Intracranial Atherosclerosis

An ultrasound study

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Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

2012

This project has been financially supported by the Norwegian ExtraFoundation for Health and Rehabilitation through EXTRA funds

Scientific environment

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Acknowledgements

My greatest gratitude goes to Professor Lars Thomassen who offered me the opportunity to carry out this fascinating project. During these years he has shown me the beauty of cerebral hemodynamics and guided me through this work with infinite enthusiasm.

I express my gratitude to Professor Ole-Bjørn Tysnes and Professor Nils Erik Gilhus for the support and the possibility to carry out this project at the Department of Neurology, Haukeland University Hospital and the Department of Clinical Medicine, University of Bergen.

I have had the privilege to work with outstanding experts in the fields of medicine, physics and engineering. Dr. Halvor Næss and Dr. Ulrike Waje-Andreassen have been a font of inspiration and have improved my work through continuous support and brilliant ideas. Dr. Titto Idicula and Dr. Jan Brøgger with their passion and in-depth knowledge of stroke research and statistics have helped me coping with several methodological and statistical challenges. I am deeply grateful to Dr. Sean Wallace and his family for the enthusiasm shared during our effort to understand basic physical principles of ultrasound. I thank Professor David Russell, University of Oslo and Professor Rainer Brucher, University of Ulm, for the possibility to join their research groups in the study of experimental ultrasound. I express my gratitude to Professor Knut Matre, University of Bergen, who has contributed with his expertise in the field of physics and ultrasound.

I thank all the Ph.D. and medical students who I have shared the office with, who have always been able to create a stimulating and relaxing work environment.

Finally, I am infinitely grateful to my family for their care and support, and last but not least, to my girlfriend Nina who has made me a happy *refugee* in this country.

Contents

List of abbrevations

A	Area
ACA	Anterior cerebral artery
AF	Atrial fibrillation
AI	Area index
BFV	Blood flow velocity
CE MRA	Contrast-enhanced MRA
CSA	Cross-sectional area
CT	Computed tomography
CTA	Computed tomographic angiography
D_i	Intrastenotic diameter
DM	Diabetes mellitus
D_p	Prestenotic diameter
DSA	Digital subtraction angiography
DSI	Doppler signal intensity
DWI	Diffusion-weighted MR imaging
ECAS	Extracranial atherosclerosis
ES	Extracranial stenosis
FI	Flow index
Hct	Hematocrit
HR MRI	High-resolution magnetic resonance imaging
Hz	Hertz
ICAS	Intracranial atherosclerosis
IS	Intracranial stenosis
MCA	Middle cerebral artery
MES	Microembolic signal
MHz	Mega Hertz

MPa	Mega Pascal
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MV	Mean velocity
NPV	Negative predictive value
PCA	Posterior cerebral artery
PPV	Positive predictive value
p_r	Acostic pressure
PSV	Peak systolic velocity
RBC	Red blood cells
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
UCA	Ultrasound contrast agents
V	Velocity

Introduction

Intracranial Atherosclerosis

Atherosclerosis is a systemic disease of the vessel wall. The atherosclerotic plaque develops due to the accumulation of lipids, inflammatory cells, smooth muscle cells, and extracellular matrix in the subendothelial space over time.[1] Atherosclerotic plaques are mainly found at arterial bifurcations, branch points and vessel curvatures, whereas straight unbranching arterial segments are generally spared.[2] Cerebral, cardiac, and peripheral atherosclerosis are leading causes of morbidity and mortality in the Western world.[3-6] Although the higher risk of ICAS in patients of Asian, African, and Hispanic ethnicity compared to Caucasians was described already in 1986,[7] ICAS has long been a rather neglected research field in Europe and North America. In Chinese patients ICAS accounts for about 33–50% of ischemic strokes and >50% of TIA.[8-10] As the majority of the world's population is represented by Asians, ICAS is the most common vascular lesion in stroke patients worldwide.[11] In Caucasians, ICAS accounts for ~1-12.4% of all ischemic stroke patients.[12-15] Research on ICAS has exponentially increased over the last two decades.[16] However, several aspects still remain to be investigated, such as the prevalence of ICAS in Northern Europe; the responsible factors for the development of atherosclerosis in different locations among ethnicities; the pathophysiological differences between extracranial atherosclerosis (ECAS) and ICAS; the optimal diagnostic screening and evaluation of ICAS lesions; the best medical treatment for patients with ICAS; the optimal candidates for revascularization therapy; and the ultimate clinical outcome in patients with ICAS.

Epidemiology

The studies reporting the prevalence of ICAS include population-based, hospital-admission-based, and autopsy studies.

Autopsy studies have the major advantage to identify minor intracranial plaques and to investigate the histopathologic features of the intracranial plaque, but are affected by selection bias. *In vivo* studies are characterized by the limitations of the diagnostic imaging methods, which may be unable 1) to detect initial stages of ICAS; 2) to differentiate ICAS from stenoses of other etiology; and 3) to provide histopathologic composition and activity of the intracranial atherosclerotic plaque.[16] One of the most striking and unresolved issue about ICAS is the large difference in prevalence among ethnicities.[11] Literature regarding ICAS epidemiology will therefore be reviewed on a geographic basis.

Asia

In the early 1980s, a Japanese autopsy study showed that ICAS was more severe in ischemic stroke patients than in patients without stroke or cerebral hemorrhage.[17] Another Chinese autopsy study showed a significantly higher prevalence of ICAS compared to ECAS, and a strong correlation between age and ICAS, with at least one ≥50% stenosis in more than 50% of the patients reaching 90 years of age.[18]

Population-based studies have been made possible by the high availability and low-cost of transcranial Doppler (TCD). Two large Chinese TCD studies have shown ICAS to be present in ~6-7% of stroke free, asymptomatic subjects.[19, 20] The prevalence of asymptomatic ICAS in the Chinese population therefore appears similar to the prevalence of asymptomatic ECAS in Caucasians, i.e. 2-8%.[21]

In Chinese patients with at least one vascular risk factor (hyperlipidemia, hypertension, diabetes mellitus) middle cerebral artery (MCA) stenosis was found in 12.6% of the subjects and the prevalence of MCA stenosis increased quadratically with increasing number of risk factors: from ~7% for one risk factor to ~30% for four risk factors.[22] In Japanese patients undergoing coronary artery bypass graft, ≥50% ECAS was found in ~17% of the patients, and ≥50% ICAS in ~21%.[23]

In studies reporting the prevalence of symptomatic ICAS in stroke patients, digital subtraction angiography (DSA) has long been considered the gold standard, but non-invasive methods have gained importance during the last years.[24] One magnetic resonance angiography (MRA) study has shown that ICAS was present in ~35% of Indian ischemic stroke patients versus ECAS ~7%.[25] In South Asians ischemic stroke patients, ICAS was found by MRA or TCD in 54% of the cases.[26] Other ultrasonographical studies from China have confirmed the high prevalence of ICAS in Chinese stroke patients, which appears to vary between 30 and 54%.[8, 26] The prevalence of ICAS in TIA patients is most likely as high as in ischemic stroke patients, although based on limited data from one ultrasonographical study reporting ICAS in 51% of Chinese TIA patients.[9]

North America and Europe

The interest towards ICAS in the Western world began to rise in the late 1950s when one of the first autopsy studies described the presence of atherosclerosis in the intracranial arteries.[27] Already in the late 1960s, several studies had shown that ICAS occurred more frequently among black people compared to white.[28-31] More recently, data from the Northern Manhattan Stroke Study have shown ICAS to be the cause of ischemic stroke in 1% of Caucasians, 6% of African Americans, and 11% of Hispanics.[12] In Europeans, ICAS appears to be the cause of ischemic stroke in ~2-7% of the cases in Germany,[13, 14] ~10% in Greece and ~12% in Spain.[15, 32]

There are limited data on the prevalence of ICAS in the general population. In asymptomatic patients referred for carotid Doppler ultrasound, ICAS was identified in ~13% of the patients by TCD.[33] In Spain, ICAS appears to affect ~9% of stroke-free Caucasians with moderate-high vascular risk.[34]

Risk factors

Ethnicity is undoubtedly the major risk for ICAS. However, it remains unclear if ethnicity is an independent risk factor for ICAS or if the heterogeneity of risk factors presented in different ethnic groups represents a confounder. Most of the conventional cardio- and cerebrovascular risk factors have been associated also with ICAS. Advanced age is a strong risk factor for ICAS, and it has been suggested that ICAS develops one decade later in life compared with ECAS.[35] Evidences about association between ICAS and gender are controversial, but some evidences suggest a male predominance in ICAS.[7, 20, 36] Gender appears also to influence the progression of the ICAS. ICAS is found earlier in men but may progress faster in women, possibly due to menopausal hormonal changes.[37] Both autopsy[35] and clinical studies[19, 22, 38-43] have found a strong association between hypertension and ICAS. Diabetes mellitus (DM) appears also to be a strong risk factor for ICAS, both in asymptomatic[43] and symptomatic ICAS,[15, 44, 45] possibly even stronger than hypertension. DM is closely associated also to both ICAS and ECAS.[46, 47] Although hyperlipidemia is a well-known risk factor for ECAS, its association to ICAS remains unclear and only few studies have found a strong association between hyperlipidemia and ICAS.[22, 38] Metabolic syndrome is another composite risk factor which has recently been found to be more strongly associated to ICAS than ECAS.[48-50]

Studies which have compared the role of risk factors in ICAS and ECAS patients have shown discordant results. Several studies have suggested that hypertension is more closely associated with ICAS than hyperlipidemia, whereas hyperlipidemia may play a greater role than hypertension in patients with ECAS.[7, 30, 40, 51] However, other

studies have failed to find difference in risk factors between these two groups of patients, and have suggested ethnicity as the only independent factor.[36, 52, 53]

Stroke mechanisms

The pathophysiology of the intracranial plaque does not substantially differ from atherosclerosis in other vessels such as coronary or carotid arteries. *In situ* thrombotic occlusion, artery-to-artery embolism, branch occlusive disease of the orifice of perforating arteries, hypoperfusion or the combination of these mechanisms lead to cerebral ischemia in patients with ICAS.[54, 55] Advances in neurovascular imaging have improved our insight into stroke mechanisms in ICAS patients.

