Ultrasound evaluation of atherosclerosis and other cardiovascular sources of cerebral embolism

Robert Wetterholm

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Department of Molecular and Clinical Medicine/Clinical Physiology,

Institute of Medicine, Sahlgrenska Academy at Göteborg University, Sweden

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Abstract

The search for embolic sources has high priority in patients presenting with suspected cerebral embolism. Non-invasive cardiovascular ultrasound is frequently used to reveal the presence of carotid stenosis and cardiac disorders with embolic potential. Transesophageal echocardiography (TEE) provides images of aortic atherosclerosis, shown to be associated with increased risk of stroke. Some guidelines claim that TEE should be reserved for younger patients. In recent years microembolic signal (MES) detection with transcranial Doppler has emerged as a tool with potential of identifying patients at high risk of recurrent embolism.

We compared the diagnostic value of transthoracic and transesophageal echocardiography in relation to age in stroke/TIA patients. We found that among 453 patients investigated with TEE during 3 years, TEE had a higher proportion of relevant findings, e.g. complex aortic arch atheromas, in patients aged > 50 years compared to those < 50 years.

Carotid stenosis is a well known cause of embolism; however the association between routinely described plaque morphology and risk of recurrent embolism is not clear. In 197 patients with symptomatic high grade carotid stenosis, we found a strong correlation between the side of symptomatic stenosis and occurrence of microembolic signals on transcranial Doppler compared to the contralateral hemisphere. The occurrence of MES, however, only tended to correlate to plaque morphology.

Complex aortic atheromas are often found in the distal aortic arch or proximal descending aorta. Therefore, we investigated if regional flow conditions, with retrograde diastolic flow, make plaques located in the aorta distal to the cerebral branches relevant as sources of cerebral embolism; we found that this possibility does exist.

Previous research has shown hyperlipidemic rabbits to be a useful atherosclerosis model. The possibility to perform serial non-invasive evaluation of the aorta in the same animal would add a new dimension in the study of pathophysiology and treatment effects. Therefore, we validated high frequency transthoracic ultrasound for repeated in vivo measurements of aortic intima-media thickness in hyperlipidemic rabbits.

To conclude, our study shows that TEE has the highest yield of relevant information in stroke/TIA patients above 50 years of age. In carotid stenosis, plaque morphology as described by Gray-Weale scaling shows only a tendency to correlate with microembolism.
Local flow conditions can allow plaques located in the aorta distal to the cerebral arteries to embolize to the brain. To facilitate future studies of aortic atherosclerosis, we developed and validated an animal model for repeated ultrasound investigations of aortic intima media thickness.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I.   Wetterholm R, Bech-Hanssen O, Blomstrand C, Volkmann R, Caidahl K.
     Cardiovascular ultrasound above and below age 50 in embolic stroke
     Submitted for publication.

II. Zuromskis T, Wetterholm R, Fréden Lindqvist J, Jatuzis D, Obelieniene D, Caidahl K, Volkmann R.
     Prevalence of microemboli in symptomatic carotid artery disease:
     A transcranial Doppler study before carotid endarterectomy
     Eur J Vasc Endovasc Surg., 2008. In press.

III. Svedlund S, Wetterholm R, Volkmann R, Broomé M, Caidahl K.
     Retrograde blood flow in the aortic arch determined by transesophageal Doppler ultrasound
     Submitted for publication.

IV. Wetterholm R, Caidahl K, Volkmann R, Brandt-Eliasson U, Fritche-Danielsson R, Gan L.
     Imaging of atherosclerosis in WHHL rabbits using high-resolution ultrasound
     Ultrasound in Med. & Biol., 2007;33; (5): 720–726

Contents

Abbreviations

ACA	Anterior Cerebral Artery
AF	Atrial Fibrillation
ASA	Atrial Septal Aneurysm
CCA	Common Carotid Artery
CEA	Carotid Endarterectomy
CHF	Congestive Heart Failure
Comp-CCA	Compression of the Common Carotid Artery
CT	Computed Tomography
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
ICA	Internal Carotid Artery
IHD	Ischemic Heart Disease
IMT	Intima Media Thickness
LA	Left Atrium
LV	Left Ventricular
MCA	Middle Cerebral Artery
MES	Microembolic Signals
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
PFO	Patent Foramen Ovale
PMD	Power M-mode
SBP	Systolic Blood Pressure
SEC	Spontaneous Echo Contrast
TCD	Transcranial Doppler
TEE	Transesophageal Echocardiography
TIA	Transitory Ischemic Attack
TTE	Transthoracic Echocardiography
VTI	Velocity Time Integral
WHHL	Watanabe Heritage Hyperlipidemic

Introduction

Historical mile stones

Hippocrates first recognized "stroke" more than 2,400 years ago. The Greeks called it apoplexy, which means "struck down by violence". There was little or no understanding of its mechanisms until the mid-1600s when Jacob Wepfer, who dissected corpses in the University of Padua in Italy, discovered that something disrupted the blood supply to the brains of people who died from apoplexy. In some cases, the arteries were blocked; in others, there had been a massive bleeding into the brain tissue [2, 3].

