in BIF in middle-aged patients, representing both the carotid segment known to be most prone to atherosclerosis, and the patient subgroup with the highest RF burden. Previous studies have established atherosclerosis to be more associated with male sex[299]. In our data, sex-related cIMT differences were inconsistent, most likely due to the low sample size.

There are several large and renowned population studies evaluating cIMT and its associations with risk factors and incident cardiovascular disease. However, comparison of our cIMT results to those of the other studies is complicated, as study population age, IMT measuring sites (Figure 5), and/ or methods for data analysis partly differ substantially.

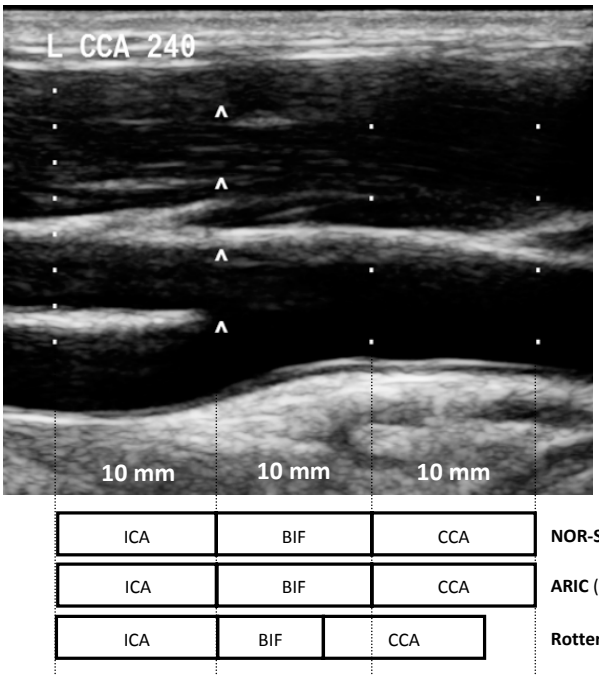

Figure 3:

Definition of multisegmental measuring sites in NOR-SYS, ARIC and the Rotterdam Study

^ Tip of the flow divider

CIMT among young stroke patients aged ≤ 49 years has previously been measured using comparable ultrasonographic methods in a Western Norwegian population as part of a retrospective study with follow-up about 12 years after the index stroke[300]. Unfortunately, acute phase cIMT data do not exist for this population. Our population may be closest comparable to participants of the ARIC study, still those were slightly older, and the young age group is missing (45-64 years, mean age 54 years in ARIC vs. 15-60 years, mean age 49 years in NOR-SYS). In ARIC, IMT was obtained from measuring sites identical with ours, but mean segmental values were analyzed, not

maximum segmental values as in our study. Hence, the values obtained in ARIC are lower (e.g. all-site IMT for CVD free population 0.68 mm in ARIC vs. 0.86 mm in NOR-SYS)[185, 225]. The methods of the Rotterdam Study match relatively well with those used in NOR-SYS, including that maximum IMT values were obtained from each carotid segment and all segments combined, but in the Rotterdam Study near and far wall values were analyzed (Figure 3). The population investigated was mainly aged ≥55 years, and first from 2006 participants aged 45-54 years were added[301]. Even recently published cIMT results have been obtained before 2006, and are thus not directly comparable to ours due to the differing population age [302, 303]. The Tromsø Study results are gathered from a population and by a method differing to a high degree from NOR-SYS. cIMT measurements were obtained from three locations of the right CCA (near and far wall) and BIF (far wall) including plaques when present, and mean IMT was calculated[304, 305]. Alternatively, mean CCA-IMT from three separate recordings was averaged, and measurements including present plaques were excluded[306]. The invited population represented all inhabitants of Tromsø aged 55–74 years and random 5-10% samples of subjects aged 20–54 years and 75–84 years, giving a mean age of about 56 years for both sexes[304, 306]. Maximum far wall CCA-IMT was 1.01 mm among males and 0.92 mm among females of the Tromsø Study[305] vs. (patients/controls) 0.89/0.82 mm among males and 0.75/0.70 mm among females in NOR-SYS. Maximum far wall BIF-IMT was 1.49 mm among males and 1.34 mm among females of the Tromsø Study[305] vs. (patients/controls) 1.39/1.34 mm among males and 1.23/1.04 mm among females in NOR-SYS. However, direct data comparison is not applicable due to differences in methods and population age.

Prevalence of carotid atherosclerosis in TOAST subtypes

As expected, we found highest mean cIMT values and highest cIMT increase compared to controls among LAA patients of all ages[307, 308]. Mean cIMT of all segments combined reached an upper pathological value (1.44 mm) among young LAA patients, and matched our plaque definition among middle-aged LAA patients (1.57 mm).

Second highest values were found among SAO patients (1.11 mm), possibly partly explained by frequent coexistence of LAA and SAO, as shown previously[26, 279]. However, SAO is an imprecise category most likely embedding non-SAO cases of lacunar appearance. It may include embolic causes from cardiac sources, or from proximal unstable atherosclerotic lesions not matching TOAST LAA criteria.

SUC patients had comparably high mean cIMT values as SAO patients (1.10 mm).

cIMT increase to ≥ 1.0 mm was in our analysis defined as pathological based on previous results indicating that cIMT ≥ 1.0 mm is consistent with subclinical arterial disease[268]. Subclinical atherosclerosis is related to future cardiovascular events[184, 185], and this association has been found across a wide age range, including young adults[229]. In our cohort, SAO and SUC patients represent 58% of the total patient population, indicating that a considerable proportion of our patients are suffering from presumed subclinical atherosclerotic disease, in addition to those with progressed atherosclerosis, matching the TOAST criteria for LAA. Middle-aged and male patients contributed most to the pathologic measurements, again confirming the associations between cIMT, age and sex. However, we further suggest that the SUC subtype may include a considerable number of patients with symptomatic atherosclerotic disease, which did not meet the TOAST criterion of ≥ 50% stenosis required for classification as LAA. Additionally, SUC includes patients with more than one equally probable causes of stroke, which LAA may be one of.

Controls´ mean cIMT values were equal to those of SOC patients (both 0.86 mm), and were below those of all other TOAST subtypes, but did not differ statistically from values obtained from CE patients (0.89 mm), SOC patients, or from patients with specified dissection (0.95 mm). We assume that those patients of our population who suffered stroke due to CE, dissection, prothrombotic state or other rare causes do not have relevant co-existing atherosclerotic carotid artery disease.

Comparison of our results to other studies is limited by the low number of available literature. We did not find any publication on investigation of all carotid segments related to stroke subtype. A French study demonstrated higher CCA-IMT in all stroke subtypes (adapted from TOAST) in a population with a mean age of 69 years, compared to controls[307], and IMT increase was highest in stroke with atherothrombotic cause. Both findings are in line with our data derived from all carotid segments. Controls´ mean CCA-IMT was 0.73 mm, identical with our controls´ mean measurements obtained from all segments combined. A Japanese study (mean age 62) demonstrated CCA-IMT increase compared to controls for atherothrombotic and lacunar stroke patients only, but "other determined" and "unknown cause of stroke" had been comprised to one subtype, limiting comparison to our results[308]. In an older Italian population (mean age 68-70 years), CCA-IMT was identified as one of two factors able to discriminate non-lacunar from lacunar stroke, classified by TOAST[309].

Further, a greater plaque burden has been associated to LAA and SAO[310]. Plaque area and plaque echogenicity are more sensitive in evaluation of the atherosclerotic burden and more predictive regarding future cardiovascular events than cIMT[211, 311-313], although both measures are highly correlated[314, 315]. Plaque evaluation may be particularly superior, when IMT values are obtained from CCA alone, where plaques are least common due to hemodynamic reasons[223, 308, 316]. Plaque characteristics are not included in the ultrasonographic examinations applied in NOR-SYS so far. However, a multisegmental approach at the carotid sites most prone to plaque development is considered to improve the evaluation of prevalent atherosclerotic

disease compared to single-segment methods[298, 317]. In NOR-SYS, a multisegmental multi-angle approach is used, and maximum values of cIMT, irregularities and plaques are evaluated. However, plaque characteristics are a field of interest in the Bergen Stroke Research Group, and we aim to develop and implement methods for specific plaque evaluation in our future work.

Impact of the presented data

Decisions concerning future treatment are commonly influenced by the assumed cause of stroke. We assume that the presented results may have an impact on the treating physicians´ sense of responsibility regarding extensive and complete investigation of young stroke patients, with the purpose to thoroughly classify the cause of stroke, including the evaluation of its multiple potential phenotypes. Latent vascular RF or prevalent atherosclerotic disease may potentially coexist with cardiac disorders, small vessel disease, dissections, or other causes of stroke. Optimal, standardized identification and aggressive treatment of modifiable RF and prevalent disease are expected to improve the long-term prognosis of individuals at risk.

Concerning atherosclerotic arterial disease, statin treatment has a strong impact on the disease´s further development as stable or unstable variant[318-320]. Young patients with undetermined cause of ischemic stroke and treated with statins have recently shown a favorable outcome[262]. Further, the treatment effect is most likely best in continuous and long-term use[262, 320]. These observations may be related to undetected prevalent atherosclerotic disease, as identified by the pathologic cIMT values in our SUC patients. Our results lead to the indication for consequent statin treatment not only of the LAA and SAO, but also of the SUC subtype, and in all patients with cIMT increase to $\geq$ 1.0 mm.

Conclusions

The results presented in this thesis add new information to our knowledge about young stroke patients concerning etiology, RF, and the prevalence of clinical and subclinical carotid artery disease. Analysis of our cohorts demonstrated that cardiovascular RF, cIMT and atherosclerosis increase with age, most particularly from mid-age. Further, our data support the conclusions drawn in previous studies regarding the varying impact of different RF on IMT development in the single carotid segments dependent on age and sex. Even though our young stroke population is comparably small, our data support the ICA to be the carotid segment with a distinct association to incident stroke.

We found substantial etiological differences across age groups. Dissection and CE mainly from low-risk sources were the leading determined causes of ischemic stroke at young age, while SAO and CE mainly from high-risk sources were the most common causes from mid-age and throughout lifetime. The constantly high proportion of about 40% undetermined causes of stroke at all ages is a known limitation of the TOAST classification. Besides LAA, particularly SAO and SUC have been identified as the stroke subtypes including a considerable number of patients suffering from atherosclerotic arterial disease in our cohort. Standardized and complete investigation is required to reveal otherwise potentially overlooked RF and subclinical vascular disease, and to identify patients at risk.

Atherosclerosis progresses slowly and mainly undetected over decades. Western Norwegian CVD patients have previously shown a high RF burden, which is associated with the development of atherosclerosis. Among patients ≥ 50 years of our NORSTROKE study population, prior cerebral, cardiac and peripheral vascular disease was frequently established at the time of the index stroke, and may only be slightly modulated further on. However, vascular RF and cIMT increase beyond that of controls were frequently prevalent also among young patients. The young represent

our main target group, for which we expect the strongest effect of life styles changes and interventional treatment on further progression of arterial disease. Opposite to former assumptions, stroke has recently been evidenced to be a severe event also when incident at young age, with serious physical, psychological, cognitive and socio-economic consequences throughout lifetime. Extensive investigation is expected to reveal additional vascular disease to a considerable degree. This, we assume, will have an important impact on further decisions concerning optimal secondary preventive treatment, including stabilization of atherosclerosis. Hence, young patients suspected to suffer from acute vascular disease implicitly need to be investigated extensively and as emergencies, with the purpose to detect prevalent vascular disease and RF at an early stage, and to initiate aggressive appropriate treatment at the first possible occasion. We expect these proceedings to slow the progression of atherosclerotic disease, and to prevent future vascular events and subsequent disability, cognitive decline and death. Unfortunately, rigid scoring systems, as e.g. ABCD2 score, are frequently implemented in acute risk estimation. Young patients with acute CD may easily be overlooked by these, and thereby miss the chance of immediate intervention[321].

Even though CVD-free controls had overall lower cIMT values than patients, subclinical atherosclerosis and vascular RF were prevalent among controls, too. Given the increasing rate of established CVD with increasing age, vascular screening has an impact on revelation of subclinical disease, even at young age, and at early stages. In accordance with recent guidelines, our data emphasize preventive strategies for individuals with signs of atherosclerotic disease at any stage.

Ultrasonography is a precise, highly accessible, rapid, safe, and cost-effective method, which depicts details of arterial pathology with high accuracy. Beyond stenosis with luminal narrowing, otherwise easily detectable by angiographic methods, ultrasonography has a great advantage particularly regarding the identification of morphological characteristics which define unstable, potentially emboligenic lesions. It is further an excellent method to detect subclinical disease, such as external

remodeling of the arterial wall, which usually stays undetected by angiographic methods. Our data demonstrate that utilization of ultrasonography improves diagnostics, reveals otherwise overlooked arterial disease, and leads to improved preventive treatment adapted on individual level.

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Errata

Thesis:

p. 29. An abbreviation error occurred in Figure 1: CAD should be CHD, as stated in the figure legend.

p. 45. An abbreviation error occurred in line 12 and 13. The correct ultrasound system reference is Philips Medical Systems, Bothell, WA, USA (not UWA).

p. 51. The following sentence should be added to the paragraph *Carotid Intima-media thickness* as it has been included and discussed in paper III and in the discussion of the thesis:
Increased ICA-IMT was associated with dyslipidemia (p=0.025) and prior stroke (p=0.009).