In situ thrombotic occlusion

Acute thrombosis superimposed on the intracranial atherosclerotic plaque is caused by the rupture of the plaque fibrous cap leading to release of tissue factors from the damaged endothelial surface.[56] Secondary artery-to-artery embolism may coexist.[57, 58] Among the stroke mechanisms in ICAS patients, *in situ* thrombotic occlusion usually leads to the largest infarcts. However, the gradual progression of atherosclerosis may allow the establishment of intracranial collaterals which may prevent a complete territorial infarction. The infarction area is therefore often limited to the striatocapsular area, borderzone area or both, and the final infarction size depends on the development of the collateral circulation.

Branch occlusive disease

Occlusive disease of deep central perforating arteries occurs when an intracranial atherosclerotic plaque protrudes and occludes the orifice of perforating arteries, leading to a subcortical lacunar-like infarct. Atheromatous branch occlusive disease must be differentiated from lipohyalinosis, which is characterized by segmental wall

disorganization along the course of perforating arteries and usually related to hypertension. Lipohyalinosis is considered the common underlying cause of lacunar infarcts.

Intracranial atheromatous branch disease has long been a "neglected, understudied, and underused concept".[59] Although branch occlusion is accurately detected only by anatomopathological studies, vascular imaging and DWI may provide some pathognomonic signs, such as coexistence of small subcortical infarct and ipsilateral intracranial stenosis. Asian studies have shown that many lacunar infarcts are caused by ICAS rather than lipohyalinosis.[60-63] Clinical course and infarction size may help distinguish between these two diseases. Infarcts caused by branch occlusive disease may be characterized by symptoms fluctuation and are usually larger than lacunes caused by lipohyalinosis.[60, 61] Occlusion of the perforating arteries may be caused by minor atherosclerotic plaques[64] which are often overlooked by the routine intracranial vascular imaging. The actual prevalence of this mechanism therefore remains undefined and probably underestimated.

Artery-to-artery embolism

Distal artery-to-artery embolism from an intracranial stenosis to peripheral arterial segments often coexists with *in situ* thrombotic occlusion. Distal embolism of atherosclerotic debris may originate from plaque ulceration, and/or from disgregation of a thrombotic occlusion. Lesions causing artery-to-artery embolism may have higher degree of stenosis than lesions causing branch occlusion.[64] High-grade stenosis may be associated with perfusion deficits in the territory of the stenotic vessel, leading to more severe distal embolic infarction through impaired emboli clearance.[65] Diffusion-weighted MR imaging (DWI) and microembolic signal (MES) monitoring are useful diagnostic tools to define a possible artery-to-artery mechanism in patients with cerebral infarction and ICAS. Coexistence of multiple small cortical infarctions at

DWI and detection of MES with TCD are highly suggestive for an underlying artery-to-artery stroke mechanism.

Hypoperfusion

The atherosclerotic plaque may progressively obliterate the arterial lumen and eventually lead to hypoperfusion, if collaterals are insufficient. In these patients, TIA and/or ischemic stroke typically occur in concomitance with conditions disturbing cerebral perfusion, e.g. high-grade ECAS and inadequate circle of Willis.

Hypoperfusion may coexist with other mechanisms. High-grade ICAS causing hypoperfusion may be unstable and undergo cap fissuring because of elevated share stress and turbulent flow. In patients with high-grade ICAS and small, multiple infarctions in the borderzone areas, hypoperfusion may contribute to infarction in these areas by reducing the emboli clearance.[66-68] High-grade ICAS may also theoretically lead to hypoperfusion in the territories supplied by perforating arteries. The smaller the arterial lumen, the higher the intrastenotic blood flow velocity.[69] According to the Bernoulli's and the continuity principle, an increase in flow velocity is associated with a decrease in pressure (Venturi effect) to satisfy the principle of conservation of mechanical energy. In case of a high-grade MCA stenosis the high intrastenotic velocity might cause low pressure and thereby hypoperfusion in perforating arteries.[70]

Diagnostic imaging

Digital subtraction angiography (DSA), magnetic resonance angiography (MRA), computed tomographic angiography (CTA) and transcranial ultrasonography (TCD/TCCS) are the available modalities for evaluation of intracranial arteries.

Digital subtraction angiography

DSA is the current gold standard for imaging of the intracranial arterial pathology, included intracranial atherosclerosis.[24] The superiority of DSA is due to high spatial and contrast resolution, which makes this modality the most accurate in the evaluation of the degree of intracranial stenosis. The standardized method used to measure the degree of stenosis with DSA defines the stenosis as percent stenosis based on prestenotic and intrastenotic diameters (D_p and D_i), i.e. $[1-(D_i/D_p) \times 100]\%$.[71, 72] Despite the superior image quality, there are several drawbacks. DSA may incorrectly diagnose high-grade stenosis in case of slow blood flow. In such hemodynamic conditions, the contrast filling of the vessel distal to the stenosis is poor and may falsely suggest arterial occlusion.[73] The bidimensional images provided by DSA may also overlook tortuosity and anatomical details which may be important in evaluation of patients addressed to stenting procedures. In TIA and ischemic stroke patients, DSA is associated with 3.0% risk of transient neurological deficit and 0.7% risk of permanent neurological deficit.[74] DSA exposes also patients to ionizing radiation, peripheral vascular complications and possible allergic reactions to contrast agent. Continuous improvement of other non-invasive modalities is challenging the role of DSA as gold standard.

Magnetic Resonance Angiography

The use of MRA in ischemic stroke patients has increased exponentially in the last decade and several techniques are now available for detection of ICAS. The most common MRA technique is the 3D time-of-flight (TOF) sequence. TOF MRA appears to be reliable in excluding the presence of ICAS. Studies investigating the diagnostic performance of TOF MRA in detection of ≥50% intracranial stenosis with DSA as gold standard have shown that TOF MRA has high negative predictive value (91-98%) and high specificity (99%), whereas positive predictive value and sensitivity were lower (59-63 and 70% respectively).[24, 73] However, TOF-MRA may overestimate the length and the degree of intracranial stenosis.[75] This limitation may

be overcome by contrast-enhanced MRA (CE MRA), which adopts gadolinium as contrast agent.[75] Even though CE MRA is promising, the necessity of contrast agents limits the routine use of CE MRA, and no study has compared the diagnostic performance of CE MRA with a gold standard.[76]

Computed Tomographic Angiography

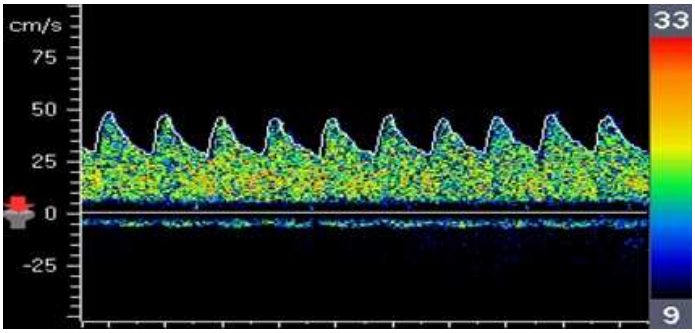
Computed tomography (CT) utilizes X-rays to produce cross-sectional, bidimensional images. CT technology has improved since Godfrey Hounsfield in 1972 developed the first scanner, which required hours to acquire images slice by slice, and days for post-processing. Modern CT acquires multiple slices in one breath hold. CT angiography (CTA) is obtained by acquisition of images during the first pass of intravenous iodinated contrast agent. Although CTA is performed quickly, expensive workstations are required to post-process the enormous image datasets. There are mainly three post-processing techniques: 1) multiplanar reformation (MPR), which creates 2-D images in arbitrary planes; 2) maximum intensity projection (MIP), which shows both contrast filling and vessel calcification; and 3) volume rendering, which provides colour 3-D images.[77] CTA has several advantages compared with MR and DSA. CTA is widely available and minimally invasive, quick to perform, less distorted from motion artifacts and less dependent from hemodynamic effects than MRA. With DSA as gold standard, CTA appears to have higher sensitivity (98% vs 70%), and higher positive predictive value (93% vs 65%) than MRA in detection of intracranial stenosis.[73] CTA may also be superior to DSA in detection of occlusion in the posterior circulation, especially in cases with slow flow.[73] CTA may therefore be the modality of choice to diagnose patients with ICAS. Drawbacks of CTA are possible artifacts deriving from extensive vessel calcification, lower resolution than DSA, and the use of iodinated contrast agents.[78] Another limitation of CTA is the lack of hemodynamic real-time assessment of the intracranial circulation.

Transcranial ultrasonography

Basic principles

The first ultrasound probe able to record blood flow velocity through the skull was introduced in 1982.[79] This ultrasound probe was based on the Doppler effect, and therefore called transcranial Doppler (TCD). The TCD machines in use today do not considerably differ from the first prototype, and still exploits a 2 MHz probe which depicts moving blood and displays the Doppler spectrum on the screen (Figure 1).

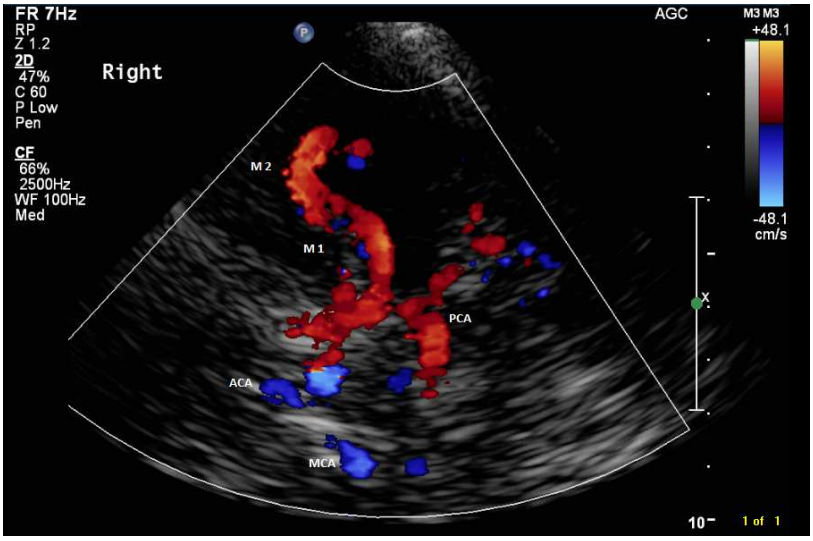
Figure 1. Transcranial Doppler spectrum of the middle cerebral artery



In the late 1980s transcranial color-coded sonography (TCCS) was introduced. This ultrasound technique utilizes a phased array transducer which combines the brightness mode (B-Mode), producing grey scale bidimensional pictures of the brain, with the Doppler mode. In TCCS the recorded Doppler shift frequencies are also exploited to create a spatial colour map of blood velocity overlaid on the B-mode image, and has therefore been defined as color Doppler mode. Thus, TCCS provides the visualization of both brain anatomy and intracranial vessels, and may both simplify and improve the examination of cerebral hemodynamics.