Another step of major importance was the development of contrast angiography in the 1920s. About thirty years later, Michael de Bakey performed the first operation to restore blood flow to the brain by surgical removal of carotid blood flow blockage, "carotid endarterectomy" (CEA) [4]. In the 1950s warfarin was used for the first time as an anticoagulant in stroke patients [5].

In the 1960s, noninvasive Doppler ultrasonography was introduced for the evaluation of occlusive arterial disease [6], and hypertension was recognized as an important treatable risk factor for stroke [7]. Ten years later, aspirin was introduced as a treatment to prevent stroke. Although new platelet anti-aggregation treatments have been introduced, aspirin remains the most frequently used antithrombotic drug [8]. Radiology techniques took major steps in improving brain imaging, including studies of brain metabolism; computed tomography (CT) and positron emission tomography (PET) scan [9].

In the 1980s cigarette smoking was recognized as a major risk factor for stroke [10]. Magnetic resonance imaging (MRI) was introduced as a contribution to the diagnostic possibilities to differentiate cerebral infarction from bleeding [11].

In the last two decades, large studies have shown that carotid endarterectomy is clinically effective in preventing stroke [12, 13]. Oral anticoagulants were proven effective to prevent stroke in patients with atrial fibrillation in several studies [14, 15]. Further, statins have been introduced in hypercholesterolemia treatment and proven to reduce the risk of stroke [16]. With access to effective treatment alternatives, adequate diagnostic procedures are particularly essential.

What is Stroke?

Stroke is a clinical condition characterized by a rapid loss of brain function due to a disturbance in the blood vessels supplying blood to the brain. It can be due to vascular thrombosis or embolism, or due to intracranial hemorrhage [17]. In analogy to the expression "heart attack", a person with an interrupted blood flow to the brain or sudden intracranial bleeding can be said to be having a "brain attack".

Brain cells die without oxygen and nutrients from the blood or when they are damaged by a sudden bleeding. This lack of blood flow with consequently impaired metabolism is called ischemia [18].

The symptoms of a brain ischemia are often dramatic and obvious. They include; sudden numbness or weakness in a limb or in both limbs on one side of the body; sudden confusion or trouble speaking or understanding speech; sudden visual disturbance involving one or both eyes; sudden trouble to walk, dizziness, or loss of balance or coordination; or sudden severe headache with no known cause. All brain ischemic symptoms appear suddenly, and often combined.

Often blood flow blockage resolves in time to avoid cell death and the symptoms disappear, mostly within minutes. This is called transitory or transient ischemic attack (TIA) if all symptoms are resolved within 24 hours. If blood flow is not restored fast enough, ischemia ultimately leads to brain infarction with persistent symptoms, and an established "stroke" [19].

Recurrent Stroke

About 25% of people who recover from their first stroke will have another stroke within 5 years. The risk of a new stroke is highest immediately after the first incident, within days, and decreases with time. Thus, about 3% will have a new stroke within the first month and a third of all recurrent strokes occur within 2 years [20, 21] .

Cerebral embolism

Stroke is a common cause of disability and death. In Sweden, the incidence of stroke is about 30,000 cases annually and another 8,000 suffer TIAs. Stroke is the third most frequent cause of death and accounts for the highest number of treatment days in Swedish hospitals, with close to 1 million treatment days annually. Stroke affects about 100,000 persons throughout the country; at least 20,000 require very extensive support services and supervision around the clock. It is an increasing problem and current prognoses indicate that it will affect about 30% more patients in 2010 than in 2005s. The overall cost for the Swedish society is over 14 billion SEK/year [22].

About 1/3 of stroke cases are considered to be embolic and therefore the search for the source of embolism has high priority in these patients. The most important reason for this is of course to prevent further ischemic events, but also to evaluate the prognosis in the individual patient.

In some of the most frequent causes of embolism, recurrent stroke can be prevented; e.g. by thrombendarterectomi in high grade carotid stenosis [12, 13]. In others, the value of available treatment options is still uncertain; e.g. closure of patent foramen ovale (PFO) [23]. Non-invasive cardiovascular ultrasound is frequently used in these patients to reveal the presence of carotid stenosis and cardiac disorders with embolic potential.

Transesophageal echocardiography (TEE) also provides images of aortic atherosclerosis, which has been shown to be associated with an increased risk of stroke, although the benefit of current treatment options in this condition is still unproven [24]. In recent years, transcranial Doppler (TCD) has emerged as a tool with potential of finding patients at especially high risk of recurrent embolism [25, 26].

Ultrasound in medicine

Pioneers in medical ultrasound got their ideas and technical solutions from the military field, where ultrasound had been developed during the 1930s. A need for a device to test ship and tank armor led to the development of the supersonic reflectoscope by Floyd A. Firestone at the University of Michigan, who in 1945 published "The Supersonic Reflectoscope, an Instrument for Inspecting the Interior of Solid Parts by Means of Sound Waves".