Paper IV:

p. 4. An error occurred in the description of *Data reliability tests*. “Kappa” should be replaced by “correlation”.

p. 8. The corrected legend for figure 2 should be:
Figure 2: cIMT distribution in TOAST subtypes and controls, stratified by age group.
A = Internal carotid artery; B = Carotid bifurcation; C = Common carotid artery; IMT = Intima-media thickness; LAA = Large-artery atherosclerosis; CE = Cardiac embolism; SAO = Small artery occlusion; SOC = Stroke of other determined cause; Diss = Dissection; SUC = Stroke of unknown cause; NA = not available; *subgroup of SOC

Paper I-IV

I

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Clinical Study

Comparison between Ischemic Stroke Patients <50 Years and ≥50 Years Admitted to a Single Centre: The Bergen Stroke Study

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Introduction. Young adults are likely to differ from old patients concerning cerebral infarction. *Methods*. We compared characteristics of patients aged under and above 50 years, admitted to the Department of Neurology with cerebral infarction between 2006 and 2009, based on prospective registration. Investigation followed one common protocol for both groups. *Results and Discussion*. One hundred patients (8.2%) were <50 years old, and the proportion of males was higher in this group (72% versus 55.8%, $P = .002$). Young stroke patients are more often current smokers (44.1% versus 23.6%, $P < .001$). Common causes for stroke in the young were cervical artery dissection (18% versus 0.6%, $P < .001$) and cardiac embolism due to disorders other than atrial arrhythmias (18% versus 5.5%, $P < .001$). Among the old, atrial fibrillation and flutter dominated (29.1% versus 5%, $P < .001$). Stroke severity and location did not differ. Old patients more often suffered from pneumonia (10.6% versus 2%, $P < .003$) and urinary tract infection (14.6% versus 2%, $P = .001$). *Conclusions*. Males dominate, and current smoking is more common in the young. Cervical artery dissection and nonarrhythmic heart disorders are frequent causes among young patients, while traditional risk factors dominate the old. Stroke severity is similar, but old patients seem more exposed for infectious complications.

1. Introduction

Cerebral infarction may have serious consequences for patients in their prime of life and influence on choice of education, vocation, and family planning. More knowledge regarding pathophysiological mechanisms and prognosis is urgently needed. Several studies have shown that risk factors and etiology differ between young and old patients. Migraine is frequently reported among young adults [1–5] whereas traditional risk factors such as hypertension and dyslipidemia are usually less frequent. Large-artery atherosclerosis is rare [3, 6] whereas cervical artery dissection is a common cause of cerebral infarction among young adults [2, 4, 6, 7]. Cardioembolic stroke is in the majority of cases caused by cardiac conditions with low to uncertain embolic risk, such as patent foramen ovale and atrial septal aneurysm [4, 8]. Methodological differences may obscure comparison between different centres. There has not been many comparisons between young and old patients treated and investigated in a single centre.

The aim of this study was to compare characteristics of cerebral infarction between young and old patients undergoing treatment and investigations according to one common protocol in a single centre.

2. Methods

2.1. Patients. All consecutive patients with acute cerebral infarction (the index stroke) admitted to the Stroke Unit, Department of Neurology, Haukeland University Hospital, Bergen, Norway, between February 2006 and March 2009, were prospectively registered in a database (The Bergen Stroke Registry). Cerebral infarction was defined in accordance with the Baltimore-Washington Cooperative Young Stroke Study Criteria comprising neurological deficits lasting more than 24 hours because of ischemic lesions or transient

Table 1: Demography of young and old patients with cerebral infarction, based on patient history recorded on admission.

	Young patients ($n = 100$)	Old patients ($n = 1117$)	P
Age (mean)	40.8 (SD 7.6)	73.4 (SD 11.8)	
Females	28 (28.0)	494 (44.2)	.002
Males	72 (72.0)	623 (55.8)	
Married	62 (62.6)	631 (57.8)	.40
Employed	81 (85.3)	236 (22.0)	<.001
Prior cerebral infarction	4 (4.0)	179 (16.2)	<.001
Myocardial infarction	4 (4.0)	155 (13.9)	.003
Angina pectoris	4 (4.0)	160 (14.4)	.002
Mechanic aortic valve	5 (5.0)	21 (1.9)	.05
Peripheral artery disease	3 (3.0)	89 (8.1)	.08
Hypertension	27 (27.0)	598 (53.8)	<.001
Paroxysmal atrial fibrillation	2 (2.0)	104 (9.4)	.009
Chronic atrial fibrillation	0 (0.0)	105 (9.46)	<.001
Diabetes mellitus	10 (10.0)	163 (14.8)	.23
Migraine	14 (17.7)	149 (19.4)	.88
Prior depression	15 (18.3)	185 (22.8)	.41
Current smoking	41 (44.1)	249 (23.6)	<.001
Never smoking	38 (40.9)	439 (41.6)	
Quitted smoking	14 (15.1)	368 (34.9)	

Data are expressed as mean or n (%). SD: standard deviation.

ischemic attacks where CT or MRI showed infarctions related to the clinical findings [9]. The patients were dichotomized into two groups: <50 years (young patients) and ≥50 years (old patients).

All patients had CT or MRI. Isolated acute ischemic lesions on CT or MRI were defined as lacunar infarctions (LI) if <1.5 cm and located as subcortical or in the brainstem. All other acute ischemic lesions were defined as nonlacunar infarction (NLI). NLI comprised subcortical and brainstem infarction ≥1.5 cm, cortical infarction, mixed cortical and subcortical infarction, and cerebellar infarction. Leukoaraiosis was defined as the presence of hypodense periventricular abnormalities on MRI (T2).

The National Institute of Health Stroke Scale (NIHSS) was used to assess stroke severity. NIHSS measurements were performed on admittance and 7 days after stroke onset or earlier if the patient was discharged earlier (NIHSS7). Likewise, modified Rankin Scale (mRS) score and Barthel Index (BI) were obtained 7 days after stroke onset or earlier if the patient was discharged earlier. Blood pressure, body temperature, and serum glucose on admittance were registered. Diagnostic workup included ECG, Holter monitoring, echocardiography, and duplex sonography of neck vessels. Holter monitoring was performed among patients with embolic stroke and no known atrial fibrillation.

Risk factors including hypertension, smoking, diabetes mellitus, myocardial infarction, angina pectoris, peripheral artery disease, and atrial fibrillation were registered on admittance. Hypertension was defined as prior use of antihypertensive medication. Current smoking was defined as smoking at least one cigarette per day. Diabetes mellitus was considered present if the patient was on glucose-lowering diet or medication. Angina pectoris, myocardial infarction, and peripheral artery disease were considered present if diagnosed by a physician any time before stroke onset. Atrial fibrillation required ECG confirmation any time prior to stroke onset. A history of prior cerebral infarction was registered. Old infarctions on CT or MRI were registered, including both clinically silent and symptomatic infarctions. Etiology was determined by the Trial of Org 10172 in Acute Stroke Treatment classification (TOAST) [10], performed by a neurologist (HN). Clinical classification was based on the Oxfordshire Community Stroke Project (OCSP) scale which includes lacunar syndrome (LACS), partial anterior circulation syndrome (PACS), total anterior circulation syndrome (TACS), and posterior circulation syndrome (POCS) [11].

ICA stenosis was defined as a percentage of area reduction in neurosonology, graded from 30–49%, 50–69%, 70–99%, to occlusion (Table 5). Calculation was performed by Phillips software, integrated in IU 22.

Complications including pneumonia, urinary tract infection, and seizures were registered.

2.2. Statistics. Chi-square test, Fisher's exact test, and student's t-test were performed when appropriate. Logistic regression was performed to analyse the effect of the two age groups (young or old patients) on outcome day 7 adjusting for sex

Table 2: Characteristics of cerebral infarction in young and old patients.

	Young patients (n = 100)	Old patients (n = 1117)	P
Classification			
LACS	20 (20.2)	281 (25.2)	.33
TACS	17 (17.2)	184 (16.5)	
PACS	38 (38.4)	458 (41.0)	
POCS	24 (24.2)	193 (17.3)	
Parameters on admission			
Systolic blood pressure (mmHg)	155	168	<.001
Body temperature (centigrade)	36.8	36.6	.47
Serum glucose (mmol/L)	6.5	6.8	.26
Scores			
On admission			
NIHSS	5.7	6.3	.45
At day 7			
NIHSS	4.4	4.9	.50
mRS 0–2	70 (70.0)	677 (60.6)	.11
mRS 3–5	26 (26.0)	408 (36.5)	
mRS 6	4 (4.0)	32 (2.9)	
Barthel Index (mean)	86.9	78.1	.01
Complications			
Nasogastric feeding	6 (6.0)	132 (11.8)	.10
Pneumonia	2 (2.0)	118 (10.6)	.003
Urinary tract infection	2 (2.0)	163 (14.6)	<.001
Seizures	4 (4.0)	40 (3.6)	.78
Etiology			
Large-artery atherosclerosis	3 (3.0)	139 (12.4)	.003
Cardiac embolism	21 (21.0)	328 (29.4)	.08
Small vessel disease	14 (14.0)	170 (15.2)	.88
Other causes	23 (23.0)	10 (0.9)	<.001
Unknown	39 (39.0)	468 (41.9)	.4

Data are expressed as mean or n (%).
NIHSS, The National Institute of Health Stroke Scale; LACS, lacunar stroke syndrome; TACS, total anterior circulation stroke syndrome; PACS, partial anterior circulation stroke syndrome; POCS, posterior circulation stroke syndrome; mRS, modified Rankin Scale.

and NIHSS score on admission. mRS score 0–2 versus 3–6 was used as dependent variable. STATA 11.0 was used for analysis.

3. Results

In total, 1217 patients were included. One hundred (8.2%) were <50 years (range: 18–49 years) and 1117 (91.2%) were ≥50 years (range: 50–98 years). The proportion of males was higher among young patients: 72% versus 55.8% (Table 1).

The following risk factors were more frequent among old patients: myocardial infarction, angina pectoris, hypertension, atrial fibrillation, and prior cerebral infarction. Mechanic aortic valves and current smoking were more frequent among young patients (Table 1).

There was no difference concerning NIHSS score on admittance or OCSP classification. Systolic blood pressure was lower among young patients on admittance: 155 mmHg versus 168 mmHg (Table 2).

Outcome on day 7 (or on discharge if discharged earlier) was similar regarding mRS score and NIHSS score, whereas mean Barthel Index was higher among young patients: 86.9 versus 78.1. Figure 1 shows mRS scores according to age. The mortality rates did not differ significantly on day 7, respectively, on discharge (P = .5). Logistic regression showed that mRS score 0–2 versus 3–6 was associated with NIHSS score on admittance (odds ratio (OR) 1.29 (95% confidence interval (CI) 1.25–1.34), P < .001), but not with sex (OR .76 (95%CI .57–1.01), P = .064) or young versus old patients (OR .69 (95%CI .40–1.20), P = .19). Subanalysis for patients >45 years and <45 years, traditionally regarded as "young" in stroke literature, did not change the results concerning stroke severity on admission (NIHSS): 6.9 in the young versus 6.2 in the old group, P = .6, neither was there a difference regarding short-term outcome at day 7: mRS 2.3 versus 2.3, P = .81.

Pneumonia and urinary tract infections were less frequent among young patients. Seizures were seen in about 4% in both groups (Table 2).

Cardiac embolism was found in 21% of the young patients versus 29.4% of the old patients and included most frequently in the young with patent foramen ovale (in 2 cases combined with atrial septal aneurysm), mechanical heart valve and paroxysmal atrial fibrillation, or combinations

Table 3: Heart disorders associated with cardiac embolism.

	Young patients ($n = 21$)	Old patients ($n = 328$)	P
Patent foramen ovale	4	9	—
Patent foramen ovale and atrial septal aneurysm	2	0	—
Patent foramen ovale and paroxysmal atrial fibrillation	1	0	—
Atrial fibrillation (paroxysmal and chronic)	3	261	<.01
Atrial flutter	0	6	.54
Atrial septal defect	1	0	—
Atrial septal defect and paroxysmal atrial fibrillation	1	0	—
Atrial septal aneurysm	0	2	—
Ventricular septal defect	1	0	—
Anterior myocardial infarction/akinesia	2	6	—
Heart valve dysfunction	0	15	—
Mechanical heart valve	4	10	—
Mechanical heart valve and prothrombotic disorder	1	0	—
Ventricular thrombus	0	2	—
Papillary fibroelastoma	1	0	—
Cardiomyopathy	0	2	—
Severe heart failure	0	3	.66
Other	0	12	—
Cardiac embolism due to atrial fibrillation/atrial flutter*	5 (5)	267 (29.1)	<.001
Cardiac embolism due to disorders other than atrial fibrillation/atrial flutter*	18 (18)	61 (5.5)	<.001

P value is given only for diagnoses where equal investigation methods were used for both groups.
*in relation to all 100 young and 1117 old patients included in the study.

Table 4: Other causes of cerebral infarction.

	Young patients ($n = 23$)	Old patients ($n = 10$)	P
Cervical artery dissection	18	7	<.001
Giant aneurysm MCA	1	0	.001
Pseudoaneurysm ICA	1	0	.001
Moya moya	1	0	.001
Prothrombotic disorder	1	1	.03
Pulmonary shunt	1	0	.001
Migraine	0	1	.76
CADASIL	0	1	.76

MCA, middle cerebral artery; ICA, internal carotid artery.

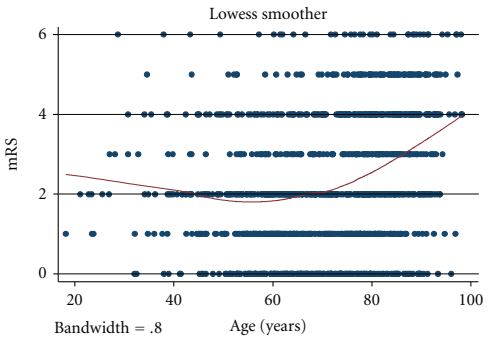

Figure 1: mRS scores, at day 7 or at discharge (if before 7 days), among patients with cerebral infarction according to age. Solid line shows mean mRS. mRS, modified Rankin Scale.

of these conditions. Other causes were found in 23% of young patients versus 0.9% of the old patients, and cervical artery dissection was the most frequent one (18%). More rare conditions included pseudoaneurysm of the ICA, giant aneurysm of the MCA, prothrombotic disorders, and Moya moya. Large-artery atherosclerosis was less frequent among young patients: 3% versus 12.4% (Tables 2, 3, and 4).

The frequency of atrial fibrillation on ECG on admittance was low among young patients compared to old patients: 2.4% versus 17.0%. Likewise the frequency of atrial fibrillation disclosed on Holter monitoring was low among young patients: 1.8% versus 17.7% (Table 5).

Based on MRI findings, there were no differences concerning location of cerebral infarction. Fewer young patients showed leukoaraiosis (7.8% versus 50.4%) or had sequels after old infarctions on MRI (10% versus 21.3%) (Table 6).

4. Discussion

The proportion of males was larger among the young patients than among the old patients. The proportion of males was also higher compared to other studies of cerebral infarction among young adults [7, 12]. Accumulation of traditional risk factors probably starts earlier in males than in females. Women have a longer life expectancy, which may play a role for the relatively larger proportion of female stroke patients in the older group. On the other hand, it is possible that a change in risk factors or life style has reduced the

Table 5: Investigations.