Insonation of the intracranial vessels can be performed through several bone "windows". The most commonly used windows are the transtemporal, the transnuchal (or transforaminal) and the orbital window. Examination techniques are similar in the two transcranial ultrasonographic technologies, but TCCS has the advantage of being guided by the color Doppler mode. Only TCCS examination technique will be presented here in detail. Transtemporal examination is performed by placing the transducer anterior to the upper insertion point of the auricle above the zygomatic arch. Insonation is performed using either axial or coronal plane. In the axial plane, several planes may be visualized tilting the transducer apically or caudally. It is recommended to start from the mesencephalic plane, which visualizes the midbrain as a hypoechogenic butterfly-like structure. In this plane, the whole circle of Willis (except the siphon) may be visualized with color Doppler (Figure 2).

Figure 2. Color-coded overview of the Circle of Willis with TCCS



M1: sphenoidal segment of the middle cerebral artery (MCA)

M2: insular segment of the MCA

ACA: anterior cerebral artery

PCA: posterior cerebral artery

The siphon may be visualized by tilting the transducer further caudally.

The coronal plane is achieved by turning the transducer 90°. The anterior coronal plane is performed by tilting the transducer forwards. In this plane, the terminal siphon segment and its bifurcation into the middle and the anterior cerebral arteries may be visualized. By tilting the transducer backwards, the top of basilar and its bifurcation into the superior cerebellar arteries and the posterior cerebral arteries may be visualized. Other insonation approaches are the transorbital examination, performed by placing the transducer on the closed eyelid, and the transnuchal examination, performed by placing the transducer suboccipitally on the midline. Using the orbital ultrasound window the ophthalmic artery and the siphon may be visualized. Through the transnuchal bone window the distal segment of the intracranial vertebral artery and the proximal segment of the basilar artery may be depicted.

Diagnostic criteria

Detection of an intracranial stenosis with ultrasound is based on the principle that reduction in the vessel lumen leads to an increase of the blood flow velocity (BFV). If a focal velocity increase is found, the degree of stenosis is defined comparing the highest BFV measurement to BFV thresholds. To define the best BFV thresholds in detection of intracranial stenosis, ultrasonographic examinations of normal and stenotic arteries have been compared with a reference method, usually digital subtraction angiography. TCD diagnostic criteria are based on mean velocity (MV) threshold values while TCCS usually employs peak systolic velocity (PSV) measurements. Several threshold values have been tested against different gold standard modalities for detection of ≥50% stenosis. A summary of the diagnostic performance of threshold values in detection of MCA stenosis by both TCD and TCCS are shown in Table 1.

Table 1. Diagnostic performance of threshold values in detection of ≥50% MCA stenosis

TCD		Sensitivity	Specificity	PPV	NPV
		%	%	%	%
MV	≥80 cm/s [a]	92	92	89	98
	≥100 cm/s [a]	100	97	88	100
	≥100 cm/s [b]	---	---	55	83
	≥100 cm/s [c]	78	93	73	94
TCCS					
PSV	≥ 220 cm/s [d]	100	100	100	100

PPV: positive predictive values NPV: negative predictive values

a) Navarro JC, Cerebrovasc Dis. 2007;23:325-330 [80]
b) Feldmann E, Neurology. 2007;68:2099-2106 [24]
c) Zhao L, Stroke. 2011;42:3429-3434 [81]
d) Baumgartner RW, Stroke. 1999;30:87-92 [82]

Several velocity thresholds have been proposed and tested to diagnose stenosis in the other segments of the circle of Willis (Table 2).

Table 2. Diagnostic performance of threshold values in detection of ≥50% stenosis in other circle of Willis segments

	TCCS[a]	**Sensitivity**	**Specifity**	**TCD[b]**	**Sensitivity**	**Specifity**
	PSV (cm/s)	%	%	MV(cm/s)	%	%
ACA	≥155	100	100	≥80	---	---
ICA[c]	≥120[c]	87[c]	83[c]	≥80[c]	93[c]	76[c]
PCA	≥145	100	91	≥50	---	---
Basilar	≥140	100	100	≥80[d]	69[d]	69[d]
Vertebral	≥120	100	100	≥80[d]	69[d]	69[d]

a) Baumgartner RW, Stroke. 1999;30:87-92 [82]
b) Alexandrov AV, Cerebrovascular ultrasound in stroke prevention and treatment. Wiley; 2011 [83]
c) You Y, J Neuroimaging. 2010;20:234-239 [84]
d) Zhao L, Stroke. 2011;42:3429-3434 [81]

The reliability of these diagnostic criteria will be discussed in the "Discussion" section.

Ultrasound contrast agents

Ultrasound contrast agents (UCA) are micron-sized bubbles containing low-soluble gas and surrounded by a thin outer layer or shell of phospholipids, sugars or polymer coating.[85] Gas-filled microbubbles scatter ultrasound much more effectively than a liquid or solid-filled microbubble of comparable size, and hence increase the magnitude of the received echo. UCA are used in all imaging modalities to increase the sensitivity of the imaging technique by increasing the image contrast between different structures.

UCA have been an active area of research since 1968, when opacification of the right cardiac ventricle was observed after injection of saline.[86] During the last three decades there has been efforts to develop stabilized microbubbles capable of trans-pulmonary passage for left side blood pool enhancement.[87] Since the introduction of UCA in neurosonology in 1993,[88] the application of UCA in this field has considerably increased. Common applications employing UCA include transcranial ultrasound studies in patients with severe hyperostosis of the skull, quantification of internal carotid stenosis in the presence of calcification, imaging of the carotid plaque angiogenesis[89] and brain perfusion.[90, 91]

Fundamental properties of ultrasound contrast agents

UCA used before commercial manufacture of UCA, i.e. agitated saline or dyes, tended to be large (>10 μm). Current UCA are comparable in size to red blood cells (2-6 μm), and are thereby able to pass through the lung capillary, arrive into the left ventricle, and be delivered to the intracranial vasculature. The behavior of UCA

within the ultrasound beam is complex and depends on several factors including shell composition, transmit frequency, and transmit power. The large difference between the acoustic impedance of plasma and the encapsulated gas generates much stronger echoes than those produced by RBC.[92] Gaseous microbubbles insonated by the ultrasound beam are forced to oscillate – contracting during the positive part of the ultrasound wave cycle and expanding during the negative part of the cycle. The degree of scattering and oscillation is maximum if the ultrasonic wave is at the resonant frequency of the microbubbles.[85] The microbubble resonance frequency depends on the size of microbubbles and lies within the clinical diagnostic frequency range of ultrasound. The reaction of microbubbles varies also according to the acoustic pressure (p_r). At low acoustic pressure (p_r<0.1 megaPascal [MPa]), the majority of microbubbles oscillates symmetrically in the ultrasound beam, contracting during the positive part of the cycle and expanding during the negative part of the cycle. The scattered ultrasound signal from the microbubble will therefore be the same as the ultrasound wave sent from the probe (incident wave). This is known as linear behavior. As the acoustic pressure increases (0.1<p_r<1.4 MPa), the expansion of the bubble during the negative phase will be different from the compression in the positive phase. During the negative phase, the bubble's radius can increase by as much as several hundred percent. During the positive phase, the gas inside the bubble rapidly stiffens as the molecules are forced closer together, and makes the bubble less compressible. This results in an asymmetric nonlinear bubble oscillation, which produces an echo with asymmetric top and bottom, instead of a sinusoidal echo. This asymmetry in the scattered ultrasound wave produces harmonics which can be exploited to differentiate the bubbles signals from the surrounding tissue. At even higher acoustic pressure (p_r≥1.4 MPa), the shell of the microbubbles may rupture, and release the gas from microbubble. The resulting free gas bubbles will strongly scatter ultrasound. However, they will rapidly dissolve into the bloodstream and will therefore only be visualized in few ultrasound frames. This strong interaction between the microbubbles and the high pressure ultrasound pulse will result in a scattered wave with a wide range of frequency components. Acoustic pressure is therefore an

important parameter when performing contrast-enhanced ultrasound. This parameter is not displayed by ultrasound scanners. However, the mechanical index (MI) can be used as an indicator of the likelihood of bubble rupture because MI is proportional to the acoustic pressure: $MI = p_r / \sqrt{f}$, where p_r is the peak rarefaction pressure of the ultrasound wave *in situ* (peak negative pressure) and f is the ultrasound frequency.

Contrast-enhanced transcranial ultrasound

Excessive signal attenuation by the skull occurs in 5% to 25% of patients.[93, 94] UCA overcome this limitation in almost all patients with poor transtemporal window.[95] The introduction of UCA therefore rendered possible otherwise failed Doppler exams.[96] UCA are administered intravenously either as bolus injection or as continuous infusion. UCA bolus injection results in a wash-in-wash-out enhancement curve characterized by a rapid increase and a short peak of strong enhancement, with subsequent decay over a few minutes. The duration of diagnostically useful enhancement is therefore relatively short (2-5 minutes), and blooming commonly occurs.[97] Blooming is an undesired saturation artifact compromising image quality and hindering Doppler measurement. It occurs when the intensity of the backscattered signal is too high, and is characterized by a false colour Doppler signal which extends outside the actual width of the vessel and covers most part of the Doppler sector. Blooming occurs commonly during the peak of contrast-enhancement after UCA bolus injection or if the rate of UCA continuous infusion is too high. Proper UCA continuous infusion prolongs the enhancement duration, avoids saturation artifacts, and provide a constant plateau of enhancement throughout the duration of infusion. In patients with poor transtemporal bone window, a stable and diagnostically useful enhancement contrast enhancement is reached by 0.4-0.5 ml/minute infusion rate and kept for 10-12.5 minutes. UCA continuous infusion should therefore be considered the method of choice.