Ultrasound was first applied for diagnostic purposes by Dr. George D. Ludwig at the Naval Medical Research Institute, Bethesda, Maryland in 1947-1949. He was able to demonstrate that gallbladder stones could be detected by an ultrasonic echo method using amplitude mode metal flaw detectors and naval sonar [16].

Transthoracic echocardiography

In 1953, at Lund University, cardiologist Inge Edler and Carl Hellmuth Hertz, who at that time was a graduate student at the department of nuclear physics, pioneered the use of ultrasound in cardiology. Edler had asked Hertz if it was possible to use radar to look into the body, but Hertz said this was impossible. However, he was familiar with ultrasonic reflectoscopes for nondestructive materials testing and suggested that it might be possible to use ultrasonography [27]. The first successful measurement of heart activity was made on October 29, 1953 using a device borrowed from the ship construction company Kockums in Malmö. Later on, they expanded their work with an improved reflectoscope construction developed by Siemens. Since then, the ultrasound imaging technique has developed immensely with innovations such as 2-dimensional phased array echocardiography [28], second harmonic imaging [29] and 3-dimensional imaging [30].

Transesophageal echocardiography

Over the last three decades, the continuing development of TEE, introducing phased array transducers in the late 1980s and biplane followed by multiplane probes in the early 1990s, has represented a major advance in our ability to visualize cardiovascular structures with ultrasound [31, 32].

TEE serves as a valuable complement to transthoracic echocardiography (TTE) by allowing: images to be obtained with less attenuation from structures such as the lung, muscle, bone, and soft tissue; high resolution visualization of structures not well seen by TTE, such as left atrial appendage, descending thoracic aorta, and prosthetic heart valves; and assessment of hemodynamics and flow disorders in greater detail.

TEE also has potential major advantages to TTE in the search for sources of cerebral embolism, since besides its ability of high resolution imaging of the cardiac anatomy, it allows visualization of the ascending aorta, the aortic arch and the thoracic part of the descending aorta. Furthermore, it is superior to TTE in identifying presence of a PFO [33-35].

Carotid Duplex Ultrasound

The first attempts to measure blood flow velocities by ultrasound occurred in the late 1950s [36], but the technique was invasive and not suitable for clinical use. The first clinical application of the transcutaneous non-invasive Doppler flowmeter was developed in 1958 in Japan, by Satomura et al, who managed to measure arterial flow velocities [37]. Strandness et al. diagnosed, in the late 1960s, various arterial diseases by spectral analysis of Doppler signals [38]. Introduction of pulsed Doppler instruments by Wells and Baker enabled for the first time, in the early 1970s, non-invasive regional measurements of blood velocity. In 1974, Baker combined pulsed Doppler with a real-time B-mode imager to form an instrument known as a duplex scanner [38]. Duplex Doppler instruments allow the Doppler angle to be determined from the B-mode image and thus, with the addition of spectral analysis, enabled the accurate measurement of blood flow velocity [39]. In 1991 two major studies; the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST) [12, 13], showed clear benefit of carotid endarterectomy (CEA) in patients with severe symptomatic internal carotid artery (ICA) stenosis. Determination of the severity of stenosis in those studies was based on angiography. Slightly different methods of measuring resulted in some difficulties to compare NASCET and ECST results.

Recently, recalculation to a common database has contributed to a lot of important new conclusions on how to handle subgroups, e.g. patients with tandem lesions. The most important development is probably the increased understanding of the importance of early intervention to avoid recurrent embolism [40, 41].

Today, angiography is rarely done due to the risk of complications. In Sweden, about 80% of CEA are performed exclusively on the basis of carotid duplex information regarding degree of stenosis [22]. Duplex ultrasound might also give an indication of plaque composition. Thus, it has been suggested that echolucent plaques causing at least 50% stenosis are associated with increased future risk of stroke in symptomatic patients [42].

Transcranial Doppler

At the end of the 1970s, it became possible to achieve a two-dimensional visualization of the intracranial structures in infants through the acoustic window of the anterior fontanel; the pulsations of the large cerebral arteries could be qualitatively evaluated.

A major breakthrough occurred in 1982, when for the first time Rune Aaslid measured flow velocities in the vessels of the circle of Willis through the skull using the TCD technique. In just a few years, this method was introduced for diagnostic purposes as well as therapeutic control of intracranial vascular disease in adults [43]. In the early 1990s, Spencer et al were able to demonstrate the occurrence of microembolic signals (MES) using TCD. Although individual MES are asymptomatic, it has been shown that presence of MES is associated with a high risk of future ischemic events [44]. In recent years, the most important diagnostic development of TCD was the introduction of microembolic detection, the sensitivity of which was further increased by a new technique, Power M-mode Doppler [45].

Why did we do this study

In the mid-1990s, it was uncertain and intensely discussed if, when and by which technique, cardiac ultrasound examinations should be done in suspected cardiovascular embolic stroke. This debate still goes on. The selection of patients for TEE at our department was at the time influenced by several factors, e.g. the opinions of the investigating and referring physicians about the importance of TEE-findings. This caused a time consuming procedure, where TEE was considered only after the TTE was done, and the patient often had to restrain from breakfast in vain. All this led to the design of paper I.