	Young patients ($n = 100$)	Old patients ($n = 1117$)	P
ECG on admission			
Total	82 (82)	1057 (94.6)	
Atrial fibrillation	2 (2.4)	181 (17.1)	<.001
Left bundle branch block	0 (0)	38 (3.6)	.11
Left ventricle hypertrophy	6 (7.3)	73 (6.9)	.82
Unspecific ST depression	7 (8.5)	232 (21.9)	.003
Acute anterior myocardial infarction	0 (0)	3 (.3)	1.00
Old anterior myocardial infarction	2 (2.4)	52 (4.9)	.42
Acute inferior myocardial infarction	0 (0)	2 (.2)	1.00
Old inferior myocardial infarction	2 (2.4)	59 (5.6)	.31
Echocardiography			
Total	63 (63)	357 (32.0)	
TTE	28 (44.4)	284 (79.6)	
TEE	35 (55.6)	73 (20.4)	
Left ventricle hypertrophy	7 (11.1)	119 (33.3)	<.001
Patent foramen ovale	10 (15.9)	14 (3.9)	.001
Sequelae anterior myocardial infarction	2 (3.2)	19 (5.3)	.75
Sequelae inferior myocardial infarction	0 (0)	16 (4.5)	.15
Holter monitoring			
Total	57 (57)	434 (38.9)	
Paroxysmal atrial fibrillation	1 (1.8)	78 (18.0)	.001
Duplex of cervical arteries			
Total	86 (86)	893 (79.9)	
ICA stenosis[1]	11 (12.8)	356 (39.9)	.000
Symptomatic ICA stenosis ≤49%[1]*	0 (0)	83 (13.9)	.002
Symptomatic ICA stenosis 50–69%[1]*	0 (0)	55 (9.2)	
Symptomatic ICA stenosis 70%–99%[1]*	2 (3.9)	34 (5.7)	
Symptomatic occlusion[1]*	5 (9.8)	29 (4.9)	
No ICA stenosis[1]*	44 (86.3)	397 (66.4)	

Data are expressed as mean or n (%).
ECG, electrocardiography; ICA, internal carotid artery.
[1]Area reduction measured by neurosonology.
*Among patients with ipsilateral infarction in the middle cerebral artery territory.

frequency of stroke among young females in recent years. Smoking has decreased among young women [13], and there has been a change regarding the use of oral contraceptives [14]. Another possible reason is better diagnostic methods of cerebral infarction because of high use of DWI. Psychogenic neurological symptoms are, for example, more frequent among females [15, 16] and may sometimes be mistaken for stroke but are easily distinguishable by DWI. Other studies showed migraine as a cause of stroke in up to 20% in the early 1990s [17], while newer studies find this in only few patients [4, 7, 18–21]. Complex migraine might have been misdiagnosed as cerebral infarction in the pre-DWI era. It is unlikely that this mistake was performed in this study because there was no difference regarding the frequency of migraine among young and old patients. The diagnosis of migraine was based on an interview by a neurologist during the hospital stay strengthening our findings. Thus, our result indicates that migraine is not particularly related to cerebral infarction among young patients compared to old patients.

Most traditional risk factors were less frequent among young patients. However, the fact of smoking made an exception. It has previously been shown that smoking is more frequent among young patients with cerebral infarction compared to matched controls [6]. In our study, the proportion of current smoking was clearly higher among the young compared to the old, and the proportion of

Table 6: MRI findings among young and old patients with cerebral infarction.

	Young patients	Old patients	P
MRI	89 (89)	848 (76.0)	.003
DWI positive	84 (93.3)	815 (96.7)	.13
Anterior circulation	68 (68)	812 (72.7)	.35
Posterior circulation	30 (30)	297 (26.6)	.48
Middle cerebral artery	66 (66)	790 (70.7)	.36
Anterior cerebral artery	3 (3)	37 (3.3)	1.00
Occipital	8 (8)	102 (9.1)	.86
Thalamus	3 (3)	79 (7.1)	.15
Mesencephalon	3 (3)	20 (1.8)	.43
Pons	2 (2)	64 (5.7)	.16
Medulla oblongata	5 (5)	24 (2.2)	.08
Cerebellum	11 (11)	87 (7.8)	.25
More than one artery domain	6 (6)	57 (5.1)	.54
Anterior and posterior circulation	2 (2)	35 (3.1)	.83
Bilateral middle cerebral arteries	4 (4)	22 (2)	.19
Leukoaraiosis (MRI)	7 (7.8)	424 (50.4)	<.001
Old infarctions (MRI)*	10 (10)	238 (21.3)	.006
Embolic infarction (MRI)	66 (79)	594 (73)	.30
Lacunar infarction (MRI)	18 (21)	223 (27)	.25

Data are expressed as mean or n (%).
MRI, magnetic resonance imaging; DWI, diffusion-weighted imaging.
*Including both silent and symptomatic infarctions.

past smoking was lower in the young patients group. The frequency of diabetes mellitus did not differ between young and old ischemic stroke patients.

Large-artery atherosclerosis was a rare cause of cerebral infarction among the young patients. Its frequency was also lower than among young patients with cerebral infarction in previous studies [6, 7]. This may indicate that symptomatic atherosclerosis has decreased among young people in recent years.

There was no difference concerning small vessel disease among young and old patients, and the frequency was similar to the findings in other studies of cerebral infarction among young adults [6, 7]. This is perhaps surprising because there is much uncertainty regarding the pathophysiological mechanisms of lacunar infarctions [22–24].

The frequency of cardiac embolism was similar between young and old patients (Table 2), and the proportion of cardiac embolism in the young is in line with other findings [3, 7, 19, 20, 25]. However, the specific cardiac sources differed between young and old patients. Atrial fibrillation was the dominating cardiac source among old patients but infrequent among young adults. In young adults the dominating heart disorders were patent foramen ovale with and without atrial septal aneurysm, followed by mechanical heart valves. This matches with the findings in other studies [7, 19], but mechanical heart valves were more frequently found as the cause of infarction in our study.

The proportion of other causes did not differ from most investigations [3, 4, 6, 7, 18, 21, 26]. Cervical artery dissection was with 18% the most common other cause among the young patients. Dissections were mostly located in unilateral ICA, less frequently in unilateral VA, and in a few cases in bilateral ICA.

Neither proportion of patients with unknown etiology was different from other studies, which is 31–62% in young patients [3, 6, 20, 27] and 35% in stroke patients overall in this category [26].

The distribution of infarctions in the anterior and posterior circulation was similar between young and old patients. The frequency of posterior circulation infarction was lower than in some other studies including young patients [7, 12]. We believe that this reflects better diagnostic precision in this study because most patients underwent DWI. Frequent MRI may also explain that we found a higher frequency of leukoaraiosis in old patients compared to recent studies [7, 12]. In our study, 7.8% among the young versus 50.4% among the old patients had leukoaraiosis. Old infarctions on MRI were found in 10% of the young patients versus 21.3% of the old ones. Multiple infarctions were common but less frequently seen in our study compared to recent publications [7, 12], and there was no difference between young and old patients.

There was no difference with respect to severity of neurological deficits on admittance between young and old patients. There was also small difference in the one-week outcome or mortality at day 7. Only Barthel Index was significantly higher among young patients whereas modified Rankin score or NIHSS score did not differ, neither was

there any difference concerning the one-week improvement among young and old patients on multivariate analyses. This may indicate that young adults in our investigation do not tackle cerebral ischemia better than old patients concerning short-term outcome, which is in contrast to recent observation made by a Swiss group [28]. Differences in methodology (e.g., stroke unit cohort versus population-based study) may account for this discrepancy. However, subanalyses suggested that patients >80 years may experience less improvement than patients <80 years (analysis not shown).

This is one of the largest studies making a hospital-based direct comparison between ischemic stroke patients <50 years and ≥50 years admitted to a single centre, which we consider to be one of its strengths. All patients underwent investigations and treatment according to one common protocol. Another strength was the frequent use of MRI which promotes high diagnostic precision. However, there are some limitations; using the Baltimore-Washington Cooperative Young Stroke Study Criteria may complicate comparison with other studies using other criteria such as the WHO criteria. However, specificity is high in our study due to the frequent use of MRI. As described in Section 2, certain risk factors were registered as present when diagnosed before stroke onset. We might have missed some patients with untreated hypertension, atrial fibrillation and diabetes here, especially in the young patient group. We did not register outcome at 3 months, which gives an incomplete impression about the patients' outcome in the different groups. Young patients may improve more in long-term outcome compared to old patients. Although investigations were thorough in most patients, not all patients underwent complete workup. We might have missed few patients with, for example, atrial fibrillation or carotid stenosis due to that fact.

In conclusion, there are important differences between young and old patients with respect to risk factors, etiology, and distribution of gender. However, severity of stroke on admittance and short-term outcome is similar among young and old patients.

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STUDY PROTOCOL Open Access

The Norwegian Stroke in the Young Study (NOR-SYS): Rationale and design

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Abstract

Background: Ischemic stroke in young adults is a major health problem being associated with a higher vascular morbidity and mortality compared to controls, and a stroke recurrence rate of 25% during the first decade. The assumed cause of infarction and the detected risk factors determine the early- and long-term treatment. However, for many patients the cause of stroke remains unknown. Risk factor profile and etiology differ in young and elderly ischemic stroke patients, and atherosclerosis is the determined underlying condition in 10 to 15%. However, subclinical atherosclerosis is probably more prevalent and may go unrecognized.
Ultrasound imaging is a sensitive method for the detection of arterial disease and for measurement of adipose tissue. The relationship between intima-media thickness (IMT), plaques, cardiovascular risk factors including visceral adipose tissue (VAT) and ischemic events has repeatedly been shown.
We have established The Norwegian Stroke in the Young Study (NOR-SYS) as a three-generation research program with the goal to increase our knowledge on heredity and the development of arterial disease and ischemic stroke. Extended standardized ultrasound examinations are done in order to find subclinical vessel disease for early and better prophylaxis.

Methods/Design: NOR-SYS is a prospective long-term research program. Standardized methods are used for anamnestic, clinical, laboratory, imaging, and ultrasound data collection in ischemic stroke patients aged ≤60 years, their partners and joint adult offspring. The ultrasound protocol includes the assessment of intracranial, carotid and femoral arteries, abdominal aorta, and the estimation of VAT. To date, the study is a single centre study with approximately 400 patients, 250 partners and 350 adult offspring expected to be recruited at our site.

Discussion: NOR-SYS aims to increase our knowledge about heredity and the development of arterial vascular disease in young patients with ischemic stroke and their families. Moreover, optimization of diagnostics, prophylaxis and early intervention are major targets with the intention to reduce stroke recurrence and other clinical arterial events, physical disability, cognitive impairment and death.
NOR-SYS is reviewed and approved by the Regional Committee for Medical and Health Research Ethics, Western-Norway (REK-Vest 2010/74), and registered in ClinicalTrials.gov: NCT01597453.

Keywords: Ischemic stroke, Stroke in the young, Atherosclerosis, Arterial disease, Ultrasound, Heredity, Vascular risk, Long-term outcome, Mortality

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Background

Cerebrovascular and coronary artery disease are the main causes of disability and death in the western world [1]. According to observational studies where TOAST criteria have been used, atherosclerosis is the underlying condition in 10 to 15% of patients with ischemic cerebrovascular events of determined etiology [2]. However, in 30-40% of cases the cause of stroke remains unknown [3]. Risk factor profile and etiology differ in young ischemic stroke patients compared to the elderly [3-5]. In addition, young patients have a higher vascular morbidity and mortality compared to healthy controls [6-9], and recurrent ischemic events are common [10,11]. Further, a significant portion of ischemic stroke patients have unrecognized atherosclerosis not only located to cervical arteries, but as well to intracranial arteries [12], to coronary arteries [13], to the aortic arch [14] and to femoral arteries [15]. As therapeutic options are limited, primary and secondary prophylaxis of atherosclerosis and generalized arterial disease should be a major target with the purpose to reduce long-term disability and death among young stroke patients.

Ultrasound imaging is a sensitive, non-invasive, and low-cost method for the detection of arterial vessel disease in major arteries [16,17]. The measurement of carotid intima-media thickness (cIMT) and plaques in B-mode ultrasound has become a tool for vascular risk prediction, as the relationship between IMT, plaques, cardiovascular risk factors and future ischemic events has consequently been shown in several longitudinal studies, predominantly in older individuals [17-27]. However, the value of IMT measurements in all carotid artery segments compared with measurements in the distal CCA alone is disputable [28], and a recent meta-analysis concluded that cIMT measurements in the CCA alone adds little to the improvement of a 10-year risk prediction [29].

NOR-SYS is a concept for the standardized gathering of anamnestic, clinical and biological data in young ischemic stroke patients, their partners, and their family members. The intention is to estimate the presence of arterial vessel disease, to determine the individual's vascular risk profile, and to offer optimal prevention.

Inclusion of the patients' partners and joint adult offspring is providing a platform for primary vascular prevention and early intervention. Stroke is a result of multifactorial causes with genetic, environmental and life-style components [30]. The combination of a standardized case-history, standardized ultrasound protocols, and a prospective long-term follow-up schedule is expected to give knowledge regarding heredity and vascular co-morbidity. The optimal goal and the major purpose of the study is to reduce vascular morbidity, disability, cognitive impairment and mortality in young ischemic stroke patients.

Methods and design

NOR-SYS is intended to be a national multicenter study, performed by co-operating neurological departments in Norway. The study was initiated at Haukeland University Hospital, Bergen, in September 2010. The inclusion period will be 5 years. NOR-SYS is designed as a three-generation study with prospective long-term follow-up design. In addition to a routine cerebro-cardiovascular work-up including clinical examination, neuroimaging, cardiac investigations, and laboratory analyses, all participating patients and relatives are investigated according to the NOR-SYS protocol (Figure 1). This includes questionnaires regarding vascular disease burden in the family, the patient's medical history and life styles. In addition, all patients are examined by transcranial, extracranial, abdominal and peripheral ultrasound, arterial stiffness measurements, and 24 hour blood pressure monitoring. Participants with undocumented but suspected coronary and/or peripheral arterial disease are referred to the Department of Cardiology and the Department of Vascular Surgery, respectively, for further appropriate investigations, including cardiac computertomography-angiography (CCTA) and CT of the thoracic aorta.