Another pitfall related to UCA is an artificial increase in measured blood flow velocity (BFV). This phenomenon has been observed in studies administering UCA in patients with optimal transtemporal bone window and comparing the BFV measured before and after bolus injection.[98, 99] A 20–24 % increase in measured peak systolic velocity (PSV) after UCA bolus injection has been reported.[98, 99] The reason for the UCA-related increase in measured BFV remains unclear and still debated. Possible explanations will be presented and discussed in the "Discussion" section. The artificial increase in measured BFV caused by UCA implies diagnostic and clinical consequences. Current neurosonographic diagnostic criteria for intracranial stenosis are based on threshold BFV.[80, 82] A falsely elevated BFV will therefore lead to false positive cases or overestimation of the degree of the intracranial stenosis.

Ultrasound measurement of the cerebral blood flow

Transcranial ultrasound provides reliable measurements of blood flow velocity (BFV) which are accurate in detection of intracranial stenosis and occlusion.[80, 82, 100] BFV may be used as a surrogate of cerebral blood flow volume. Relationship between BFV and cerebral blood flow volume is defined by the formula $F = V * A$, where F is the cerebral blood flow volume, V is the BFV, and A is the area of the cerebral vessels. If the area is constant, flow volume and velocity change proportionally. However, cerebral arteries are extremely reactive and changes in the vascular bed make BFV no longer directly proportional to the cerebral blood flow volume. Thus, it is challenging to interpret changes in velocity without knowledge of the area of the vascular bed. TCCS provides anatomical visualization of the brain in the brightness-mode (B-Mode). The resolution with this technology is, however, too poor to provide sufficient anatomical information of the intracranial vessels, and it is therefore not possible to measure the diameter of the intracranial vessels. However, it has long been known that blood flow volume can be estimated by analyzing the Doppler spectrum.[101] Blood velocity measurements are based on the Doppler shift. The Doppler spectrum is the

result of many red blood cells (RBC) moving in the Doppler sample volume at different velocity. The received ultrasound signal therefore contains not just one frequency, but a spectrum of frequencies. Despite moving at different velocities, the received signal from each RBC has equal power. The power of the reflected Doppler signal is thus proportional to the number of RBC, and to the cross-sectional area of Doppler sample volume. Considering that 1) blood flow velocity may be expressed by Doppler shift frequency, 2) the power of the reflected Doppler signal is proportional to the cross-sectional area and 3) $F = V * A$ (see above), an estimation of the blood flow volume, expressed as a flow index, can be calculated from the weighted sum of each Doppler frequency (fi), and the corresponding power signal (Pi), i.e. flow index (FI) = $\sum fi * Pi$. The flow index does not provide an absolute value of the cerebral blood flow but a series of instantaneous estimations which may detect relative changes in flow volume. Once the flow index has been calculated, an estimation of the cross-sectional area (area index, AI) may be derived by the formula: $AI = FI / V$. However, several factors may distort the insonating ultrasound beam and the reflected Doppler signal, and hinder the correct calculation of the flow index. The major disturbing factor is represented by the heterogeneity of tissues lying between the transducer and the vessel, and most specifically the temporal bone, which causes attenuation and refraction of the ultrasound beam and non-uniform insonation of the vessel.[102, 103]

The development of a feasible and reliable estimation of the blood flow volume may have important clinical applications and is therefore a focus for continuous research.

List of publications

This thesis is based on the following papers:

I Logallo N, Naess H, Waje-Andreassen U, Thomassen L.
Intracranial Atherosclerosis in Norway. Preliminary Results from the Norwegian – Intracranial Atherosclerosis Study.
Submitted

II Logallo N, Lind J, Naess H, Idicula TT, Brogger J, Waje-Andreassen U, Thomassen L.
Middle Cerebral Artery Stenosis: Transcranial Color-Coded Sonography based on Continuity Equation versus CT-Angiography.
Ultraschall in der Medizin. E-print August 2012

III Logallo N, Fromm A, Waje-Andreassen U, Thomassen L, Matre K.
Effect of Microbubble Contrast on Intracranial Blood Flow Velocity assessed by Transcranial Doppler.
Submitted

IV Wallace S, Logallo N, Faiz KW, Lund C, Brucher R, Russell D.
Relative Blood Flow Changes Measured using Calibrated Doppler Spectral Power at varying Hematocrit Levels: an *in vitro* Study.
Submitted

Aims of the thesis

1. To assess the prevalence of symptomatic and asymptomatic intracranial atherosclerosis in a Norwegian, community-based, ischemic stroke and TIA population.

   We hypothesized a relative low prevalence of intracranial atherosclerosis. This hypothesis is discusses in Paper I

2. To assess the diagnostic performance of an ultrasonographic method based on continuity equation in detection and grading of intracranial stenosis, with CTA as reference method.

   We hypothesized the continuity equation method to be superior to the cut-off velocity method in detection of intracranial stenosis. This hypothesis is discussed in Paper II.

3. To assess the effect of both UCA continuous infusion and gain reduction on transcranial Doppler measurements of blood flow velocity.

   We hypothesized that UCA continuous infusion leads to an increase in measured blood flow velocity and that optimal gain reduction is able to restore the blood flow velocity measured at baseline. This hypothesis is discussed in Paper III.

4. To assess whether analysis of Doppler spectrum signal may detect relative changes in blood flow volume.

   We hypothesized that a flow index calculated from the weighted sum of each Doppler frequency and the corresponding power signal detects relative changes in blood flow volume. This hypothesis is discussed in Paper IV

Material and Methods

The data for papers I, II, III were obtained from a cohort of the Bergen NORSTROKE study. The study period was 18 months for paper I and 10 months for paper II. All patients with diagnosed ischemic stroke or TIA were included in Paper I. Patients with intracranial stenosis undergoing both CT-angiography (CTA) and transcranial color-coded sonography (TCCS) were included in paper II. Ten patients with TIA or minor stroke with normal CTA were included in paper III.

The data for paper IV were obtained from *in vitro* experiments on a closed-loop phantom. *In vitro* experiments were carried out at the Department of Medical Engineering, Hochschule Ulm, University of Applied Sciences, Ulm, Germany.

Bergen NORSTROKE study

The Bergen NORSTROKE study is a prospective study which includes all ischemic stroke and TIA patients admitted to the Centre for Neurovascular Diseases, Department of Neurology, Haukeland University Hospital, Bergen, which serves a well-defined population of 244 170 inhabitants.[104] After obtaining patient-signed informed consent, demographical and clinical data, laboratory and radiological findings and outcome were registered. The study was approved by the local ethics committee.

Diagnostic work-up

In paper I, II, and III, the patients diagnostic work-up included ECG, Holter monitoring, echocardiography, and extracranial Duplex ultrasonography. Intima-media thickness was measured in the right distal carotid common artery. Extracranial

carotid artery stenosis was defined as <50% or ≥50% based on percentage of area reduction at ultrasonography. A multimodal, non-invasive approach including TCCS, CTA and MR-Angiography (MRA) was chosen to investigate the presence of ICAS.

Transcranial color-coded sonography

TCCS was performed in all patients on the day after admission (day 1). All TCCS examinations were performed by the Ph.D. candidate (N.L.) using a S5-1 probe (iU22, Phillips Medical Systems, Bothell, WA, USA). The intracranial internal carotid artery (ICA), the middle cerebral artery (M1 and M2 segments), the anterior cerebral artery (A1 and A2 segments) and the posterior cerebral artery (P1 and P2 segments) were insonated by the transtemporal bone window bilaterally. Peak systolic velocity (PSV) was measured from the origin to the distal part of each segment with a 2 mm sample volume, a stepwise depth decrement of 1 mm, and a stepwise optimal angle correction of Doppler sampling. The localization of the Doppler sample volume was confirmed by updating the color flow image at every step.

Transcranial Doppler and contrast-enhanced transcranial Doppler

With the patient in supine position, TCD examinations were performed using a 2 MHz probe (Companion III Nicolet Vascular, Madison, WI, USA) secured to the patient's head using a head band. The right MCA was insonated with a 15-mm sample volume centered at 52 mm insonation depth. SonoVue (Bracco, Italy), a microbubble contrast agent containing sulphur hexafluoride gas stabilized by phospholipids, was used as ultrasound contrast agent (UCA). UCA was administered via antecubital fossa venous access using an infusion pump (VueJect, Bracco, Italy).

CT and CTA

CT was performed in all patients on admission. If not contraindicated, CTA was performed on admission in patients admitted within 6 hours from stroke symptoms,

whereas in patients admitted after 6 hours from stroke symptoms CTA was performed if TCCS and/or MRA identified ICAS. The CT scanners used were GE Lightspeed Ultra or Toshiba Aquillion 64. Contrast injection rate was 5 ml/s iv with a total contrast volume of 80 ml for the GE Lightspeed ultra and 70 ml for the Toshiba Aquillion 64. Either Visipac 320 mg/ml or Iomeron 350 mg/ml was used in all cases. The same intracranial segments investigated with TCCS were evaluated with CTA for stenosis and occlusions. All measurements were done using AGFA IMPAX 4.5 workstation for 3D reconstructions with volume rendering technique to aid in detection and characterization of stenosis.

After identification of a candidate stenosis, reduction in luminal caliber was documented on the 2D grayscale MPR images. Prestenotic (D_p) and intrastenotic (D_i) diameters were measured by an electronic ruler. Percent stenosis was calculated as: $[1-(D_i/D_p) \times 100]\%$.[71] All image interpretation and measurements were made by a neuroradiologist (J.L.) who was aware of the presence of stenosis, but was blinded to site and degree of stenosis. CTA represented the reference method in Paper II.

MRI and MRA

Diffusion weighted MRI and time-of-flight MRA were performed as part of a routine MRI protocol for stroke patients on Siemens Magnetom 1.5 Tesla (Symphony) in all patients on day 1 if not contraindicated. The investigated arterial segments were the same as for TCCS and CTA. The degree of stenosis was defined following the WASID method[72] and grouped into <50% and ≥50%.

Diagnostic criteria for intracranial stenosis and intracranial atherosclerosis

CTA and MRA diagnostic criteria for intracranial stenosis (IS) used in paper I and II have been presented in the CT-CTA and MRI-MRA section.