Furthermore, many patients had to wait for weeks to have their carotids scanned. At this time, little was known about the urgency of early intervention on patients with symptomatic carotid stenosis. Even though the benefit of intervention was proven, further risk stratification through evaluation of plaque morphology was routinely performed with standard ultrasound equipment and equivocal results. Then, the possibility of MES detection gave us the tool to study embolic potential discriminating between left and right hemisphere, thereby indicating possible plaque instability. This gave us the idea to perform study number II.

In paper I, we found a lot of complex plaques just distally to the arteries supplying the brain. This led to the question if backward flow in the aortic arch would allow retrograde embolization, and which factors might influence the presence or magnitude of retrograde flow. In paper III, therefore, we applied Doppler technique in TEE investigations of patients with different conditions to clarify this question.

The awareness of atherosclerosis as a major cause of stroke, and the challenges in measuring plaque thickness in paper I, was a reason to validate the ultrasonic technique. This was done in an experimental study, paper IV.

AIMS OF THE THESIS

the objectives of the study were to:

I.   Compare the diagnostic value of transthoracic and transesophageal echocardiography in relation to age in stroke patients.

II.   Evaluate the association between severe carotid stenosis and plaque morphology with the presence and side location of cerebral microembolism.

III.   Investigate if flow conditions in the aortic arch make plaques located distal to the left subclavian artery relevant as sources of cerebral embolism.

IV.   Validate ultrasonic measurements of aortic wall thickness by an in vivo model of atherosclerosis prone WHHL rabbits.

Material

To evaluate the prevalence of findings which could be extracranial cardiovascular embolic sources in stroke patients of different age, we studied (paper I), all patients (n=867) who during a 3 year-period were referred from the internal medicine and neurology departments at Sahlgrenska University hospital for echocardiography and carotid ultrasound after suspected stroke or a transitory ischemic attack. To identify these patients we used a computed patient register covering all investigations at our unit. All patients underwent transthoracic echocardiography (TTE) and carotid duplex ultrasound, and 453 patients also underwent transesophageal echocardiography (TEE). There was a wide age span from 18 to 89 years at time of inclusion. Patients without TEE were slightly but significantly older, mean 62.4 compared to 56.9 years.

To determine whether TCD can detect micro-embolization on an individual basis, we studied (paper II) 214 consecutive patients (mean age 68.4±8.4) who were prospectively recruited after referral for a preoperative evaluation of an ICA stenosis of 70-99% and a history of ischemic events within the ipsilateral anterior circulation. In 17 (7.9 %) patients the transtemporal TCD investigations were inconclusive because of absent temporal windows on the symptomatic side. The remaining 197 patients consisted of 161 males and 36 females (mean age 69.5 ± 8.6). 178 asymptomatic contralateral ICA vessels were monitored by power M-mode TCD, (19 patients had absent window on the asymptomatic side. The ischemic symptoms included recent amaurosis fugax, TIA or minor stroke. All patients were on antiplatelet therapy (aspirin and/or dipyridamole and/or clopidogrel).

Exclusion criteria were: 1) agitated, severely demented or non-cooperative subjects; 2) severely disabled stroke patients; 3) atrial fibrillation or flutter; 4) mechanical or biological valve prosthesis; 5) evidence of other sources of embolism (cardiogenic or paradoxical); 6) intracranial hemorrhage or brain tumor on cranial CT/MRI.

To determine by Doppler the physiological basis for retrograde embolization from aortic plaque situated distal to the cerebral artery orifices, we recruited (paper III) fifty-six patients of various age and with different referral diagnoses. Criteria for inclusion were:
1. The ultrasound examination of the patient had to be performed by one experienced investigator and be complete with respect to the blood flow velocity profiles of interest.
2. The angle between the Doppler transecting line and the assumed flow direction had to be small enough to enable high-quality antegrade and retrograde flow signals.
3. The quality of the image and Doppler signal had to be acceptable and the patient should have no significant aortic regurgitation.

Of the 56 patients, 17 were diagnosed as having aortic plaques ≥ 4 mm as a potential source of embolization.

To determine the possibility to evaluate aortic arch atherosclerosis by transthoracic echocardiography, we studied (paper IV) 34 Homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits from Harlan Interfauna, UK, at the age of 10 weeks and later. Twentyfour animals were sacrifized at 35 weeks, and 10 at 60 weeks of age.

Early progression of atherosclerosis was evaluated in additional 13 animals at the age of 21 and 36 weeks respectively, one operator, blinded to the identity of the animals, analyzed all the measurements offline on one occasion.

In addition, in 44 consecutive WHHL rabbits, used in our laboratory for various studies, one investigator performed blinded ultrasound measurements of the same plaque on two different days to establish the day-to-day intra-individual measurement variation. The inter-observer variability was evaluated by comparing IMT measurements from six WHHL rabbits of different ages scanned by two independent observers (LG and RW).