Anthropometric variables, such as height, weight, and waist-hip ratio are measured and EDTA-blood and serum samples are collected to a biobank. The patients' partners and biological offspring aged ≥18 years are being offered investigations as shown in Figure 1. Data on medical history and life styles are collected from the patients' biological parents, partners, and offspring by standardized questionnaires. For deceased first-degree relatives, the patient will be asked about their cardio-vascular clinical events and the achieved information will be verified by medical records and data from the Norwegian Cause of Death Registry.

NOR-SYS will be carried out in two phases. In the first, cross-sectional phase, a comprehensive stroke data base on vascular risk factors, arterial ischemic events, and clinical and subclinical atherosclerotic disease burden in the study population is being established.

The second, longitudinal phase will constitute long-term follow-ups, at 5, 10 and 15 years from the time of inclusion for patients and their partners, and at 10 and 20 years from time of inclusion for offspring. The purpose of the follow-up is to observe the biological development of atherosclerosis and vascular disease over time, and to optimize primary and secondary medical prophylaxis. The complete work-up is shown in Figure 1.

Subject selection

Study participation is offered to all patients with Norwegian residency aged 15 to 60 years, with radiologically documented acute cerebral infarction. All study participation is based on informed written consent. Patients of non-

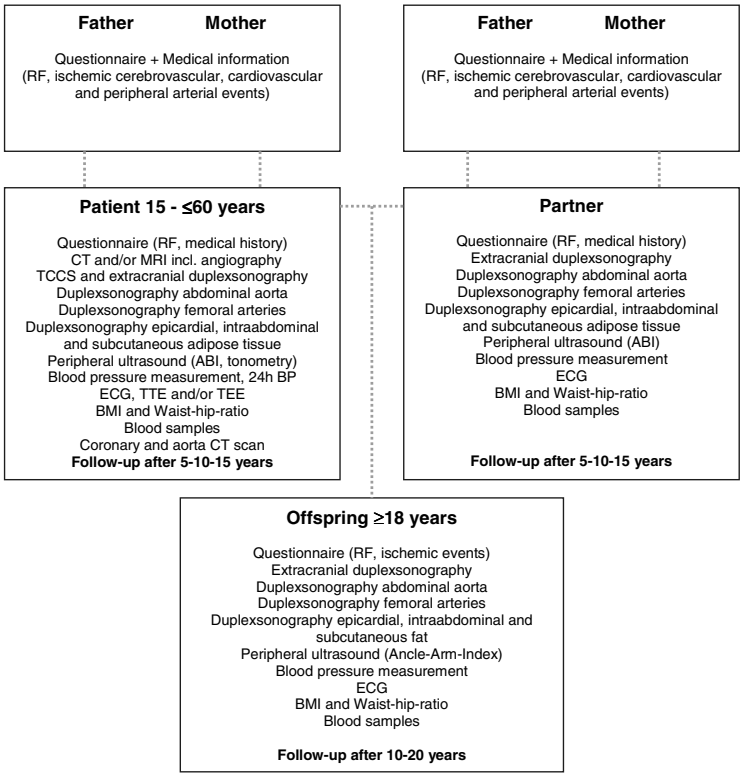

Figure 1 NOR-SYS Assessment chart. RF = Risk factors; CT = Computed tomography; MRI = Magnetic resonance imaging; TCCS = Transcranial color-coded sonography; ABI = Ankle-Brachial Index; ECG = Electrocardiogram; TTE = Transthoracic echocardiography, TEE = Transesophageal echocardiography; 24 h BP = 24 hour blood pressure monitoring; BMI = Body Mass Index.

western European ethnicity are investigated in agreement with the NOR-SYS protocol, but are not included in statistical study analyses. Patients with ischemic stroke due to a traumatic cause or subarachnoidal bleeding are excluded from study participation. Spouses and partners of included patients are offered participation as control persons and as reference persons to participating joint offspring. Parents of patients and partners are invited to return standardized questionnaires. All participants are asked for permission to review their relevant medical records from hospitals, specialists or general practitioners for verification.

Baseline procedures at study inclusion

A. Oral and written study information and signing of informed written consent
B. Anamnestic data collection by standardized questionnaires, including socio-demographic variables, history of previous vascular disease, history of vascular risk factors, data on life styles and nutrition habits, history of other disease, allergies, recent infections, current medication, and circumstances around stroke onset.
C. Neurosonology and Duplex/Doppler ultrasound examinations.
Duplex sonography studies are performed using a iU22 Philips Medical Systems, Bothell, WA, USA. Neurosonologists are trained and certified by the most experienced neurosonologists at Haukeland University Hospital (LT and UWA) in collaboration with the University Medical Centre of Utrecht (RM), The Netherlands. Intra- and interobserver variability investigations are performed. Continuous ECG monitoring during the ultrasound examination is done in order to perform standardized carotid and femoral IMT-measurements in the enddiastolic phase of the

cardiac cycle. Except for cardiac ultrasound examination, the subject is being placed in supine position.

a) *Transcranial color-coded sonography (TCCS).* The intracranial arteries are systematically assessed following the protocol established by Logallo et al. [31]. A 5–1 MHz sector array probe (iU22 Philips Medical Systems, Bothell, WA, USA) is used for bilateral insonation of the sphenoidal segment (M1) and the insular segments (M2) of the middle cerebral arteries (MCA) in the axial planes. Peak systolic velocity (PSV) is measured from M1 origin to distal M2 segments with a 2 mm sample volume, by stepwise depth decrement, and stepwise optimal angle correction of Doppler sampling.

b) *Carotid artery ultrasonography.* The carotid arteries are examined by use of a 9–3 MHz linear array transducer (iU22 Philips Medical Systems, Bothell, WA, USA).

b1) Overview, stenoses, hemodynamics and velocities: For an orientating overview, initial B-mode scans in transversal and longitudinal plane are performed to visualize the common carotid artery (CCA), carotid bifurcation (BIF), and internal carotid artery (ICA). Segments of interest are stored as frozen images, or as video loop. Observation of carotid atherosclerotic plaques, stenosis, occlusion, dissection or fibromuscular dysplasia are noted. In case of stenosis, according to the international consensus statement [32], geometric lumen reduction is assessed by calculation of area reduction in the cross-sectional plane, using the combination of B-mode and color flow. Measurement of area reduction is considered independent from morphological configurations of the stenosis. Further, color and power Doppler modes are used for evaluation of hemodynamic effects in longitudinal plane, such as orthograde or retrograde flow, color aliasing phenomenon or turbulence. Blood flow velocities are measured using Pulsed-Wave (PW) Doppler under optimal angle correction. The maximum peak systolic velocity (PSV) is noted for the distal CCA, for the carotid bifurcation under visualization of the proximal ICA, and for the proximal ICA, of which frozen pictures are stored.

b2) IMT-measurements: Vertical markers in a horizontal distance of 10 mm each are used to define the distal CCA, the bifurcation (BIF), and the proximal ICA segment in longitudinal view, using the tip of the flow divider (TFD) as internal landmark for placement of the second vertical marker (Figure 2). The CCA segment is defined 20–10 mm proximally to the TFD, the BIF segment is defined as 10–0 mm proximally to the TFD, and the ICA segment is defined as 0–10 mm distally to the TFD. Intima-media thickness (IMT) is visualized in longitudinal view on the far and, if possible, on the near wall of each segment, to ensure center position of the scan plane in the artery. Meijer's Carotid Arc® (Figure 3) is used for standardization of the scan angles, performed at 180°, 150°, 120° and 90° in the right CCA segment, and at 180°, 210°, 240°, and 270° in the left CCA segment. IMT-measurements in BIF and ICA segments are bilaterally performed at the angle representing the most significant pathological finding, respectively. All measurements are performed in the enddiastolic phase of the cardiac cycle. Frozen pictures are stored for each measurement. IMT-analysis is performed by Philips QLAB-software after completed examination. IMT measurements are done over a distance of 10 mm for each far wall segment (Figure 2), and are stored as a mean value. In case of irregular IMT or presence of plaques, measurements of the maximum IMT or plaque thickness are additionally performed. Due to updated Mannheim Carotid Intima-Media Thickness Consensus criteria, plaques are defined as focal IMT measurements >1.5 mm [33]. Plaque surface is being evaluated as smooth, irregular or ulcerated.

c *Ultrasonographic epicardial adipose tissue (EAT).* EAT measurements are performed by use of a 5–1 MHz sector array transducer (iU22 Philips Medical Systems, Bothell, WA, USA). The subject is placed in left lateral decubitus position. Epicardial fat thickness is measured on the free wall of the right ventricle from parasternal short-axis view during end-systole. EAT is defined as the echo-free space between the outer layer of the myocardial wall and the visceral layer of the pericardium [34,35] (Figure 4). The mean of three maximum value measurements is calculated.

d *Ultrasonographic visceral abdominal adipose tissue (VAT).* VAT measurements are performed by use of a 5–1 MHz curved array transducer (iU22 Philips Medical Systems, Bothell, WA, USA). All measurements are performed in longitudinal view on umbilicus level, and the distance between the

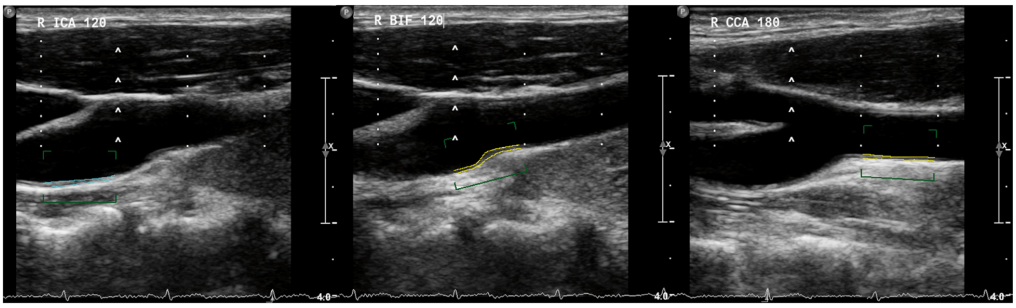

Figure 2 Ultrasonographic IMT measurement in the proximal ICA (left), the bifurcation (middle) and the distal CCA segment (right) by QLAB software.

external face of the rectus abdominis muscle/the peritoneum and the lumbar spine is used [36] (Figure 4). All measurements are performed at the end of expiration and without distortion of the abdominal cavity due to compression. The vertebral column is positioned horizontally. VAT is measured in frontal median position, 10 cm laterally to the left and 10 cm laterally to the right for the median position, and the mean value of these three measurements is calculated.

e *Ultrasonographic subcutaneous abdominal adipose tissue (SAT).*
SAT measurement is performed by use of a 9–3 MHz linear array transducer (iU22 Philips Medical Systems, Bothell, WA, USA).The transducer is positioned transverse 1 cm above the umbilicus. SAT is defined as the distance between the cutis and the external face of the rectus abdominis muscle tendon plate (linea alba) (Figure 4), and is measured under maximum decompression of the skin.

f *Abdominal aorta ultrasonography.*
The abdominal aorta is examined by use of a 5–1 MHz curved array transducer (iU22 Philips Medical Systems, Bothell, WA, USA). Infrarenal vessel lumen is measured in longitudinal view, and external diameter measurement is repeated in transversal view. Infrarenal diameter >30 mm is suspect for aneurysm and considered for additional vascular surgical investigations [37]. Hemodynamically significant stenosis is

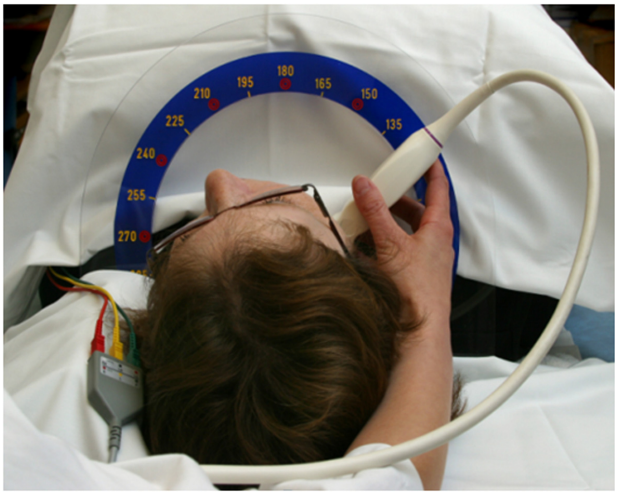

Figure 3 Meijer's Carotid Arc® (publication with written informed consent by the patient).

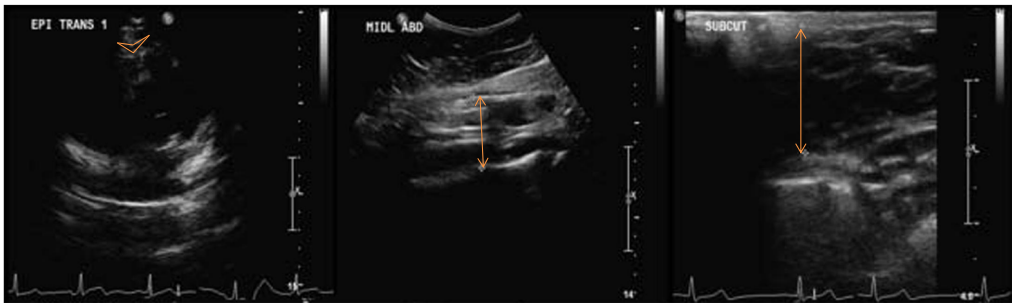

Figure 4 Ultrasonographic epicardial (left), intraabominal visceral (middle) and abdominal subcutaneous adipose tissue (right) in B-mode ultrasonography.

assumed when PSV is ≥ 200 cm/s. Severity of atherosclerotic lesions is evaluated.

g *Femoral artery ultrasonography*.
The femoral arteries are examined by use of a 9–3 MHz linear array transducer (iU22 Philips Medical Systems, Bothell, WA, USA). Transversal view is used for identification of the common femoral artery (CFA) and localisation of the femoral artery bifurcation. Femoral IMT (fIMT) measurements are bilaterally performed in longitudinal view over a distance of 10 mm in the distal CFA (Figure 5) and in the proximal 10 mm of the superficial femoral artery (SFA), respectively (Figure 5). Frozen pictures are stored for each measurement. As for cIMT, fIMT is analyzed by Philips QLAB-software after completed examination, and performed over a distance of 10 mm for each far wall segment and stored as a mean value. In case of irregular IMT or presence of plaques, measurements of the maximum IMT and plaque thickness are additionally done.

h *Ankle Brachial Index (ABI).*
ABI is measured after a resting period of at least 5 to 10 minutes by Ultrasonic Doppler Flow Detector, Model 811-BTS, Parks Medical Electronics, Inc., Aloha, OR, USA. Bloodpressure measurements are performed bilaterally in the radial, the dorsalis pedis, and the posterior tibial artery. ABI ≤ 0.9 at rest is defined as the cut-off point for peripheral artery disease (PAD). ABI 0.7-0.9 is considered as mild, 0.4-0.7 as moderate and <0.4 as severe arterial disease. ABI >1.4 may be explained by medial sclerosis or other conditions leading to arterial incompressibility [38]. Suspect subjects and participants with known diabetes mellitus are reported to the respective departments of vascular surgery for further investigation.