An IS was indicated by a focal increase in blood flow velocity (BFV). The focal increase in BFV was defined as $[1-(PSV_{prestenotic}/PSV_{intrastenotic}) \times 100]\%$;[105] PSV = peak systolic velocity. This formula has been derived from the continuity equation principle.[69] The diagnostic threshold for IS was set at 26%. Each stenosis was therefore defined as percent area reduction between 26% and 99%. $PSV_{intrastenotic}$ was defined as the highest velocity measured in the area of focal velocity increase. $PSV_{prestenotic}$ was defined as the most distal velocity measurement before focal velocity increase.

IS were also divided in < or ≥50% stenosis according to three different methods: a) continuity equation, i.e. $[1 - (PSV_{prestenotic}/PSV_{intrastenotic}) \times 100]\%$; b) published and widely used[82] velocity cut-off values which define stenosis as <50% if $PSV_{intrastenotic}$ ≥155 cm/s and <220 cm/s, or as ≥50% if $PSV_{intrastenotic}$ ≥220 cm/s; c) cut-off velocity value which provided the highest sensitivity and specificity for MCA stenosis ≥50% in our study, i.e. 180 cm/s. IS was diagnosed if detected by either TCCS, CTA or MRA.

IS was considered atherosclerotic (ICAS) if possible or probable cardioembolic causes or other defined causes (e.g. vasculitis, dissection) were ruled out by clinical and diagnostic work-up. ICAS was considered symptomatic if located in the arterial segment supplying the infarct area.

In vitro closed-loop phantom

The *in vitro* experiments investigating the feasibility and reliability of a software designed to calculate a flow index from the sum of frequency-weighted calculations of the Doppler power (page 27-28) were performed using a closed-loop system of silicon tubes containing saline and human whole blood.

Blood was obtained from the local transfusion bank, having exceeded its clinical usage date the previous day. Forward flow was generated using a digital roller pump, (Ismatec, MCP Process Pump, Glattburg, Switzerland). The blood was heparinized, kept in constant flow and continuously filtered using a micro-filter to prevent contamination by either gas bubbles or solid micro-particles. A Windkessel function was built into the system. A constant temperature of 32°C was maintained within the closed-loop system by passing the tubes through a heated water bath and the temperature was monitored using a digital thermometer (BBC, Goerz metrawatt, M4051, Austria).

Four different silicone tubes with inner diameters of 1.5, 2, 3 and 4 mm and a wall thickness of 0.5 mm were insonated with a 2 MHz TCD probe (DWL Compumedics, Singen, Germany). Each of the four tubes was insonated through a 5 mm thick Plexiglas and a water bath, and examined with constant power and gain settings. The probe was secured at a 45° angle of insonation with a specially designed Plexiglas holder. The sample volume length and the insonation depth were varied until the maximum Doppler power signal was obtained. By maximizing the received signal power, it can be assumed that the maximum beam intensity lies within the central region of the parabolic flow in the tube being insonated, i.e. the area of highest flow velocities. Maximizing the signal power is also necessary to ensure that the insonating beam intensity distribution is approximately equal for each tube studied. The high-pass frequency filter was set to a standard setting of 100 Hz. Frequency-weighted first

moment calculations of the Doppler power were made using specifically designed software. The measurements were recorded and averaged over a ten second period. Calculated flow index and velocity values were then vertically shifted to pass through the zero intercept with a velocity value of zero corresponding to flow index value of zero (off-set corrected). To enable calculation of relative changes (percentage) in both flow and area indices the flow index value in the largest tube (4 mm diameter) was designated to be 100%. Flow index measurements were made at flow rates of 150, 240 and 320 ml/min.

Heparinized whole blood with an initial hematocrit (Hct) value of approximately 60% was used for all recordings. The closed-loop system was initially filled with 0.9% saline and all gas bubbles removed. Heparinized whole blood was added gradually to the system and the saline removed. Extreme care was taken not to introduce gas bubbles as blood was injected. Blood was allowed to flow through the system for at least 3 minutes before recordings. Increasing Hct values were measured directly using a centrifuge (Hemokrit 4, Lic Instruments, Stockholm, Sweden) and Hct graph (Heræus sepatech, Osterode/Harz, Germany). Doppler measurements were then carried out at the 3 different flow rates above and at Hct values of 10, 20, 29 and 42%. The Hct value was controlled at the end of each set of recordings for each flow value, to ensure it had not fallen.

Results

Prevalence of intracranial atherosclerosis (Paper I)

The prevalence of intracranial atherosclerosis was calculated by screening 607 consecutive ischemic stroke or TIA patients which were admitted to our Neurovascular Centre over an 18-month study period. MRA was performed in 533 (87.8%) and CTA in 227 patients (37.4%). TCCS was performed in all patients and a fair/good transtemporal bone was found in 485 patients (79.9%). At least one vascular imaging method was available in 575 patients (94.7%). Out of the 607 patients admitted with an ischemic event, 562 had ischemic stroke and 45 TIA. Intracranial stenosis was found in 54 patients (8.9%; 95% CI: 6.6% - 11.1%). Seven patients presenting intracranial stenosis had a possible or probable cardioembolic cause, and intracranial atherosclerosis was therefore diagnosed in the remaining 47 patients (7.7%; 95% CI: 5.6% - 9.8%). ICAS was symptomatic in 30 patients (4.9%; 95% CI: 3.2% - 6.7%). Concomitant asymptomatic ICAS was found in 13 of the 30 patients with symptomatic ICAS.

The total number of ICAS lesions was 69, 30 symptomatic and 39 asymptomatic. Among the symptomatic lesions, 8 (27%) were <50%, 20 (66%) were 50-69%, and 2 (7%) were ≥70%. Among the asymptomatic lesions, 22 (56%) were <50%, 17 (44%) were 50-69%, and none was ≥70%. Moderate-high degree of stenosis (≥50%) was significantly associated with symptomatic ICAS (OR 7.27; 95% CI: 1.63-32.41; p<0.01). Diabetes mellitus was the only risk factor significantly associated with symptomatic ICAS (OR 2.55; 95% CI: 1.03-6.31, p=0.04).

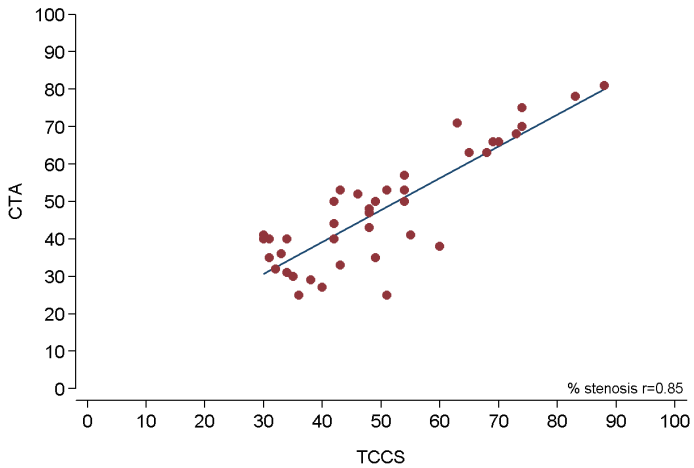
Diagnostic performance of transcranial color-coded sonography (Paper II)

Screening for intracranial stenosis was performed on 278 consecutive patients with adequate transtemporal bone window and confirmed ischemic stroke or TIA over a 10-month study period. At least one M1 or M2-MCA stenosis was diagnosed in 25 patients (9%) by the continuity equation TCCS method. Four patients were excluded because of contraindication to CTA. Twenty-one patients matched the inclusion criteria [14 men, 7 women; age (±SD) 74.2 years (12.3)]. All stenosis were confirmed by CTA. The 21 patients included into the study presented 40 MCA stenoses, 29 in the M1 and 11 in the M2 segments.

TCCS continuity equation method vs CTA

The overall correlation coefficient for degree of MCA stenosis between TCCS and CTA was 0.85 ($p<0.0001$), Figure 3.

Figure 3. Correlation between degree of stenosis: TCCS vs CTA



The overall correlation coefficient for M1 stenosis was 0.87 ($p<0.0001$), and for M2 stenosis 0.71 ($p<0.01$). Correlation coefficient for MCA stenosis defined with CTA as ≥50% was 0.94 ($p<0.0001$).

Continuity equation vs cut-off velocity method

Continuity equation method showed a sensitivity of 78% (14/18) and a specificity of 86% (19/22) in detection of ≥50% MCA (M1 and M2) stenosis. Cut-off velocity method based on previously published criteria,[82] showed a sensitivity of 50% (9/18) and a specificity of 91% (20/22) in detection of ≥50% MCA stenosis, and failed to detect 16 stenoses, 4 defined by CTA as ≥50% and 12 as <50%. The most accurate $PSV_{intrastenotic}$ value for detection ≥50% MCA stenosis in our patients cohort was 180 cm/s and provided a sensitivity of 67% (12/18) and a specificity of 86% (19/22). When only M1 was considered, continuity equation method showed a sensitivity of 85% (11/13) and a specificity of 81% (13/16) in detection of ≥50% M1 stenosis. Cut-off velocity method based on previously published criteria,[82] showed a sensitivity of 54% (7/13) and a specificity of 94% (15/16) in detection of ≥50% M1 stenosis, and failed to detect 12 stenoses, 2 defined by CTA as ≥50% and 10 as <50%. The most accurate $PSV_{intrastenotic}$ value for detection of ≥50% M1 stenosis was 180 cm/s and provided a sensitivity of 77% (10/13) and a specificity of 86% (14/16).