All the animals were housed at AstraZeneca R&D Mölndal in separate cages (Scanbur) with free access to water and standard chow. A constant temperature of between 17-18°C and relative humidity between 50-70% were maintained during the experiment period. The investigation was carried out in accordance with international guidelines for the care and use of laboratory animals.

Methodological considerations

Evaluation of cardiovascular embolic source
A large number of possible cardiac sources of emboli have been suggested [46], Table 1. Several of these may be treated and warrant detection [47]. Since long ago, it has been obvious that echocardiography is the most suitable method when searching for cardiac embolic sources [48]. TTE allows detection of valvular defects, such as mitral stenosis, and myocardial dysfunction, as well as LV thrombi and myxoma [49]. It is also a good means to determine cardiac dimensions [50]. With intravenous contrast, it is possible to detect PFO [51]. Moreover, some early TEE studies did not prove a clear advantage of TEE in all age-groups [52, 53]. With some relevant findings predominantly identified by TEE, such as complex aortic atheroma [54], the therapeutic implications remain unclear [55]. At many sites, therefore, TTE has become a tradition in the search for a cardioembolic source. Further, disappointment over few findings on TTE has at some sites reduced the interest in search for cardioembolic sources altogether. We therefore evaluated a consecutive material referred for ultrasound evaluation of embolic source, to find out the prevalence of possible sources of emboli. All patients were evaluated by TTE and about half of the patients also by TEE.

Further, all patients were evaluated by Duplex Ultrasound of the carotid arteries as it has long been accepted that carotid stenosis or intra- and extracerebral atherosclerotic arteries can lead to ischemic stroke through embolization or hypoperfusion [56]. Another way to identify patients at high risk of recurrent stroke is to apply TCD, which can detect MES as an indication of ongoing embolization [25].

Table 1. Possible causes of stroke found on echocardiography by Chambers et al [46].

Left ventricle:
 Anterior myocardial infarction
 Dilated left ventricle
 Hypertrophic cardiomyopathy (with atrial fibrillation)
 Thrombus
 Ventricular septal defect with pulmonary hypertension

Valves:
 Mitral stenosis
 Replacement heart valve (aortic or mitral)
 Endocarditis
 Fibromyoma

Left atrium:
 Large left atrium/left atrial spontaneous contrast
 Thrombus
 Atrial septal aneurysm
 Patent foramen ovale
 Atrial septal defect
 Myxoma

Aorta:
 Aortic atheroma (mobile or $\geq$ 4 mm, [1])
 Dissection

Transthoracic echocardiography (Paper I, IV)
Standardized TTE protocol for possible cardioembolic source in suspected ischemic stroke: All patients (Paper I) had a TTE examination (Acuson Sequoia or Acuson XP, Siemens-Acuson, Mountain View, CA), or HP-Sonos 5500 (Philips Medical Systems, Andover, MA), from parasternal (long- and short-axis views) and apical (4-, long- and 2-chamber) views. Chamber dimensions and left ventricular (LV) hypertrophy were determined visually or measured, as was LV ejection fraction. By color Doppler and continuous wave Doppler, valvular leakage and stenosis were ruled out or graded. Diastolic function was evaluated by pulsed wave Doppler.

Experimental TTE for evaluation of aortic arch atherosclerosis (see also below): All ultrasound examinations were performed using a 15-MHz linear probe (HDI 5000, ATL, Philips med). All animals were examined according to a standardized protocol. After pre-sedation with 5 mg of diazepam i.m., the animals were anesthetized with intravenous pentobarbital at a rate of 15 mg/kg/min. The cranial parasternal chest wall was mechanically and chemically shaved for optimal image quality. A right parasternal image window was used to obtain a long-axis view, visualizing the entire ascending aorta, the innominate and the left common carotid arteries in the same plane. From the same position, a short-axis view of the aorta was obtained.

Transesophageal echocardiography (Paper I and III)

All TEE examinations in paper III were performed using a multiplane probe (Sequoia, Acuson Corp., Mountain View, or Vivid 7, GE). All examinations were performed by one and the same experienced physician and digitally stored on MO disks. In paper I either a biplane or multiplane transducer were used (Acuson Sequoia or Acuson XP, Siemens-Acuson, Mountain View, CA), or HP-Sonos 5500 (Philips Medical Systems, Andover, MA). TEE was performed in the left lateral supine position. Before insertion of the TEE probe, the epipharynx was anesthetized using lidocaine hydrochloride spray. Sedation with midazolam i.v. was given when needed. Cardiac chambers were carefully examined for presence of thrombi, tumors, aneurysms, akinetic segments or other pathology in multiple planes. The atrial wall was inspected for atrial septal aneurysm (ASA), color Doppler used to exclude or confirm shunts. Presence of PFO was evaluated with repeated injections of agitated Haemaccel with and without preceding Valsalva maneuver. Left atrial appendage was inspected in 0° and 90°, flow velocities recorded. The thoracic aorta was investigated for the presence of atheroma. In paper III, we measured the flow profile in the aortic arch by means of pulsed wave Doppler.