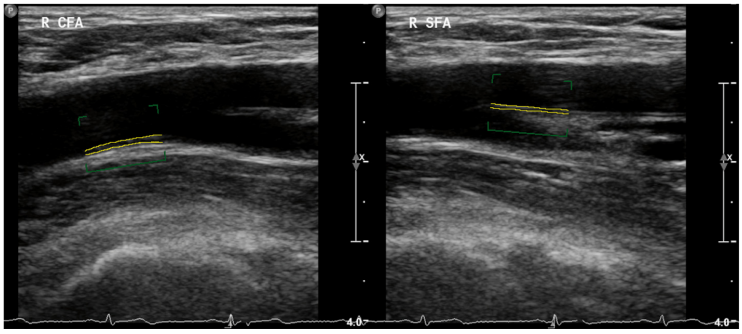

Figure 5 B-mode ultrasonography and IMT measurement in the distal CFA (left) and the proximal SFA segment (right) performed by QLAB software.

D Anthropometric variables, electrocardiography (ECG), and blood pressure measurements.
Current height and weight are measured and Body mass index (BMI) is calculated as indicator for nutrition and body fat. Waist-hip ratio (WHR) is calculated from the respective circumference measurements. Systolic and diastolic blood pressures are measured bilaterally in the subject's upper arm after a resting period. ECG is performed in all actively participating subjects.

E Neuroradiology (patients only).
Routine initial neuroimaging includes a cerebral CT scan and CT-angiography (CTA), and is performed in all patients with acute stroke symptoms at hospital admission. Magnetic resonance imaging (MRI) may be the method of first choice in some patients. In absence of contraindications, MRI including axial FLAIR, MR-angiography (MRA), diffusion-weighted imaging (DWI) and susceptibility weighted imaging (SWI) is performed within 24 hours after admission.
Intra- and extracranial artery stenoses are described as minor (≤50%), moderate (51-70%) and severe (71-99% maximum actual area reduction (AAR)) or occlusion.

F Arterial stiffness measured by aplanation tonometry (patients only).
Carotid-femoral pulse wave velocity is measured using aplanation tonometry (SpygmoCor, AtCor medical, West Ryde, Australia) operated by a trained technician following a standardized program with inborn quality control assessment. Pulse waves from the carotid and femoral arteries are obtained with the tonometer and the pulse wave velocity is calculated taking the distance between the two measure points into account. From the carotid pulse wave, central (aortic) blood pressure is estimated.

G Ambulatory blood pressure monitoring.
Twenty-four hour ambulatory blood pressure measurement is performed using a non-invasive ambulatory blood pressure monitor Diasys Integra II (Novacor, Cedex, France), set to auscultatory mode. The device is mounted on the non-dominant arm with an appropriately sized cuff, and the patients are instructed to relax their arm when readings are initiated. Blood pressure is pre-set to be measured every 20 minutes during daytime and every 30 minutes during night-time, giving an average of 78 measurements per 24 hours. Daytime is defined as the fixed period between 7 a.m. and 22 p.m.. The recording is accepted when > 70% of the measurements are technically valid, and otherwise will be repeated.

H CCTA including CT of the thoracic aorta (patients only).
CCTA and CT of the thoracic aorta is performed in those patients found to have plaques in the femoral arteries and/or pathological ABI. For ECG-triggered CT-scanning, a Siemens dual FLASH scanner (Siemens Somatom Definition FLASH; Erlangen, Germany) is applied. Due to administration of I-contrast agents, patients with reduced glomerular filtration rate (GFR < 30 mL/min/1.73 m^2) are excluded. Calcium scoring of coronary arteries is assessed before administration of intravenous contrast, whereas, the lumen and wall of the coronary arteries as well as the occurrence of aorta pathology is evaluated after intravenous contrast administration.

I Study of biomarkers and genetic analyses.
Samples of serum and EDTA-plasma are collected, processed, coded and stored at −80°C until analyzation for each participating subject. Analyses are scheduled after completion of the 5-years inclusion period. Biomarkers to be investigated will be determined at the time of analyses according to the most relevant biomarkers known at that time point. GWAS, exone sequencing or any newer technology, relevant and feasible at the time of genetic analyses, will be applied.

Primary and secondary prevention strategies

Additionally to stroke treatment and secondary prevention in the patient population, all subjects are being evaluated concerning the presence and severity of established clinical and sub-clinical cardiovascular disease and modifiable vascular risk factors. An evaluation sheet is given to all patients at discharge, issues where improvement is recommended are pointed out and intervention is initiated as soon as possible during hospital stay. For family members, a short report is being sent to their respective general practitioner, in which clinical and anamnestic results are discussed and recommendations for intervention or further investigation are given. A modified Essen Stroke Risk Scale is applied for all participants [39].

Prospective follow-up

During a standardized telephone interview one week after discharge performed by a study nurse, patients are asked to evaluate the information they received concerning their stroke, investigation results, and individual vascular risk factors, as well as their hospital stay in general. Three months follow-up is performed at the out-patient clinic and includes standardized questionnaires concerning recurrent ischemic events, seizures, pain, cognitive function, psychological disorders, tolerability of medication, quality of life, employment/

education after the stroke, sick leave, as well as changes with respect to life styles and modifiable risk factors after discharge. Clinical and functional scoring by NIHSS, mRS, and Barthel index are performed, and weight and blood pressure measurements are repeated. One-year follow-up is performed by telephone interview as short standardized questionnaire update on changes concerning modifiable risk factors.

For long-term follow-up, examinations C. a-h and D. will be repeated after 5, 10 and 15 years or after 10 and 20 years from inclusion regarding patients and partners or their offspring, respectively.

Study endpoints

Primary endpoints are death and documented cerebral, coronary and/or peripheral arterial events. Secondary endpoints are the long-term development or progression of atherosclerosis and the failure of therapeutic goal achievement (tobacco cessation, well-regulated blood-pressure, dyslipidemia and diabetes mellitus, and normal weight or slight overweight). Data validation will be done by medical record information.

Statistics

All obtained data are registered in the NOR-SYS Research Registry. Statistical analyses are performed by' STATA/SE for Windows'and 'R' in cooperation with a biostatistician.

Discussion

Long-term follow-up studies of young stroke patients have shown high mortality and vascular morbidity compared to healthy controls [6-10,40]. Hence, a prospective cohort follow-up based on thorough investigation of clinical and sub-clinical vascular disease and risk factors is necessary in order to achieve a better long-term outcome.

Ultrasound imaging has been proved to be a sensitive and cost-effective method for the detection of arterial vessel disease in major arteries [16], as well as for the evaluation of adipose tissue [35,41]. For this reason, ultrasound was chosen as the predominating tool for the investigations in the NOR-SYS protocol. IMT increases are dependent on age, sex and cardiovascular risk [42]. However, the increase and prevalence of atherosclerotic lesions vary among different anatomical segments. Moreover, increased IMT has repeatedly been associated with cardiovascular risk factors and the incidence of cardiovascular events [19,43], and has been validated as a surrogate marker of atherosclerosis [44,45]. Atherosclerotic lesions are not distributed circumferentially, but develop asymmetrically [46], and their prevalence varies in the different artery segments [47]. In our study, Meijer's Carotid Arc® is used for standardized imaging at defined angles [47,48], and cIMT and plaque measurements are aquired bilaterally in three carotid segments: the distal CCA, the bifurcation and the proximal ICA [47]. We suppose that this approach will improve the individual risk classification, as recently suggested [49]. It has also been suggested that the presence of carotid artery plaques may be even more representative for CVD prediction than increased cIMT itself [50]. Hence, plaque measurements are performed in addition to the standardized IMT measurements at all three carotid sites, if present.

Atherosclerosis is a systemic disease, and lesions are often to be found in several locations of the vasculature, such as in the peripheral arteries. Intermittent claudication is a frequent condition in western European populations [51,52] and associated with symptomatic CAD and cerebrovascular events [53,54]. Acute death due to PAD has been shown in 9% [40], compared to 45% and 42% due to cerebrovascular and coronary death, respectively [55]. The CFA has been reported as the segment most prone to IMT increase and plaque formation [42] compared to the SFA and the carotids. CFA IMT has beyond that been related to coronary angiographic [56] and echocardiographic parameters [57]. It is considered suitable for long-term observations concerning the natural development of atherosclerosis in healthy participants, and for the observation of treatment effects in a participant group requiring intervention [42]. For these reasons, IMT measurements are additionally performed bilaterally in the distal CFA and the proximal SFA segment, and included in study analyses. Atherosclerosis in the abdominal aorta is leading to aortic stenoses and PAD. Abdominal aortic aneurysms are also considered to be a manifestation of advanced atherosclerosis [58], and are frequently observed in patients with carotid stenoses, cardiovascular events and PAD [59]. Therefore, in NOR-SYS the abdominal aorta is evaluated with respect to atherosclerotic lesions, stenoses, and aneurysms. The ABI is performed in all participants as it is a well-established tool in investigation for peripheral artery disease and adds valuable information to vascular risk prediction [60,61].

Standard screening for a cardiac embolic source, including 24 hour heart rhythm registration and echocardiography is carried out in order to diagnose left ventricular hypertrophy, abnormal left ventricular geometry, and dilated left atrium as they are well-known predictors of stroke, both in the general as well as in the hypertensive population [62]. Blood pressure is measured after hospital discharge as an ambulatory 24-hour measurement as it has been proven to be closer associated with cardiovascular target organ damage and incident cardiovascular events than clinic pressure [63]. Ambulatory blood pressure measurements identify hypertension more accurately than clinic blood pressure measured during an acute stroke.

Measurement of arterial stiffness by carotid-femoral pulse wave velocity by aplanation tonometry may be useful in identifying arterial disease which is not captured by routine carotid ultrasound visualization [64].

NOR-SYS includes CCTA and CT of the thoracic aorta because of the well-known association between peripheral and coronary disease [65]. In addition, aortic arch atheroma or other wall disease of the ascending aorta or the aortic arch might cause the index-stroke or recurrent stroke [66].

Obesity is an increasingly prevalent disorder [67] which is associated with atherosclerosis and cardiovascular disease. Particularily abdominal obesity has been associated with metabolic syndrome [68], pre-clinical atherosclerosis [69], cardiovascular events [70] and mortality [70].

Epicardial adipose tissue (EAT) has its embryologic origin in common with mesenteric and omental fat, and all these are accordingly classified as visceral adipose tissue (VAT) [71,72]. Associations between VAT and cIMT [73], metabolic syndrome [74,75] and cardiovascular disease [76,77] have been reported in several studies. Release of free fatty acids due to the proximity to the portal circulation leading to direct lipotoxicity [78,79], and release of pro-inflammatory and pro-atherogenic cytokines and hormones with impact on endothelial function [80,81] are related issues. The accumulation of VAT has therefore been found to be an independent vascular risk factor, even within the normal range of BMI [82]. Accordingly, the anatomical relationship of EAT to the heart is providing local interaction with modulation of the coronary arteries and the myocardium, which may subsequently affect cardiac function and morphology [83-85]. On the other hand, subcutaneous adipose tissue, which is a non-portal fat type with less metabolic activity [86], has previously shown only a weak relationship to increased cIMT [73]. Its evaluation related to the amount of VAT and anthropometric parameters is assumed to be relevant for risk prediction and for that reason included in NOR-SYS. Anthropometric parameters such as BMI and WHR are simply applicable clinical tools and widely used in obesity evaluation. They are as well associated with ultrasonographic visceral adipose tissue measurements [41,87], and applied in NOR-SYS.

In conclusion, the major objective of NOR-SYS is the standardized gathering of anamnestic, clinical, and biological data concerning life styles, medical history, and clinical and subclinical vascular disease at several sites of the vasculature including body fat composition and anthropometric measurements in young ischemic stroke patients and their families. Standardized questionnaires and standardized ultrasound examinations combined with detailed clinical data are assumed to increase the precision in diagnostics and risk estimation, and generate a solid basis of decision-making concerning secondary prophylaxis after acute ischemic stroke.

Further investigation and evaluation of vascular risk factors and sub-clinical artery wall disease in young ischemic stroke patients' family members provide a platform for primary prophylaxis and early intervention.

NOR-SYS aims to reduce co-morbidity, disability, recurrent stroke, cognitive impairment and mortality in young patients with acute ischemic stroke. We expect that a comprehensive work-up and long-term observation, combined with biological, genetical and clinical information gathered from three family generations, will give the opportunity to improve our basic knowledge concerning preclinical atherosclerosis in families with a vascular disease burden.

NOR-SYS is reviewed and approved by the Regional Committee for Medical and Health Research Ethics, Western-Norway (REK-Vest 2010/74), and registered in ClinicalTrials.gov: NCT01597453.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the study design in their respective professional field, helped to draft the manuscript, and read and approved the final manuscript.

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III

Risk factors and carotid IMT in young ischemic stroke patients and controls: The Norwegian Stroke in the Young Study.

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ABSTRACT

Background

Vascular morbidity and mortality due to cardiovascular disease (CVD) are high after ischemic stroke at a young age. Data on carotid intima-media thickness (cIMT) as marker of atherosclerosis are scarce for young stroke populations. In this prospective case-control study, we examined the burden of vascular risk factors (RF) and their impact on cIMT, aiming to detect clinical and sub-clinical arterial disease.

Methods

This study was conducted in 150 patients aged 15-60 years and 84 controls free of CVD. We related RF to ultrasonographic B-mode cIMT-measurements obtained from 12 standardized multiangle measurements in the common carotid artery (CCA), carotid bifurcation (BIF) and internal carotid artery (ICA).