Effect of ultrasound contrast agents on velocity measurements (Paper III)

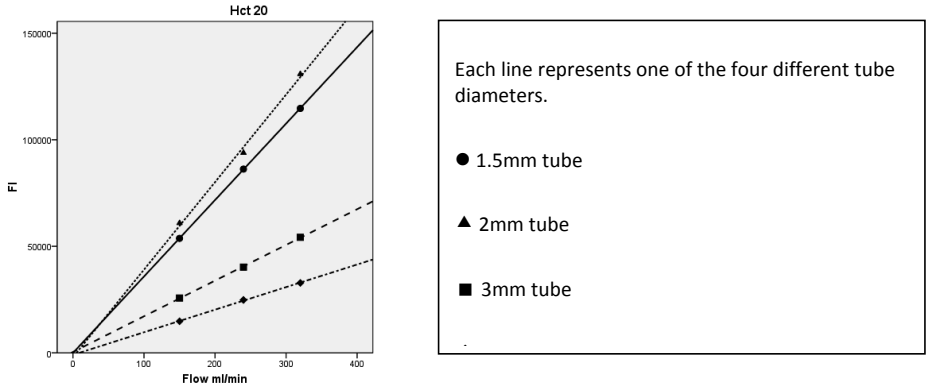
Ten patients were examined (8 male; median age 56.5 years; age range 35-67 y). Blood flow velocity (BFV) measured during UCA infusion with no gain adjustment was significantly higher than baseline BFV (PSV: 85.1 ± 19.7 vs. 74.4 ± 19.7 cm/s, $p<0.0001$; MV: 56.5 ± 11.8 vs. 50.2 ± 12.3 cm/s, $p<0.0001$). The percent increase in measured PSV was 16 ± 9% (range 6-33), and the percent increase in measured MV

was 14 ± 10% (range 4-33). BFV measured during UCA infusion with gain reduction was not significantly higher than baseline BFV (PSV: 74.3 ± 18.9 vs. 74.4 ± 19.4 cm/s, p=0.8; MV: 49.4 ± 11.0 vs. 50.2 ± 12.3 cm/s, p=0.8). No artefact hindering velocity measurement was noted during the recordings.

In vitro blood flow volume measurements (Paper IV)

The correlation between the calculated flow index (FI) against the absolute flow was strong for each tube size at each hematocrit (Hct) values (r>0.98, p=0.01).

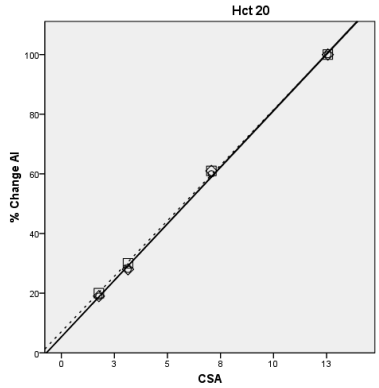
Figure 4. Correlation between the calculated flow index and the absolute flow



The correlation between the calculated area index (AI) against the absolute cross-sectional area (CSA) was strong for each absolute flow volume at each Hct values (r>0.99) (Figure 5).

The effect of varying Hct on flow index is shown in Figure 6.

Figure 5. Correlation between the calculated area and absolute the cross-sectional area



The absolute cross-sectional area (CSA) was calculated from the diameter of each tube. The 4 mm tube (CSA = 12.56 mm^2) was defined as 100% Area Index (AI). Pearson linear correlation value is >0.99.

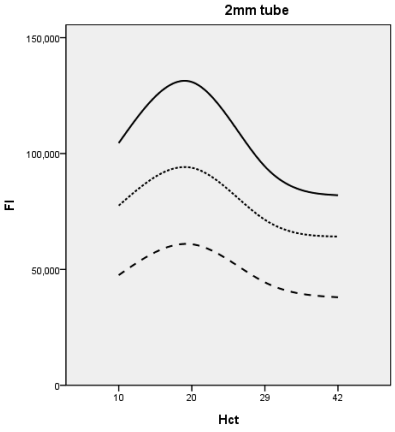
Each absolute flow is represented by different-shape points:

○ 150 ml/min

◊ 240 ml/min

□ 340 ml/min

Figure 6. Relationship between the flow index and hematocrit



Plot of Flow Index (FI) against rising haematocrit (Hct) in presence of constant flow volume and tube size.

Each curve represents a different absolute flow volume:

—— 320 ml/min
····· 240 ml/min
- - - - 150 ml/min

Discussion

Prevalence of Intracranial Atherosclerosis in ischemic stroke and TIA patients

The focus on intracranial atherosclerosis (ICAS) has dramatically increased over the last two decades, and it is now settled that ICAS is one of the leading causes of ischemic stroke and TIA worldwide.[11] Several studies have shown that the prevalence of ICAS is strongly influenced by ethnicity. In Asian ischemic stroke patients, ICAS is found in up to half of the cases,[11, 106-109] and in African Americans and Hispanics in 6% and 11% respectively.[12] In Caucasians, the prevalence of ICAS varies from 1% in White-American[12] to 12.4% in Spanish ischemic stroke patients.[15] Studies from Netherland and Germany reported a prevalence of symptomatic ICAS in ischemic stroke patients of 3% and 6.5% respectively.[13, 110]

Asians are at the highest risk to develop ICAS, and predisposition toward hypoadiponectinemia in this ethnicity may represent a possible explanation.[111] Adiponectin is an adipose tissue-specific cytokine found to be reduced in obesity, diabetes mellitus type 2, insulin resistance, hyperlipidemia, metabolic syndrome, and cardiovascular diseases.[112, 113] Adiponectin appears to have a protective role against atherosclerosis by inhibiting neointimal formation and suppressing the expression of inflammatory cytokines and adhesion molecules.[114] Asians appear to have lower adiponectin levels than Caucasian subjects of similar age, body mass index and total adiposity.[111] Low adiponectin levels may therefore play an important pathogenic role in development on atherosclerosis, but it is still unclear why low adiponectin levels may selectively lead to atherosclerosis in the intracranial vessels.

It is also unclear why there is a discrepancy in ICAS prevalence among patients from different European countries. The heterogeneity in diagnostic criteria, imaging modalities and study populations may be a possible explanation. Diagnostic performance in detection of ICAS varies not only between different modalities, but also within a specific modality depending on technique, setting and/or diagnostic criteria. Diagnostic performance also depends on the investigated vascular territory. MRA and ultrasonography both have lower sensitivity in detection of ICAS in the posterior circulation, especially in the vertebrobasilar system, compared to the anterior circulation,[81, 115] and CTA should be considered the posterior circulation gold standard in absence of DSA. It may therefore be expected that studies using different modalities will report discordant results about the distribution of ICAS lesions in the anterior and the posterior circulation. However, there are few and inconclusive data answering this hypothesis. One ultrasonographic based study has shown that the 72% of symptomatic ICAS lesions were located in the anterior circulation, versus 62% reported by one CTA based study.[110] This difference was due to the more frequently detected ICAS lesion in the posterior cerebral artery by CTA (12.5% vs 4.7%), whereas the percent of vertebrobasilar stenosis was similar between the studies (CTA 25%; TCD 23%).

In our cohort of patients, ~8% presented at least one ICAS and ~5% had symptomatic ICAS. Our results are not directly comparable with other studies since the vertebrobasilar system was not investigated. However, assuming a 25% rate of symptomatic ICAS in the vertebrobasilar system, as suggested by earlier studies,[32, 110] a prevalence of symptomatic ICAS of 6.4% could be expected in our population, which is in line with a study from Germany (6.5%)[34] (Table 3). In our study, the diagnosis of ICAS did not require confirmation by a reference method if one of the three modalities detected ICAS, and low-grade symptomatic stenoses (<50%) were also included. This approach may theoretically overestimate the prevalence of symptomatic ICAS. However, our results are lower than results from studies in Southern Europe (9.2 and 12.4%).[15, 32] These studies may have underestimated the

prevalence of ICAS because of methodological design (e.g. TCD based studies excluding patients with poor transtemporal bone window, and requiring confirmation of ≥50% stenosis with MRA/CTA). Methodological variability among European studies may therefore not fully explain the regional differences in prevalence of symptomatic ICAS.

Table 3. Distribution of ICAS lesions in a Greek study and our study

	Greece†		Bergen	
	n=43	%	n=49*	%
M1	15	35	14	40
M2	6	14	5	15
Anterior	2	5	0	0
ICA	8	18	4	12
Posterior	2	5	7	20
Vertebral	6	14	5.4*	14
Basilar	4	9	3.6*	9
Total ICAS prevalence	9.2%		6.4*%	

* Values estimated by the prevalence of vertebrobasilar stenosis reported in the Greek study†.
† Tsivgoulis G, J Neuroimaging. 2012

Community-based or hospital-based studies performed in the western part of the world have not distinguished between ischemic stroke and TIA patients when calculating the prevalence of ICAS. One study from China[9] has detected ICAS in ~50% of TIA patients, thus suggesting that the prevalence of ICAS may not differ between TIA and ischemic stroke patients. In our cohort of patients with acute cerebral ischemia screened for ICAS, 45 had TIA, of which 5 (11%) presented ICAS and 2 (4.5%) symptomatic ICAS. Although the limited sample size does not allow to draw definite conclusion, ICAS may occur equally in both ischemic stroke and TIA patients also in a Caucasian population.

Diagnosis of Intracranial Atherosclerosis

The Consensus Conference on Intracranial Atherosclerotic Disease held in 2009[116] suggested to use the term "intracranial atherosclerosis" for lesions defined by autopsy studies, and "intracranial stenosis" for lesions defined by other methods.[117] Even though both clinical and autopsy studies have the same object of investigation, i.e. the intracranial plaque, intracranial atherosclerosis is still an exclusion diagnosis when based on vascular imaging. Routine diagnostic methods, i.e. digital subtraction angiography, CTA, MRA and ultrasound are accurate in defining intracranial stenosis but are not able to visualize the intracranial atherosclerotic plaque. Emerging methods for non-invasive visualization of the intracranial atherosclerotic plaque, such as high-resolution MRI (HR MRI)[118] and intravascular ultrasound,[119] are promising but seldom available.

There is no consensus on the criteria which intracranial stenosis must fulfill to be defined as atherosclerotic, nor any studies comparing the accuracy of the imaging diagnosis of intracranial atherosclerosis with the "real" gold standard, i.e. autopsy. Inspired by the TOAST classification,[120] most studies have defined as atherosclerotic those intracranial stenoses which were ≥50%, with negative work-up for cardiac embolism, no severe extracranial stenosis, and no other possible etiology for the stenosis, e.g. MoyaMoya disease, vasculitis, or dissection.[13, 14, 121-124] This approach has some limitations. Intracranial stenoses <50% are excluded because considered as "non-significant".[120] However, evidences from both cardiac and carotid atherosclerosis have shown that several histological and morphological characteristics of the plaque, such as large lipid core, thin fibrous cap, and plaque vascularization, may predict plaque rupture independently from the degree of stenosis.[89, 125, 126] Even though an accurate grading of the intracranial stenosis is important in risk stratification of patients,[127] it is incorrect to consider a low-grade stenosis as a non-

threatening lesion. In our study, ~30% of patients with symptomatic intracranial atherosclerosis presented lesions which were <50%.