Carotid ultrasound (Paper I & II)

The severity of the carotid stenosis was defined by color-coded ultrasound, Sequoia 512, Siemens Acuson Corp.) using linear transducers (6-8 MHz) to measure the intra-stenotic peak systolic and end-diastolic velocities (12-14). The local techniques of carotid duplex had been previously validated against selective carotid angiography with outcomes comparable with other Swedish materials [57, 58]. Patients were subdivided with respect to the maximum systolic blood flow velocities into groups with <70%, ≥70% diameter reductions or ICA occlusions and high-grade stenosis with string flow pattern. Peak systolic velocities of >1.9 m/s (Doppler beam angulations 40°), or >2.5 m/s (Doppler angulations of 60°) [57] were the cut-off velocities for ICA stenosis of ≥70% [13].

Transcranial Doppler (Paper II)

MES detection was performed according to Moehring et al. [45], using the transcranial Power M-mode (PMD) technique (PMD100, Spencer Technologies) with 2MHz transducers. Transducers were fixed bilaterally over the temporal window on each side to insonate the distal ICA siphon, the middle cerebral artery (MCA) and the anterior cerebral artery (ACA). MES were monitored within a multigated PMD spectrum using 33 sample volumes arrayed within a depth range of 28-82 mm from the temporal bone. An additional sample volume channel was used to present the blood flow velocity spectrogram at a depth ranging between 50-60 mm. The patients underwent continuous 30-minute monitoring on the days before carotid end-arterectomy (CEA). MES detection within the Doppler spectrum was based on basic identification criteria of Doppler micro-embolic signals [59] and according to embolic signatures within the Power M-Mode spectrum, which should be visible at least 3 dB higher than the highest spontaneous PMD display of background blood flow signal; and reflect motion in one direction. MES within the MCA move towards the probe with a positively sloping track. MES within the ACA move away from the probe with a negatively sloping track [60].

In order to test the collateral compensation through the circle of Willis and to verify the flow of MCA and ACA, a manual compression of the proximal common carotid artery (comp-CCA) was performed during 10 heartbeats [61].

Evaluation of extracranial atherosclerosis as possible cause of embolic stroke

Aortic arch atherosclerosis (Paper I, III and IV)

By TEE, the aorta was visualized in various views (Paper I, III). From a 4-chamber position, the aortic valve and ascending aorta were studied in 135° views for the presence and location of plaques. The transducer was then rotated to visualize the descending aorta in the longitudinal view (90°) and transverse (0°) sections Fig. 1, position 2) until the appearance of the left subclavian artery, which was studied at around 45°. The aortic arch was carefully scrutinized for plaques in the transverse and longitudinal views and in the latter the flow profile was studied by color Doppler. Color guided pulse wave spectral Doppler velocities were obtained as parallel to the flow as possible, (Fig. 1, position 1).

Measurements were made offline using an Echopac workstation or on the ultrasound platform. The flow profiles in both systole and diastole were outlined manually to measure the maximum flow velocity and the velocity time integral (VTI). Three consecutive beats were measured and the mean diastolic/systolic ratio was calculated, Fig. 2. The prevalence and distribution of aortic plaques were noted.

Plaque is a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrates a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface [62, 63].

In stroke patients with aortic atheroma, plaque measurements and description is crucial to define since plaques with a thickness ≥4 mm, or plaques with a mobile component regardless of size, have been shown to be associated with a high risk of recurrent stroke [1, 34, 54].
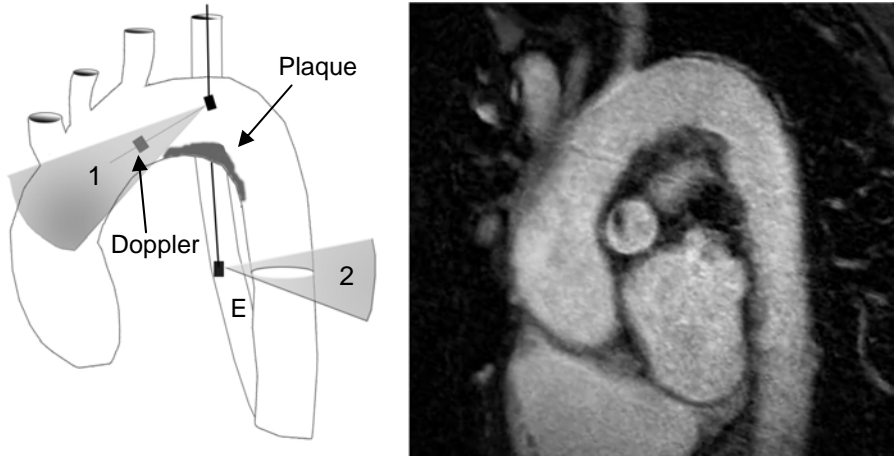