Results

RF burden was higher among patients than among controls ($p<0.001$). In multivariate analyses of all 234 participants, increased cIMT was associated with age in each carotid segment. Incident stroke was associated with increased ICA-IMT. ICA-IMT increase was associated with a family history CVD among patients aged 15-44 years, and with RF at mid-age. The overall cIMT difference between patients and controls

was 12% for CCA, 17% for BIF and 29% for ICA. Further, increased CCA-IMT was associated with male sex and hypertension. Increased BIF-IMT was associated with dyslipidemia, coronary heart disease and smoking. Increased ICA-IMT was associated with dyslipidemia and stroke.

Conclusions

Young stroke is associated with increased ICA-IMT, related to a family history of CVD among the youngst patients and to increasing RF burden with increasing age. Preventive strategies and aggressive RF treatment are indicated to avoid future cardiovascular events.

INTRODUCTION

High rates of recurrent stroke, vascular morbidity and mortality due to cardiovascular disease (CVD)[1-3] clarify the need to detect risk factors and incipient atherosclerosis at early stages. Carotid intima-media thickness (cIMT) is a surrogate marker of atherosclerosis[4, 5], and ultrasound screening a valuable tool for cardiovascular risk prediction[6, 7]. Nevertheless, cIMT data obtained from young stroke populations are scarce. We aimed to assess the prevalence of clinical and subclinical carotid artery atherosclerosis and the impact of vascular risk factors (RF) among young ischemic stroke patients compared to CVD-free controls in a prospective case-control study.

METHODS

The Norwegian Stroke in the Young Study (NOR-SYS) is a prospective three-generation study with longitudinal follow-up design. NOR-SYS combines medical history and RF ascertainment by standardized questionnaires with clinical, laboratory, neuroradiological, cardiological and complex ultrasonographic data[8]. This analysis contains data from 150 patients and 84 controls included in NOR-SYS between September 2010 and June 2012.

Approvements, registrations and consents

NOR-SYS is conducted according to the Declaration of Helsinki, approved by the local ethics committee and registered in ClinicalTrials.gov (NCT01597453). Written informed consent was obtained from all participants or their legal representatives.

Subject selection

Patients aged 15-60 years with documented acute ischemic stroke and residency in Hordaland county, Norway, were included. Two patients refused study participation. Seven patients were excluded; three due to incomplete neurosonographic data set, and four non-Caucasian patients. Patients´ partners served as controls due to their function as reference persons for joint offspring in future analyses. Of 123 available partners, 63 (70.8%) females and 21 (61.8%) males participated. Seven controls with prior cardiovascular events were excluded from statistical analysis.

Risk factors

Medical history of prior stroke, coronary heart disease (CHD: myocardial infarction, angina pectoris) and peripheral artery disease (PAD) was defined if diagnosed before admission or revealed during hospitalization for the qualifying stroke. Family history of CVD (stroke, CHD and/or PAD) was considered positive if reported for parents and/or siblings. Hypertension and diabetes mellitus were defined by diagnosis and/or treatment before hospital admission, or when revealed and treated during hospitalization for the qualifying stroke (blood pressure >140/90 mmHg; HbA1c >6.4%). Dyslipidemia was defined as prior statin use, or when revealed during hospitalization (total cholesterol >5.0 mmol/L and/or low-density lipoprotein (LDL) >3.0 mmol/L and/or high-density lipoprotein (HDL) <1,0 mmol/L and/or triglycerides >2.5 mmol/L). Smoking was categorized as never-smoking or previous/ current smoking. Alcohol consumption was categorized as never used, low (0-12 units/ week) or high (>12 units/ week). Body-mass index (BMI) was dichotomized as normal or >25. RF burden was defined as the number of RFs present (0-10).

Neurosonology and Duplex / Doppler ultrasound examinations

Extracranial high-resolution sonography of the carotid arteries was performed with Philips iU22 and 9-3 MHz linear array transducer. Two patients were examined at the intensive care unit with a portable Phillips CX50 ultrasound system and 12-3 MHz linear array transducer (both systems Philips Medical Systems, Bothell, WA, USA). Patients and controls were examined by two sonographers (AF, UWA), which both are trained and certified for the NOR-SYS duplex sonography research protocol in collaboration with the Vascular Imaging Centre, University Medical Centre, Utrecht, The Netherlands.

Data reliability tests:

Reproducibility testing of cIMT measurements within (intra-observer) and between (inter-observer) sonographers of the research group, and between ultrasound equipment (inter-equipment) was performed applying both ultrasound systems. The intra-observer correlation of sonographers was 0.78-0.98 (mean absolute cIMT difference 0.02-0.08 mm). The inter-observer correlation of sonographers was 0.83-0.93 (mean absolute cIMT difference 0.04-0.11 mm). The inter-equipment correlation for iU22/CX50 was 0.94 (mean absolute cIMT difference 0.04 mm). These results correlate with previously published studies[9-12].

cIMT-measurements:

The methods of the cIMT measurements were previously described[8]. In total 12 far-wall cIMT measurements in the common carotid artery (CCA), the carotid bifurcation (BIF) and the internal carotid artery (ICA) were performed in each participant in the end-diastolic phase of the cardiac cycle, and mean cIMT values were acquired using Philips QLAB® (Philips Medical Systems, Bothell, WA, USA). In case of intra-segmental irregularities or plaques, maximum IMT or plaque thickness were measured additionally. Maximum segmental IMT values were used in statistical analysis. IMT values were defined as normal when <0.8 mm, as suspect for arterial disease when 0.8-0.99 mm[13, 14], and as pathological when ≥1.0 mm[15, 16]. Plaques were defined as focal IMT measurements >1.5 mm[17].

Statistical analyses

To allow for comparison to other studies[18-20], and in order to assess the influence of age and sex on IMT, our study population was grouped according to age (younger, 15-44 years; middle-aged, 45-60 years) and sex. Statistical analysis was performed using R version 3.0.0, and data were formatted in STATA version 12.1. A t-test was applied when testing for differences between groups. The unadjusted analysis was conducted applying a univariate linear regression. Because each individual had two measurements per segment (right/left), a random intercept approach was used, utilizing the R-function lmer() from the lme4 package. Dependent variables (cIMT) were skewed towards low values, and a base 10 log transform was applied to meet the assumptions of a linear regression. Hence, the relative change (RC) in cIMT per unit change in the independent variables (typically 0 or 1) could be obtained. Adjusted analyses were carried out accordingly, and multivariate logistic regression was applied. Simulations were used to determine the power to detect differences between controls and patients for RC ranging from 1.00 to 1.50 across each carotid segment. Fisher's exact test (based on simulations when appropriate) was applied when comparing tables or rows within tables.

RESULTS

Population demographics are given in Table 1. Of patients, 30.0% were aged 15-44 at study inclusion, and 32.7% were female. Of 84 controls, 25.0% were aged 15-44 at study inclusion, and 75.0% were female.

Risk factors

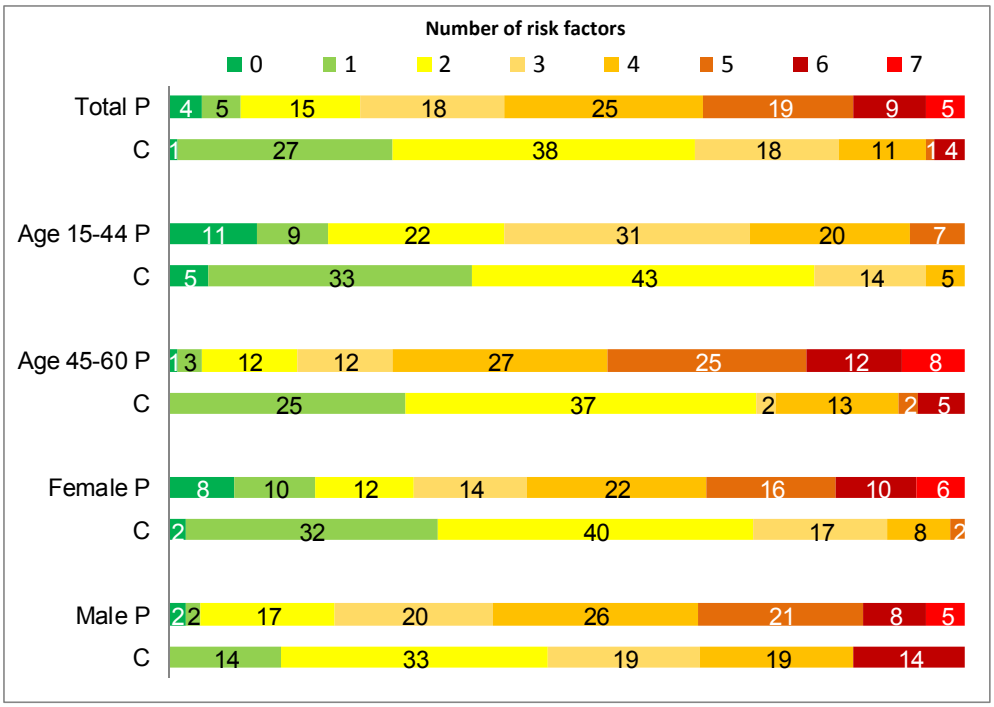
Patients had a higher RF burden than controls in analysis of the entire study population ($p<0.001$) and in subgroup analyses (age 15-44: $p=0.027$; age 45-60: $p<0.001$; females: $p<0.001$; males: $p=0.021$), visualized in Figure 1. Hypertension and dyslipidemia were more frequently present among patients. Prior stroke, CHD, family history of CVD, hypertension, dyslipidemia and smoking were less prevalent among young than among middle-aged patients, and smoking and high alcohol consumption were more common among male than among female patients.

Table 1. Patient and control characteristics. Data presented as number(percentage) or mean.

		All	*15-44 y*	*45-60 y*	*Females*	*Males*	*Age (p)*	*Sex (p)*
Patients	**N**	**150(100)***	**45(30)***	**105(70)***	**49(32.7)***	**101(67.3)***		
Age (mean)	150	48.5	35.8	54.0	46.3	49.6	<0.001	0.075
Prior Stroke	150	13(8.7)	1(2.2)	12(11.4)	6(12.2)	7(6.9)	0.017	0.325
CHD	150	15(10.0)	1(2.2)	14(13.3)	4(8.2)	11(10.9)	0.006	0.589
PAD	150	8(5.3)	1(2.2)	7(6.7)	2(4.1)	6(5.9)	0.181	0.617
Family CVD	150	78(52.0)	9(20.0)	69(65.7)	29(59.2)	49(48.5)	<0.001	0.222
Diabetes	150	16(10.7)	2(4.4)	14(13.3)	7(14.3)	9(8.9)	0.053	0.357
Hypertension	150	101(67.3)	23(51.1)	78(74.3)	30(61.2)	71(70.3)	0.009	0.282
Dyslipidemia	150	114(76.0)	27(60.0)	87(82.9)	36(73.5)	78(77.2)	0.007	0.623
Smoking	150	104(69.3)	23(51.1)	81(77.1)	28(57.1)	76(75.2)	0.003	0.033
BMI>25	144	99(68.8)	28(62.2)	71(67.6)	30(61.2)	69(68.3)	0.941	0.542
Alcohol	141							
None		9(6.4)	5(11.1)	4(3.8)	4(8.2)	5(5.0)	0.173	0.476
Low		119(84.4)	37(82.2)	82(78.1)	41(83.7)	78(77.2)	0.947	0.252
High		13(9.2)	2(4.4)	11(10.5)	1(2.0)	12(11.9)	0.137	0.011
Controls	**N**	**84(100)***	**21(25)***	**63(75)***	**63(75)***	**21(25)***		
Age (mean)	84	49.3	36.6	53.5	48.8	50.6	<0.001	0.453
Family CVD	84	49(58.3)	9(42.9)	40(63.5)	35(55.6)	14(66.7)	0.112	0.372
Diabetes	84	9(10.7)	1(4.8)	8(12.7)	5(7.9)	4(19.0)	0.218	0.249
Hypertension	84	16(19.0)	3(14.3)	13(20.6)	7(11.1)	9(42.9)	0.502	0.012
Dyslipidemia	84	12(14.3)	1(4.8)	11(17.5)	8(12.7)	4(19.0)	0.066	0.52
Smoking	84	57(67.9)	12(57.1)	45(71.4)	40(63.5)	17(81.0)	0.26	0.11
BMI>25	80	45(56.3)	11(52.4)	34(54.0)	31(49.2)	14(66.7)	0.872	0.147
Alcohol	81							
None		4(4.9)	0(0.0)	4(6.3)	3(4.8)	1(4.8)	0.045	0.989
Low		74(91.4)	20(95.2)	54(85.7)	56(88.9)	18(85.7)	0.399	0.817
High		3(3.7)	1(4.8)	2(3.2)	2(3.2)	1(4.8)	0.79	0.757

* Percentage of total population. y = years; CHD = Coronary heart disease; PAD = Peripheral artery disease; CVD = Cardiovascular disease; BMI = Body mass index

Figure 1: Risk factor burden. Data referred in percent. P = patients; C = controls



Carotid Intima-media thickness (cIMT)

Values of mean IMT and RC are presented in Table 2 and significance of all tests applied is shown in Table 3. Mean IMT values were in all subgroups of patients and controls lowest in CCA and highest in BIF, and lower in young than in middle-aged participants. Sex-related differences were inconsistent. Mean values <0.8 mm were mostly restricted to the young population and to controls. Mean values ≥1.0 mm were found in BIF in all patient and control subgroups but the young, and in ICA in middle-aged patients and male patients and controls. Mean values ≥1.5 mm were solely found in middle-aged patients.

The RC between patients and controls was in all subgroups but males most distinct in ICA (20-35%). Only middle-aged patients showed IMT increase compared to controls in CCA (19%) and BIF (23%). Statistical simulation suggested the need of RC > 12%

for CCA, RC > 22% for BIF, and RC > 25% for ICA to detect cIMT-differences ≥80% between patients and controls, which matches our results fairly well.

Details on categorized segmental cIMT distribution are shown in Figure 2 and Table 3. IMT distribution was higher than that of controls in all patient subgroups but males in ICA. IMT distribution was further higher among middle-aged patients in CCA, and among middle-aged and male patients in BIF.