Detection of low-grade stenosis is not free of pitfalls and challenges. Intracranial vessels have smaller diameter and are less accessible than extracranial arteries, and the resolution of non-invasive method may not be high enough to detect minor irregularities of the vessel wall.[128] However, applying continuity equation to transcranial ultrasonography may ensure detection of low-grade stenosis. The continuity equation states that each reduction in the area of the vessel corresponds to a change in blood flow velocity. In theory, even the smallest stenoses should be detectable by ultrasound. In practice however, it is not possible to differentiate between physiological and pathologically velocity changes if the changes are small, since tortuosity, bifurcation, and perforating arteries may lead to physiological increase in velocity. We therefore set the minimal detectable area reduction at 26%, which corresponds to ~35% focal increase in velocity. All low-grade stenoses detected by TCCS applying the continuity equation were confirmed by CTA, suggesting that this method is highly accurate. However, remodeling of arteries may limit the accuracy of this method.[129] This phenomenon, described originally in coronary arteries, consists of a compensatory enlargement of the vessel wall which allows the plaque to grow outward without compromising neither vessel lumen nor blood flow velocity.

In our patients cohort, 14 out of 114 (12.3%) patients with atrial fibrillation (AF) presented >50% extracranial stenosis (ES). Atherosclerosis is therefore not uncommon in patients with AF. In case of AF and ES, atherosclerosis is easily recognizable with ultrasound, and diffusion-weighted MRI may often help to define which of the two is the probable underlying cause of ischemic stroke. An intracranial stenosis in patients with AF may represent either a recanalizing cardiac thrombus or an intracranial plaque. Excluding *a priori* patients with AF may therefore underestimate the number of ICAS lesions. However, it is difficult to differentiate

between these two conditions with the currently available routine modalities. The detection of rapidly improving stenosis at daily ultrasonographic follow-up may suggest the presence of a recanalizing thrombus, whereas a more "stable" lesion may indicate the presence of ICAS.

It is also challenging to define whether ICAS lesions are symptomatic or not. Our current understanding of the intracranial plaque is limited by the absence of standardized methods to detect plaque instability, and is still based on indirect clues, such as the location of intracranial stenosis and infarct, and the exclusion of other plausible causes. One case-report has shown that HR MRI may visualize intraplaque hemorrhage in an intracranial plaque.[130] Another study investigating a possible role of gadolinium plaque enhancement in patients with severe MCA stenosis, failed to show that plaque enhancement is a marker of symptomatic or unstable plaque.[131] However, the small sample of the study (n=6) does not permit definite conclusions.

A hopefully higher availability of HR MRI in the future will 1) increase the sensibility of non-invasive method in detecting intracranial plaque, especially in an early stadium, 2) detect non-stenosing plaque, i.e. plaque undergoing remodeling, 3) ensure a better understanding of plaque stability, and 4) help to differentiate a recanalizing cardiac embolus from an *in situ* atherosclerotic plaque.

Role of ultrasound in the diagnosis of intracranial stenosis

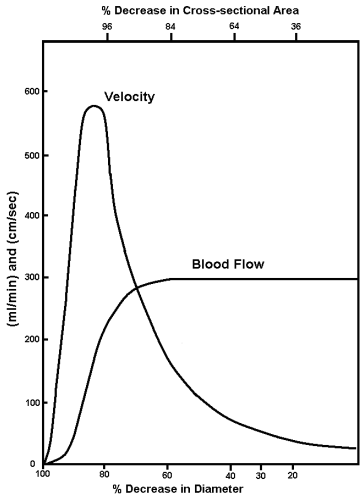
Transcranial ultrasonography is a non-invasive, bed-side, convenient, and inexpensive modality for the evaluation of the intracranial arteries.[132] Diagnostic criteria for intracranial stenosis are based on the detection of blood flow velocity (BFV) exceeding predefined "normal" velocity values. As shown in Tables 1 and 2, threshold values appear to provide high diagnostic performance. However, these results may not reflect the actual diagnostic performance of TCD and TCCS in an

unselected ischemic stroke population. Most studies are affected by selection and verification bias, as well as publication bias in favor of the best results. The most common verification bias is represented by the availability of the reference method only in patients were TCD or TCCS suspected pathology. There is also a considerable difference between studies performed in the early 1990s,[80] reporting high diagnostic performance values, and more recent studies,[24, 81] showing lower values, and in particularly very low positive predictive values (PPV) (36%).[24] There are very few studies which have tested TCCS against DSA and current TCCS diagnostic criteria for intracranial stenosis are mainly based on one study performed in the late 1990s[82] reporting 100% sensitivity, specificity, PPV and negative predictive values of threshold velocity ≥220 cm/s (PSV) for detection of ≥50% MCA stenosis. Both selection and verification bias may have led to such extremely good results. Patients included in that study had symptomatic ICAS detected at TCCS (degree ≥50% may be assumed) and/or ≥70% extracranial stenosis (ES). Patients with ICAS were therefore already selected by TCCS, whereas those patients with no ICAS had ≥70% ES, and thus low velocity in the MCA. The difference in velocity between stenotic and normal MCA may have been increased by these selection criteria.

Intracranial velocities vary among subjects depending on age, state of the extracranial arteries, hematologic and endocrine disorders. The threshold method may therefore either overlook stenosis in subjects with low intracranial velocity or provide false positive results in subjects presenting high intracranial velocities. Low intracranial velocities are often found in older patients and/or in presence of extracranial high-grade stenosis. False positive results may be found in patients with disturbed autoregulation, in conditions with general increase in blood flow volume and velocity such as arteriovenous malformations, anemia or hyperthyreosis, and in case of improper angle correction or use of ultrasound contrast agents. The use of absolute threshold values may therefore be misleading because it does not take into account cerebral hemodynamics as a whole. Other hemodynamic aspects, such as flow direction, pre- intra- and poststenotic velocity, comparison of velocity with the

contralateral artery and the other ipsilateral arteries, musical murmurs, bruits and pulsatility index may help in detection and characterization of the stenosis, and possibly provide better diagnostic results. An illustration of how blood flow velocity and blood flow volume are influenced by different degree of stenosis is shown in Figure 7.

Figure 7. Correlations between blood velocity, blood flow and stenosis grading

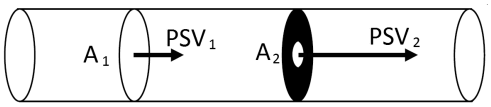


Adapted from Spencer MP. Stroke 1979, 10(3):326-330

This hypothetical flow model, also known as the Spencer curve,[69] is based on the Hagen-Poiseuille law and the continuity equation, and applies to an axis-symmetric and smooth-surface arterial stenosis in a straight vessel with no bifurcations. Although in a clinical setting most stenoses are axis-asymmetric, have irregular surface, variable length and compliance, this model represents a "milestone in understanding cerebral hemodynamics with long-lasting practical and research implications".[133] The curve shows that blood flow velocity increases exponentially

and reaches a top which corresponds to ~80% diameter stenosis. As long as the increase in velocity is able to keep flow volume constant, it is correct to assume that the product of the cross-sectional areas (A) time the blood velocity (V) is constant throughout the vessel, as stated by the continuity equation: $A_1*V_1 = A_2*V_2$ (Figure 8).

Figure 8. The continuity principle



A = cross-sectional area
PSV = peak systolic velocity

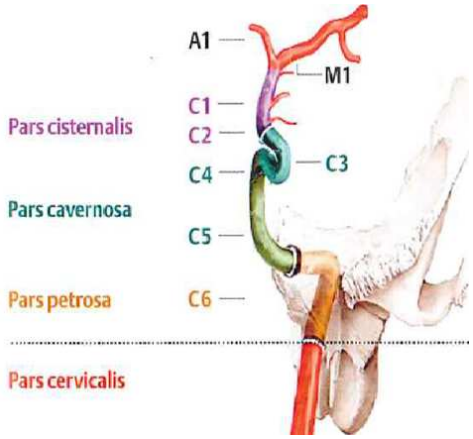
The degree of stenosis may be calculated as $[1–(PSV_{prestenotic}/ PSV_{intrastenotic})x100]\%$. This formula ensures an exact calculation of degree of stenosis, and our study (Paper II) has shown that this method is accurate when compared to CTA. Since the risk of stroke in the territory of an intracranial stenotic artery appears to be directly proportional to the severity of the stenosis,[127] accurate calculation of the degree of stenosis may have clinical implications. According to the continuity equation, a doubling of velocity, which may also be expressed as a ratio between intrastenotic and prestenotic velocities of 2, corresponds to 50% stenosis. A ratio ≥2 between intrastenotic and prestenotic velocity may therefore be more accurate than threshold values in detection of ≥50% stenosis. However, a recent TCD study[81] has failed to show that the use velocity ratio, or the use of either ratio or threshold values, are better than threshold values alone in detection of ≥50% MCA stenosis. This is in contrast with our study showing that the use of continuity equation has higher accuracy than threshold values in detection ≥50% MCA stenosis. Although both studies are based on continuity equation, the use of two different ultrasound technologies, i.e. TCD vs. TCCS, may have led to discordant results. TCD based

studies mostly use mean velocities (MV), whereas TCCS based studies commonly use peak systolic velocities (PSV). *In vitro* experiments have shown that calculating the degree of stenosis with continuity equation provides the most exact results when PSV rather than MV are used.[134] TCCS may also provide the most accurate velocity measurements because color-coding warrants both optimal placement of small sample volume and angle correction all through the artery. Continuity equation may therefore be an excellent tool for evaluation of intracranial stenosis, but it cannot be applied to the whole spectrum of stenosis. Near occlusive stenoses are characterized by abnormal low velocity and blunted Doppler waveforms. "Low velocity findings", defined as reduction ≥30% in MV compared to the non-stenotic side, plus the presence of blunted Doppler waveform, have been proposed as specific diagnostic criteria for these stenosis and appear to have high sensitivity.[81]

The continuity equation method may theoretically be applied also to other segments of the Circle of Willis. The M1 segment is often straight and almost parallel to the ultrasound beam, so that small degree of angle correction is required. However, the anatomy of the other segments creates challenges. The carotid siphon is extremely tortuous and, besides the proximal C5 segment (pars petrosa), is perpendicular to the ultrasound beam, thus not allowing angle correction (Figure 9). It is therefore not possible to achieve reliable velocity measurements throughout the length of this artery. It may be hypothesized that using velocity measurements from the corresponding contralateral siphon may be more accurate than ipsilateral prestenotic velocity measurements.