Fig.1. Illustration of aortic anatomy, to the right is an MRI of the thoracic aorta and to the left the corresponding sketch. (Note that cervical arteries origin more proximally than described in many human atlases, this is frequently the case). In the sketch we see the TEE-probe positions. Doppler registrations were made in position 1.
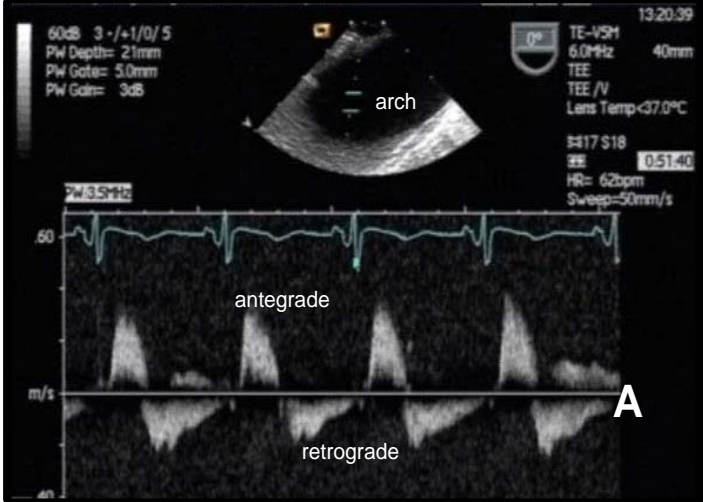

Fig. 2. Pulsed Doppler from the aortic arch obtained by TEE showing antegrade and retrograde velocity time integrals

Interobserver variability in aortic plaque measurements applying TEE (Paper I). In a smaller cohort of 16 aortic plaques from patients in paper I, mean inter-individual variation between two experienced viewers was 0.5 mm, Fig. 3. One plaque was differently categorized; as complex by one viewer (>4 mm thick) and non complex by one viewer (3.5 mm thick). There was no disagreement on plaque mobility, (3 plaques). This compares well with the results from Weber et al who found an agreement of 84-88% in ability to distinguish between plaque thickness above or below the important limit of 4 mm [64].
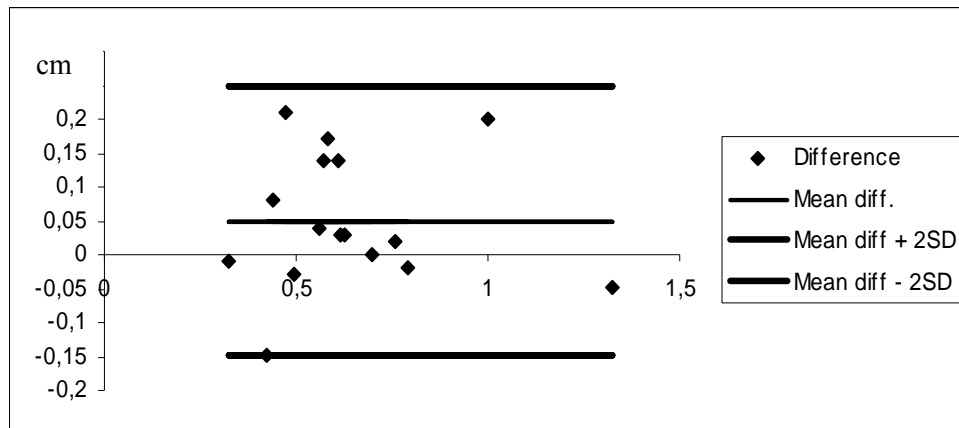

Fig. 3. Bland-Altman plot illustrating the comparison of transthoracic echocardiography and histology in terms of difference between the two investigators vs. their mean values.

Experimental TTE comparison to histopathology (Paper IV). It has been demonstrated that aortic arch atherosclerosis can be determined with reasonable success through the suprasternal approach [65]. However, according to our experience, the success rate of plaque evaluation by this technique is inferior to TEE. It is also difficult to compare techniques as a gold standard is lacking. To determine how precise measurement of plaque recorded by TTE could be, we studied atherosclerosis in rabbits by TTE and histopathology.

In intima media thickness (IMT) measurements done in WHHL rabbits the leading-to-leading edge principle was used; IMT is a double-line pattern visualized on both walls of the common carotid artery (CCA) in a longitudinal image. It is formed by two parallel lines, which consist of the leading edges of two anatomical boundaries: the lumen-intima and media-adventitia interfaces [62, 63]. IMT immediately proximal to the innominate artery branch site can be easily measured in the right parasternal long-axis view, when using the arterial branching points as anatomic landmarks. Thereafter, a right parasternal short-axis view was used to obtain cross-sectional images of the ascending aorta. The IMT proximal to the innominate artery branch site was measured. A two-second cine loop was stored digitally in each view for offline measurements. Special care was taken to identify the arterial branches in the arch and to measure IMT and arch diameter at exactly the same plaque location from which histopathological specimens were later collected.

Measurements were made in the short-axis view by measuring the maximum IMT using the leading-to-leading edge principle.

The aortic arches of 50- to 60-week-old WHHL rabbits were examined with ultrasound at the origin of the innominate artery. Plaques in that area were characterized by ultrasound and then located within the 2D image by ultrasound-guided needle insertions using a three cm long acupuncture needle with a diameter of 160 μm. The chest of the rabbit was then opened with the needle in situ and the exact needle position site was identified *ex vivo*.