Table 2: Relative change in IMT between patients and controls and mean IMT values, sorted by carotid segment, age group, and sex.

	NA	Total	15-44 y	45-60 y	Females	Males	Age (p)	Sex (p)
CCA								
Relative change (%)		12 (4-22)	3 (-9-15)	19 (8-30)	4 (-6-15)	6 (-9-23)		
Mean IMT patients	1	0.85	0.63	0.94	0.75	0.89	**<0.001**	**0.003**
Mean IMT controls	0	0.73	0.61	0.77	0.7	0.82	**<0.001**	**0.008**
BIF								
Relative change (%)		17 (2-34)	11 (-9-36)	23 (7-43)	9 (-9-31)	8 (-16-38)		
Mean IMT patients	11	1.34	0.84	1.54	1.23	1.39	**<0.001**	0.079
Mean IMT controls	2	1.12	0.7	1.26	1.04	1.34	**<0.001**	0.067
ICA								
Relative change (%)		29 (12-49)	20 (-2-46)	35 (15-58)	28 (7-53)	1 (-23-31)		
Mean IMT patients	30	0.97	0.63	1.1	0.9	1.0	**<0.001**	0.312
Mean IMT controls	14	0.73	0.47	0.83	0.64	1.06	**<0.001**	**0.021**

IMT presented in mm. NA = Not available

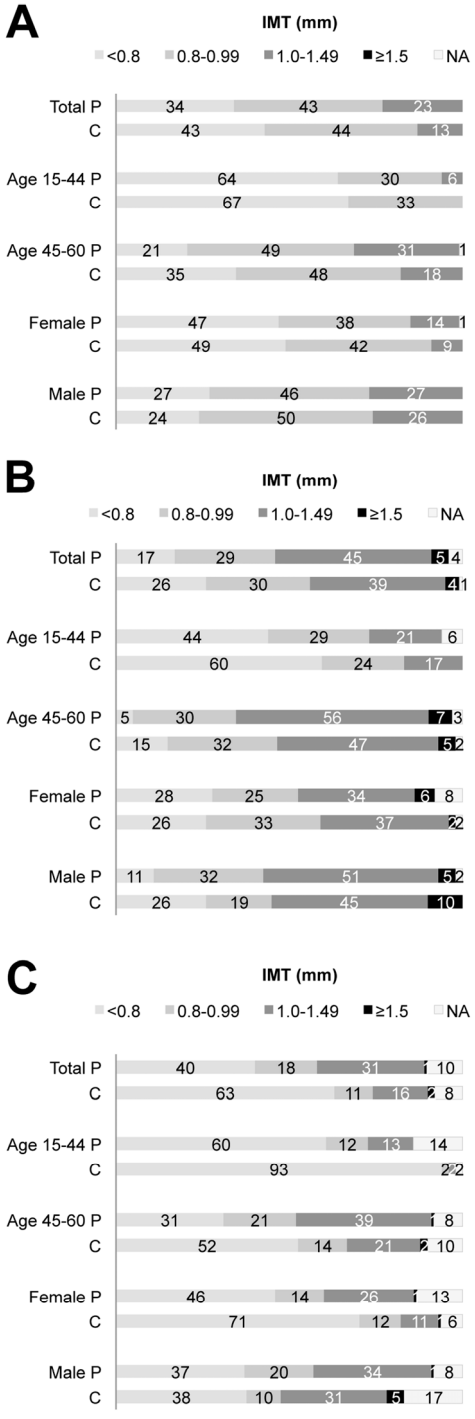


Figure 2:

Segmental cIMT distribution in Common carotid artery (A), Carotid bifurcation (B) and Internal carotid artery (C).

Data referred in percent.
P = patient; c = control

Table 3: Statistical significance for IMT increase in patients compared to controls in unadjusted analysis, t-test, and Fisher´s exact test, sorted by carotid segment, age group, and sex.

	Total	15-44 y	45-60 y	Females	Males
CCA					
Relative change (unadjusted)	**0.005**	0.669	**<0.001**	0.404	0.465
Mean IMT (t-test)	**<0.001**	0.501	**<0.001**	0.151	0.161
IMT distribution (Fisher´s exact test)	**0.018**	0.383	**0.003**	0.418	0.906
BIF					
Relative change (unadjusted)	**0.024**	0.299	**0.005**	0.344	0.544
Mean IMT (t-test)	**0.006**	**0.030**	**0.005**	0.108	0.760
IMT distribution (Fisher´s exact test)	0.111	0.447	**0.010**	0.202	**0.027**
ICA					
Relative change (unadjusted)	**<0.001**	0.079	**<0.001**	**0.008**	0.947
Mean IMT (t-test)	**<0.001**	**0.003**	**0.001**	**0.004**	0.741
IMT distribution (Fisher´s exact test)	**<0.001**	**0.005**	**<0.001**	**0.003**	0.155

Impact of risk factors on cIMT

Table 4 shows analysis of all participants (n=234). Associations between RF and IMT increase varied in the different carotid segments, and across age and sex subgroups. With one exception (ICA in middle-aged participants), IMT increase was associated with age in all carotid segments among all subgroups. On the other hand, ICA in males was the only carotid segment in any subgroup, where patients´ RF had a stronger impact on IMT increase than controls´ (p=0.038).

Table 4: Associations between vascular risk factors and segmental carotid IMT increase

Participant group	Segment	Risk factor associations
Entire study population (n=234)	CCA:	Age (p<0.001); male sex (p=0.023); hypertension (p<0.001)
	BIF:	Age (p<0.001); dyslipidemia (p=0.018); CHD (p=0.017); smoking (p=0.012)
	ICA:	Age (p<0.001); dyslipidemia (p=0.025); prior stroke (p=0.009)
Age 15-44 years (n=66)	CCA:	Age (p=0.001); hypertension (p=0.008)
	BIF:	Age (p<0.001); hypertension (p=0.009)
	ICA:	Age (p=0.002); family history of CVD (p=0.039)
Age 45-60 years (n=168)	CCA:	Age (p=0.05); male sex (p=0.037); hypertension (p=0.016)
	BIF:	Age (p=0.022); dyslipidemia (p=0.033); smoking (p=0.016)
	ICA:	Dyslipidemia (p=0.022); prior stroke (p=0.037)
Females (n=112)	CCA:	Age (p<0.001); hypertension (p=0.031)
	BIF:	Age (p<0.001); hypertension (p=0.049)
	ICA:	Age (p<0.001)
Males (n=122)	CCA:	Age (p<0.001); hypertension (p=0.012)
	BIF:	Age (p<0.001); dyslipidemia (p=0.003); CHD (p=0.031)
	ICA:	Age (p<0.001); dyslipidemia (p=0.006), PAD (p=0.028), prior stroke (p=0.004)

DISCUSSION

Our study presents cIMT data obtained from young and middle-aged patients after acute ischemic stroke. Previous studies on multisegmental RF-cIMT associations related to incident stroke did either not include participants <45 years[21, 22], or did not provide acute phase cIMT data[23]. By combination of three statistical methods, we identified increased ICA-IMT as distinctly associated with incident stroke not only

among middle-aged adults, as others described before[21], but also among young adults aged 15-44 years. Patients aged 15-44 showed a surprising 20% ICA-IMT increase compared to controls. Only a family history of CVD was found to be associated with increased ICA-IMT in the younger subgroup, which may reflect a genetic predisposition[24].

Female patients represent the best-controlled subgroup in this study. We found an unexpected ICA-IMT increase of 28% compared to female controls. The increase was surprisingly high compared to a 4% increase in CCA-IMT, and a 9% increase in BIF-IMT. In comparison, we found rather low and constant segmental differences (1-8%) from controls in male patients, though their control group was less representative. The only factor related to ICA-IMT increase in females was age. Our results may reflect hormonal influences on the development of atherosclerosis[25-27]. ICA-IMT among male patients did not differ from controls´. However, male controls are insufficiently represented, what implies limitations for the interpretation of comparing results among males.

Our study shows a higher RF burden among patients, but also a high prevalence of RF among presumably healthy controls. Other studies have reported increasing risk of vascular events[28] and higher mortality proportional to RF burden[28-30], and recommended early preventive treatment. Our data strongly support broad preventive initiatives in families at risk.

We found an increasing RF burden with age, and the three most frequent RF were dyslipidemia (76 %), smoking (69%) and hypertension (67%). This is in accordance with other studies[19, 31]. Our RF rates among patients are, however, higher than previously reported[31], as e.g. cholesterol levels among the Norwegian population remain high despite improvement during the last decades[32]. Our data further support that cIMT depends on age, sex and cardiovascular risk[15, 33-36]. We found pronounced cIMT increase in middle-aged patients, which is in line with a recent young stroke study demonstrating substantial clinical and subclinical atherosclerosis[20].

The major strengths of NOR-SYS are the inclusion of CVD-free controls and the standardized ultrasound protocol. However, our study has limitations. The size of patient subgroups varies as a consequence of stringent stratification of our patient population. Accordingly, controls (the patients´ partners) are unequally represented. Due to overall low case numbers, RF associations are in parts calculated with small sample sizes, which may affect our results. Further, our data may be valid only for Caucasians, and may be influenced by the high risk profile of our population.

We did not account for multiple testing. However, as we performed approximately 100 tests, Bonferroni correction would yield a corrected significance level of about 0.05/100=0.0005, rounded to $p<0.001$ in our study. Hence, associations with $p<0.001$ would survive a Bonferroni correction. Bonferroni correction further reduces the number of type I errors at the cost of increasing the number of type II errors, and p-values above 0.0005 may still be indicative of an association.

CONCLUSIONS

Stroke is associated with increased ICA-IMT already at a young age, related to a family history of CVD among the youngest patients and to RF burden increasing with age. Also in CVD-free controls, RFs and subclinical atherosclerosis are prevalent. Our data suggest that vascular screening reveals established clinical and sub-clinical arterial disease, requiring broad and aggressive treatment to prevent progressing CVD after ischemic stroke at a young age.

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DISCLOSURES

None.

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IV

Atherosclerosis in TOAST subtypes of young ischemic stroke. The Norwegian Stroke in the Young Study (NOR-SYS).

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ABSTRACT

Background

Ischemic stroke patients subtyped as of undetermined cause (SUC) usually outnumber those with determined cause subtypes. Etiological stroke classifications may lead to neglect of parallel, non-causative findings. Atherosclerosis progresses over decades and is associated with high morbidity and mortality in young stroke long-term follow-up studies. We compared the prevalence of carotid atherosclerosis in all TOAST subtypes among young patients with acute ischemic stroke.

Methods

We investigated 150 patients aged 15-60 years with documented acute ischemic stroke, and 84 controls free of cardiovascular disease. Stroke etiology was classified according to TOAST criteria. Carotid intima-media thickness (cIMT) measurements were obtained from 12 standardized multi-angle measurements in the common carotid artery (CCA), carotid bifurcation (BIF) and internal carotid artery (ICA).

Results

The causes of stroke were 5.3% large-artery atherosclerosis (LAA), 26.7% cardioembolism (CE), 21.3% small artery occlusion (SAO), 10% stroke of other determined cause (SOC), and 36.7% SUC. cIMT was increased in patients with LAA

(1.56 mm, p=0.002), SAO (1.11 mm, p=0.006) and SUC (1.10 mm, p=0.004) compared to controls (cIMT 0.86 mm).

Conclusions

Atherosclerotic disease is prevalent in the majority of young ischemic stroke patients, requiring determined investigation and aggressive treatment of modifiable risk factors.

INTRODUCTION

There is evidence for substantial differences in stroke etiology dependent on age[1, 2]. Etiological classifications aim to identify the causative subtype of stroke during a decision-making process by integration of symptom characteristics, risk factors and results of stroke investigation[3], but are neglecting relevant parallel findings finally judged to be non-causative. The most widely applied classification is the Trial of Org 10172 in Acute Stroke Treatment Subtype (TOAST) classification, which organizes stroke into five etiological subtypes: large-artery atherosclerosis (LAA), cardioembolism (CE), small artery occlusion (SAO), stroke of other determined cause (SOC) and stroke of undetermined cause (SUC)[4]. Large-artery atherosclerosis has been the reported cause of stroke among young adults in 3-21%[5-7]. Long-term follow-up data 12 years after stroke revealed a stroke-recurrence rate of 25%, a 5-fold increased rate of vascular co-morbidity, 10-fold increased mortality, mainly associated with cardiovascular disease, and an 8-fold increased rate of memory impairment compared to controls[8]. Carotid intima-media thickness (cIMT) as surrogate marker of atherosclerosis is frequently used for vascular risk evaluation[9]. The Norwegian Stroke in the Young Study (NOR-SYS) ultrasonographic research protocol includes multisegmental multi-angle measurements of cIMT with the purpose to evaluate clinical and sub-clinical atherosclerotic arterial disease regardless of the cause of stroke[10].

We aimed to compare the prevalence of atherosclerosis in each TOAST subtype among young and middle-aged ischemic stroke patients to that of controls free of cardiovascular disease (CVD).

METHODS

NOR-SYS is a prospective three-generations long-term follow-up study. Details of the study protocol have recently been published[10]. Medical history, vascular risk factors and clinical, laboratory, neuroradiological and cardiological results are combined with ultrasonographic data gathered according to the research protocol. The presented analysis is based on data from 150 patients and 84 controls included in NOR-SYS between September 2010 and June 2012.

Approvements, registrations and consents

NOR-SYS is conducted according to the Declaration of Helsinki, approved by the local ethics committee and registered in ClinicalTrials.gov (identifier NCT01597453). Written informed consent was obtained from all participants or their legal representatives.

Subject selection

Patient inclusion criteria were documented acute ischemic stroke, age 15-60 years, and residency in Hordaland county, Norway. Two patients refused study participation. Seven patients were excluded from statistical analyses: three due to incomplete neurosonographic data set, and four due to non-Caucasian origin. Partners of included patients served as controls due to their function as reference persons for joint offspring in future analyses. Of 123 available partners, 63 (70.8%) caucasian females and 21 (61.8%) caucasian males with no prior cardiovascular events participated.

Stroke subtype classification by TOAST

Etiological classification was performed by one experienced stroke neurologist (HN) according to the TOAST criteria[4], blinded for results derived from the NOR-SYS ultrasonographic research protocol.