The pars precommunicalis (A1) of the anterior cerebral artery (ACA) may be difficult to visualize in one single axial plane and it may therefore be difficult to define whether velocity increase is focal or not. Ultrasound scanning in the coronal plane may visualize the siphon and the origin of both A1 and M1 in one plane, and may therefore clarify if there is a diffuse velocity increase in these segments. Velocity increase in A1 is more often due to other conditions than intracranial stenosis, such as

Figure 9. Anatomical segment of the intracranial carotid internal artery (siphon)



From Valdueza J. Neurosonology and neuroimaging of stroke. Thieme; 2008 [135]

contralateral internal carotid artery occlusion, stenosis in the ipsilateral C1/C2 segments, and/or near-occlusive stenosis in the ipsilateral M1. These conditions should therefore be ruled out before considering the presence of A1 stenosis.

The pars infracallosa (A2) of the ACA is usually straight and perpendicular to the ultrasound beam, and measurements in this segment must therefore be performed without angle correction. However, the low resolution of the color-mode in most of the cases does not permit to differentiate between the right and the left segments since both segments are visualize as one single segment.

Continuity equation may theoretically be applied to the P1 segment and the proximal part of P2 since they are parallel to the ultrasound beam and optimal angle correction is possible. The distal P2 segment is tortuous and does not allow angle correction. As for the siphon, using "normal" contralateral velocity may be more accurate than ipsilateral prestenotic velocity measurements.

Ultrasound contrast agents

Transcranial ultrasonography is hindered by hyperostosis of the skull, a condition which most frequently occurs in elderly subjects, especially females. Due to the predominance of elderly among ischemic stroke patients, the lack of transtemporal bone window is a problem for neurosonographers working in stroke units. The rate of poor transtemporal bone window in our study population (~20%) was in line with data reported in earlier studies.[93, 94]

The use of modern ultrasound contrast agents (UCA) in patients with poor transtemporal bone window ensures adequate ultrasonographic examination in virtually all cases.[96] However, UCA bolus injection can cause a 20–24 % increase in measured peak systolic velocity (PSV),[98, 99] and may thereby lead to false-positive detection of intracranial stenosis (page 48).

UCA is usually administered as bolus injection. This way of administration allows short and varying contrast enhancement which often leads to artifacts, such as blooming, hindering blood flow velocity (BFV) measurements (page 26). The use of an infusion pump ensures stable and artifact-free contrast-enhancement not interfering with velocity measurements (see Paper III).

Our study has shown that UCA continuous infusion also leads to an increase (16%) in measured BFV. This increase appears to be lower than the one reported for UCA bolus injection (20–24%). However, it is not possible to compare these results because different ultrasound technologies and UCA were used.

It is strongly debated why UCA lead to an increase in measured BFV.[136, 137] UCA have no effects on heart rate or blood pressure,[138] and the increase in measured BFV is therefore not due to an increase in cardiac output induced by UCA. Microbubbles

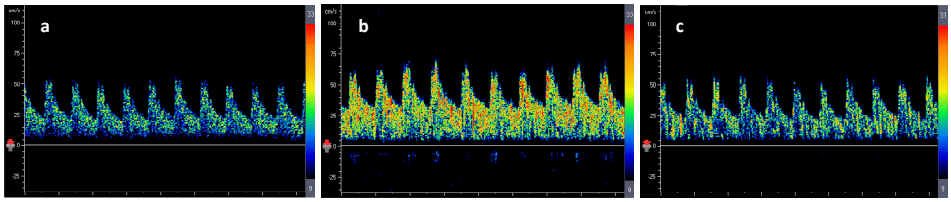
have intrinsic properties which may influence the back scattered Doppler shift. *In vitro* studies[139, 140] have shown that acoustic radiation force may displace microbubbles in the ultrasound wave propagation direction and that this can produce spectral broadening.[140] Spectral broadening has also been observed *in vitro* when microbubbles are ruptured.[141] Although these phenomena *in vivo* may not be as evident as *in vitro*,[141, 142] spectral broadening may represent a mechanism behind the UCA-related increase in measured BVF.[143] Another possible explanation is that UCA disclose areas of the blood flow spectrum previously hidden by background noise. The laminar blood flow has a parabolic shape with few red blood cells (RBC) moving at the highest velocity. The back scattered signal from the fastest RBC may therefore be weak and not distinguishable by the background noise. By adding scatterers to the blood flow, UCA may disclose areas of the blood flow spectrum previously not measurable, such as the fastest flow lamina, but also the slowest flow lamina, and hence lead to broadening of the Doppler spectrum. This hypothesis is supported by *in vivo* studies showing that UCA lead to increase in measured BFV only in deeply located vessels,[144] but not in superficial vessels,[145] suggesting that if optimal Doppler signal intensity (DSI) is obtained, a further increase in the signal-to-noise ratio does not modify BFV, since the highest velocity lamina is already disclosed.

None of the earlier clinical studies have been able to overcome the UCA-related increase in measured BFV. In our study, gain reduction performed during stable contrast-enhancement restored the BFV measured at baseline (Figure 10). Gain was reduced so that the DSI matched the intensity of the Doppler signal recorded at baseline. This was qualitatively performed by looking at one picture of the Doppler signal saved at baseline.

It remains to demonstrate if gain reduction may restore baseline BFV also in patients with poor transtemporal window, since no information about the baseline BFV and the baseline DSI are available. Our experience with administration of UCA in patients with poor temporal bone window suggests that infusion rate of 0.4 – 0.5 ml/min

allows artifact-free visualization of the circle of Willis. The Doppler spectrum detected in such conditions has higher intensity compared to subjects with optimal transtemporal bone window and no UCA infusion. It may be hypothesized that gain reduction restoring the Doppler signal to low intensity may neutralize the effect of UCA on measured BFV also in patients with poor temporal bone during stable contrast-enhancement. This hypothesis might be tested by simulating the presence of skull hyperostosis with aluminium foil in patients with optimal transtemporal bone window.

Figure 10. Doppler signal at different recording phases



a) Baseline Doppler signal. Peak systolic velocity (PSV) = 53 cm/s

b) Doppler signal during UCA infusion with no gain reduction. PSV = 69 cm/s

c) Doppler signal during UCA infusion after gain reduction. PSV = 54 cm/s

Blood flow volume measurements

Transcranial ultrasonography infers cerebral hemodynamics from blood flow velocity measurements. However, the relationship between blood velocities and blood volume is complex, and velocities alone may in some cases incorrectly evaluate the intracranial vessels and the cerebral blood flow volume. Flow impairment is detected by velocity only in cases of near-occlusive stenosis (Figure 7, page 49; “low findings criteria”, page 51). In case of less severe stenosis (approximately >60%) velocity

alone may lead to ambiguous results for both flow volume and degree of stenosis (Figure 7). Besides the degree of intracranial stenosis, flow volume is influenced by several other factors, such as the coexistence of extracranial stenosis and the collateral status. Neither degree of stenosis nor velocities are therefore sufficient to define which stenosis causes blood flow volume impairment, i.e. “really” is significant. Measurements of the cerebral blood flow volume may therefore provide a deeper understanding of the cerebral hemodynamics and have diagnostic, therapeutic and prognostic implication in acute ischemic stroke patients.

Our study (Paper 4) has shown that blood flow volume may be accurately estimated by analysing the power Doppler and the Doppler shift. This method may detect relative changes in flow volume, but does not provide absolute flow values. One possible clinical application of this method may be represented by monitoring of the flow index before and after manoeuvres or agents inducing changes in the peripheral resistances. This may be an accurate and sensitive method to test the cerebrovascular reserve capacity (vasoreactivity) in patients with acute ischemic strokes, and also in other neurological conditions.

The feasibility and accuracy of this method depend on the quality of the Doppler signal. The advent in the near future of probes continuously and automatically searching for the optimal Doppler signal may further improve our ability to measure relative changes in blood flow volume.

Conclusions

The epidemiological data on ICAS provided by our study add information about the prevalence and distribution of this disease in Europe. Although the prevalence of ICAS appears to be relative low, an in depth etiological and pathophysiological understanding of ischemic stroke and TIA has important clinical and prognostic consequences. Patients with ICAS have high recurrence risk (up to ~40% of the patients within a 2-year period)[146] and therefore need aggressive secondary medical prevention. Screening for ICAS should therefore be part of the routine work-up in all ischemic stroke and TIA patients. Transcranial color-coded sonography (TCCS) is the perfect tool to perform ICAS screening in stroke units. TCCS is inexpensive, non-invasive, and gives accurate information on the intracranial vessels. Knowledge of basic hemodynamic and physical principles is necessary to fully exploit the potential of TCCS. This work has shown that the application of continuity equation may increase the accuracy of TCCS in both detection and grading of ICAS compared to conventional diagnostic criteria. Degree of stenosis is the most important prognostic factor in ICAS patients,[127] and accurate grading of ICAS is therefore essential in the evaluation of these patients.

This work has also focused on patients in which optimal TCCS examination is hindered by hyperostosis of the skull. This problem occurs in 20% of ischemic stroke patients, but may be overcome in virtually all patients by ultrasound contrast agents (UCA). However, UCA cause an unexplained increase in blood flow velocity which may bias diagnostic results. This work has shown that the use of infusion pump and optimal gain reduction may overcome the undesired increase in blood flow velocity related to UCA administration.

Finally, advanced analysis of the Doppler spectrum has been investigated aiming to provide information of the cerebral hemodynamics beyond mere velocities. Our *in*

vitro experiments have shown that analysis of the Doppler signal power provides reliable measurements of relative changes in blood flow volume. This may represent a first step towards a more accurate evaluation of the cerebral autoregulation, possibly providing a deeper hemodynamic understanding of the intracranial atherosclerotic plaque.

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