Neurosonology

Ultrasonography was performed with Philips iU22 and 9-3 MHz linear array transducer. Two patients were examined at our intensive care unit with a portable Phillips CX50 ultrasound system and 12-3 MHz linear array transducer (both systems Philips Medical Systems, Bothell, WA, USA). The studied subjects were examined by two sonographers (AF, UWA), both trained and certified for the NOR-SYS duplex sonography research protocol[10] in collaboration with the Vascular Imaging Centre, University Medical Centre, Utrecht, The Netherlands.

Data reliability tests: Reproducibility testing of cIMT measurements within (intra-observer, kappa=0.78-0.98) and between (inter-observer, kappa=0.83-0.93) sonographers of the research group, and between iU22 and CX50 (inter-equipment, kappa=0.94) were in accordance with other studies[11-14].

cIMT-measurements: A total of 12 far-wall cIMT measurements in the common carotid artery (CCA), carotid bifurcation (BIF) and internal carotid artery (ICA) were performed in each participant in the end-diastolic phase of the cardiac cycle. Mean cIMT values were acquired using Philips QLAB® (Philips Medical Systems, Bothell, WA, USA). Maximum IMT or plaque thickness was measured additionally in case of intra-segmental irregularities.

Statistical analysis

All statistical analyses were conducted by a statistician (ØAH). To facilitate comparability to other studies[6, 15], and in order to assess the influence of age and sex on IMT, our study population was grouped according to age (younger, 15-44 years; middle-aged, 45-60 years) and sex. Data was formatted using STATA version 12.1, and statistical analyses were conducted using R version 3.0.1. Differences in means between groups were evaluated applying a standard t-test (Table 1). For analysis of segmental cIMT distribution, cIMT was categorized as <0.8 mm, 0.8-0.99 mm, 1.0-1.49 mm, and ≥1.5 mm. Fisher's exact test was utilized when considering differences in distributions between groups (Table 2). To account for dependence between cIMT

measurements in different segments within the same patient, the mean cIMT value across CCA, BIF, ICA or all segments was used when groups of patients were compared with each other. This way, a single patient only contributed once in each analysis.

RESULTS

Details on the study population, etiology of stroke and cIMT values are shown in Table 1. Overall, 150 young (15-44 years) and middle-aged (45-60 years) ischemic stroke patients and 84 controls were included in the present analysis. Acute ischemic stroke was documented by MRI.

Stroke subtypes

The stratification of our patient population regarding TOAST subtypes is shown in Table 1. Out of 10% SOC, 8% were diagnosed as dissections. CE, SOC, dissections and SUC were most frequent in patients aged 15-44 years (CE: $p=0.008$; SOC: $p<0.001$; dissections: $p=0.01$; SUC: $p<0.001$). CE and dissections were predominant in males compared to females (CE: $p=0.017$; dissections: $p=0.047$).

cIMT in TOAST subtypes

Comparing mean cIMT in each TOAST subtype to mean cIMT in controls (Figure 1), we found increased IMT measurements in patients with LAA (mean 1.56 mm vs. 0.86 mm, $p=0.002$), SAO (mean 1.11 mm vs. 0.86 mm, $p=0.006$) and SUC (mean 1.10 mm vs. 0.86 mm, $p=0.004$). In patients with CE (mean 0.89 mm vs. 0.86 mm, $p=0.775$), SOC (mean 0.86 mm vs. 0.86 mm, $p=0.974$) and, among SOC, in patients with dissection (mean 0.95 mm vs. 0.86 mm, $p=0.476$), cIMT was comparable to that of controls. Among those aged 15-44, only LAA patients differed significantly from controls (mean 1.44 mm vs. 0.59 mm, $p<0.001$). Among those aged 45-60, the pattern was similar to that of the total patient population (LAA: mean 1.57 mm vs. 0.95 mm, $p=0.011$; SAO: mean 1.23 mm vs. 0.95 mm, $p=0.005$; SUC: mean 1.2 mm vs. 0.95 mm, $p=0.008$).

Table 1. Stroke etiology by TOAST classification, and cIMT distribution.

		Total	15-44 y	45-60 y	Females	Males	Age (p-value)	Sex (p-value)
Patients	N / NA	150 (100)*	45 (30)*	105 (70)*	49 (32.7)*	101 (67.3)*		
Age (mean)	0	48.5	35.8	54.0	46.3	49.6	**<0.001**	0.075
Etiology								
LAA	0	8 (5.3)	1 (2.2)	7 (6.7)	4 (8.2)	4 (4.0)	0.057	0.178
CE	0	40 (26.7)	17 (37.8)	23 (21.9)	9 (18.4)	31 (30.7)	**0.008**	**0.017**
SAO	0	32 (21.3)	7 (15.6)	25 (23.8)	13 (26.5)	19 (18.8)	0.09	0.144
SOC	0	15 (10.0)	10 (22.2)	5 (4.8)	4 (8.2)	11 (10.9)	**<0.001**	0.442
Diss.†	0	12 (8.0)	7 (15.6)	5 (4.8)	2 (4.1)	10 (9.9)	**0.01**	**0.047**
SUC	0	55 (36.7)	10 (22.2)	45 (42.9)	19 (38.8)	36 (35.6)	**<0.001**	0.602
cIMT								
All segm.	42	1.05	0.7	1.2	0.96	1.09	**<0.001**	**0.011**
CCA	1	0.85	0.63	0.94	0.75	0.89	**<0.001**	**0.003**
BIF	11	1.34	0.84	1.54	1.23	1.39	**<0.001**	0.179
ICA	30	0.97	0.63	1.1	0.9	1.0	**<0.001**	0.312
Controls	N / NA	84 (100)*	21 (25)*	63 (75)*	63 (75)*	21 (25)*		
Age (mean)	0	49.3	36.6	53.5	48.8	50.6	**<0.001**	0.453
cIMT								
All segm.	16	0.86	0.59	0.95	0.79	1.07	**<0.001**	**0.001**
CCA	0	0.73	0.61	0.77	0.7	0.82	**<0.001**	**0.008**
BIF	2	1.12	0.7	1.26	1.04	1.34	**<0.001**	0.067
ICA	14	0.73	0.47	0.83	0.64	1.06	**<0.001**	**0.021**

Data presented as number (percentage of respective group) or mean. NA = Not available;

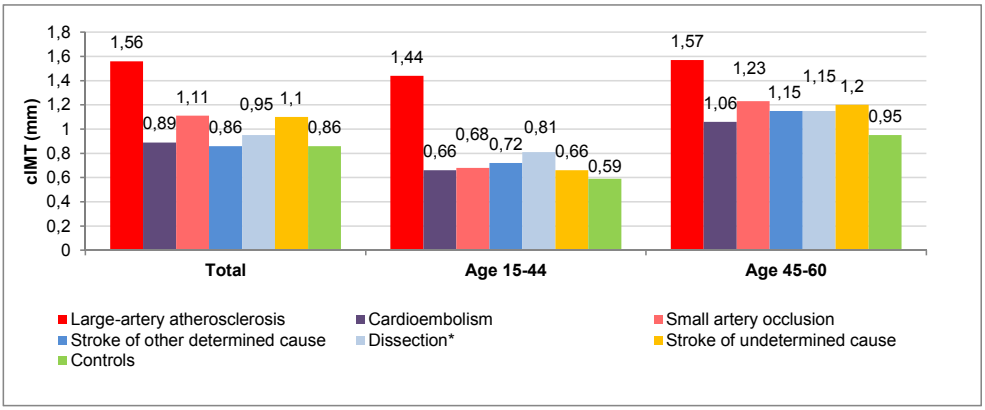
* Percentage of total population; † out of SOC; y = years; LAA = Large-artery atherosclerosis;

CE = Cardioembolism; SAO = Small artery occlusion; SOC = Stroke of other determined cause;

Diss. = Dissection; SUC = Stroke of undetermined cause; cIMT = carotid Intima-media thickness;

segm. = segments; CCA = common carotid artery; BIF = carotid bifurcation; ICA = internal carotid artery

Figure 1: cIMT distribution in TOAST subtypes and controls, stratified by age group.



cIMT = Carotid intima-media thickness; * subgroup of `Stroke of other determined cause` population

cIMT distribution in carotid segments by TOAST subtypes

cIMT distribution in each stroke subtype compared to CVD-free controls is presented in Figure 2 for each carotid segment. Table 2 shows the results of Fisher's exact test for differences in cIMT distribution across age groups and sexes in the stroke subtypes. IMT of LAA patients did not differ significantly across age groups or sexes in any segment. Middle-aged CE patients had higher IMT in CCA and BIF than young ones. Middle-aged SAO patients had higher IMT than young patients in all three carotid segments, and males had higher CCA-IMT than females. Middle-aged patients with SOC or specified dissection had higher ICA-IMT than young patients, and no sex differences were found. Middle-aged SUC patients had higher IMT than young patients in BIF and ICA, and males had higher IMT than females in CCA and BIF.

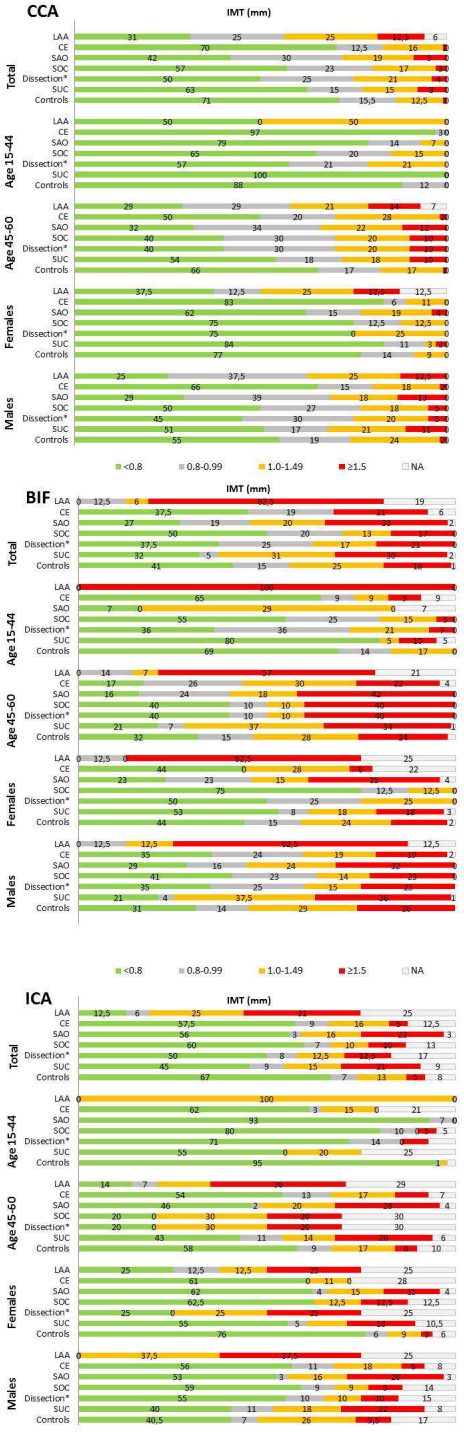


Figure 2: cIMT distribution in TOAST subtypes and controls, stratified by age group.

cIMT = Carotid intima-media thickness; * subgroup of `Stroke of other determined cause` population

Table 2: Differences in cIMT distribution across age groups and sexes in stroke subtypes.

	CCA		BIF		ICA	
	Age	Sex	Age	Sex	Age	Sex
Large-artery atherosclerosis	1	0.888	NA	1	NA	0.437
Cardioembolism	**<0.001**	0.599	**<0.001**	0.052	0.173	0.444
Small artery occlusion	**0.026**	**0.04**	**<0.001**	0.765	**0.003**	0.767
Other determined cause	0.368	0.804	0.12	0.425	**0.003**	1
Dissection	0.727	0.689	0.218	0.797	**0.011**	0.344
Undetermined cause	NA	**0.004**	**<0.001**	**0.002**	**0.02**	0.408

cIMT measurements were sorted into categories (<0.8 mm, 0.8-0.99 mm, 1.0-1.49 mm, ≥ 1.5 mm) for each stroke subtype. Fisher's exact test was applied to detect whether patient subgroups (age, sex) distributed differently across the cIMT categories. Data presented as p-values. NA indicates too few observations to properly run the test.

DISCUSSION

Of all TOAST subtypes, cIMT increase compared to CVD-free controls was highest in LAA- patients, in line with previous studies relating CCA-IMT to stroke subtypes in older populations[16, 17]. CIMT increase in SAO patients (1.1 mm) may partly be explained by coexistance of LAA and SAO[18]. An uncertain proportion of SAO may be of cardio- or arterio-arterial embolic origin regardless of their lacunar appearance[19]. However, we found comparably increased cIMT in patients with SUC (1.0 mm). cIMT increase to ≥1.0 mm is consistent with subclinical arterial disease[20], with impact on future cardiovascular events[21-23]. SAO and SUC, representing 58% of our patients, reached this value, and middle-aged and male SUC patients contributed most to the pathologic measurements. Our results indicate that a considerable number of patients with atherosclerotic disease may be hidden in the SUC and SAO subtypes.

TOAST SUC criteria include patients with cryptogenic stroke, patients with several potential causes, and patients with incomplete diagnostic work-up. LAA requires rigidly occlusion or stenosis ≥50% in the related artery. However, there is evidence for emboligenic unstable atherosclerotic plaques causing stroke regardless of the severity of stenosis[24, 25], even from low-grade or moderate degree[26] which remain unrecognized as LAA by TOAST and most likely contribute to SUC. All these factors result in oversizing the SUC subtype to approximately 40% of all strokes, as in our study, representing one major limitation of TOAST[27].

LAA is accompanied with the highest risk of recurrence within 3 months[28], 5 years[29, 30], and even 12 years[31]. Decisions concerning future treatment are influenced by the assumed cause of stroke. Statin therapy has high relevance for the further development of atherosclerotic disease[32]. A recent long-term investigation found favorable outcome among young SUC patients continuously treated with a statin[33]. Our data on increased cIMT values among SUC patients support these results.

The inclusion of controls and cIMT measurements at standardized sites are major strengths of this study. Interpretations may be limited by group imbalances regarding sex, and small groups regarding stroke subtypes.

CONCLUSIONS

Our data indicate that atherosclerotic disease is prevalent in LAA, SAO and SUC subtypes of young ischemic stroke.

Determined ultrasonographic investigation for atherosclerosis is strongly recommended despite the actual cause of stroke, to ensure optimal and aggressive secondary preventive treatment including statins.

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DISCLOSURES

None.

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