Specimens for histology were taken from this position. IMT measurements were made from the histological preparations and the 2D-ultrasound images of the same plaque location.

Specimens were obtained at the level of the innominate artery using the perfusion fixation technique and staining with Miller's elastin and Picro-Sirius red. Histological measurements were made using computerized morphometry. The intima media area (IMA) was obtained by measuring the external elastic lamina (EEL) area and lumen area: $IMA = EEL \text{ area} - \text{lumen area}$. The average IMT ($IMT_{hist\ mean}$) was calculated according to the following formula: $IMT_{hist\ mean} = (EEL\ area/\pi)^{0.5} - (lumen\ area/\pi)^{0.5}$. Since *ex vivo* specimens partly collapse into an irregular structure with a concomitant increase in IMT thickness, we also presented normalized IMT values, $IMT_{hist\ korr}$, based on the following formula: $IMT_{hist\ korr} = (IMA/\pi + R_u^2)^{0.5} - R_u$, where R_u was the ultrasound-assessed *in vivo* aortic lumen radius.

Aortic strain

Aortic strain has been shown to correlate with atherosclerosis [66]. We studied strain and its effect on aortic flow direction. Strain was assessed as $(\chi SA - \chi DA)/\chi DA$, where χSA is the three heart beat average of the aortic luminal area in systole and χDA the corresponding average area in diastole [67]. The trailing edge to leading edge technique was used to measure the aortic luminal area by circular regions of interest placed between the calipers to outline the aortic lumen [68], Fig. 4.
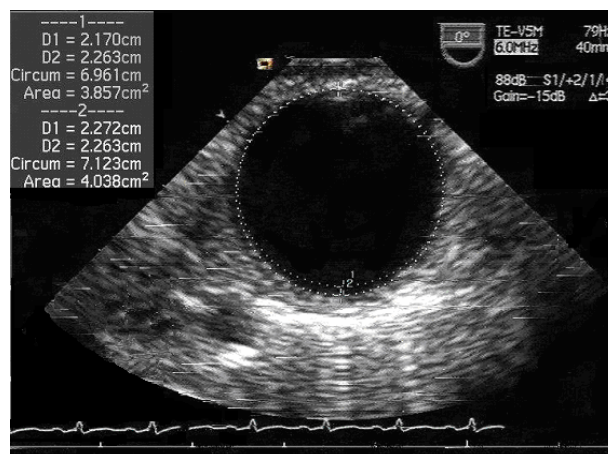

Fig. 4. Aortic internal borders are outlined in systole and diastole for area calculations.

Carotid plaque characteristics

Gray-Weale scale applied to carotid plaque. In carotid occlusive disease it has been shown that degree of stenosis correlates with risk of recurrent stroke [12, 13] However also plaque morphology is of interest, in particular ultrasound imaging gives information about plaque echogenicity which in some studies correlates to increased risk of recurrence [69]. A frequently used scale for plaque characteristics were assessed by Gray-Weale scaling as modified by Geroulakos & Sabetai [70, 71]:

Type 1: predominantly echolucent lesions with a thin echogenic cap;

Type 2: intermediate echolucent lesions with small areas of echogenicity;

Type 3: intermediate echogenic with small areas of echolucency (< 25%);

Type 4: uniformly echogenic lesions (equivalent to homogeneous);

Type 5: plaque with acoustic shadowing artifact.

Evaluation according to this scale has been clinical routine at our department. In the current study, grading was performed by two trained investigators independently of the TCD outcomes.

Analysis and statistics

Statistical analysis was performed using SPSS v.11.0 data analysis package. Normally distributed variables were reported as absolute values and percentages, or mean values and standard deviations. The significance of differences between groups was tested using Student's t-test. Pearson's linear correlation coefficients and Fisher's test were computed to illustrate relationships. The Bland-Altman test was used to show individual variations compared with the average values of both methods.

Variables that do not follow the normal distribution were expressed as median and interquartile range (IQR). McNemar test was applied evaluating the difference of microembolic activity on symptomatic and asymptomatic sides. Spearman's rank correlation coefficient was used to assess correlation between number of MES and number of days since the last ischemic event. Nonparametric Kruskal-Wallis and Mann-Whitney U tests were applied comparing variables that did not follow normal distribution. A *p*-value <0.05 was considered to prove statistically significant difference.

Results

Ultrasonic findings in a representative consecutive group of patients with possible ischemic stroke (Paper I)

Stroke population characteristics

There was a wide age span, 18 to 89 years, among patients referred for ultrasound evaluation of possible embolic source. Totally, 198 out of 867 patients were less than 50 years old.

Representativeness of the TEE group

Patients in the 'non-TEE-group' were older, mean 62.4 years compared to 56.5 years in the 'TEE-group'. There was no difference regarding gender or blood pressure at admission. Myocardial infarction and ischemic heart disease in total were more common in the 'non-TEE-group', while hypertension, atrial fibrillation and cholesterol levels did not differ. Those with significant carotid stenosis or plaque were less frequently subjected to TEE.

Age related differences within the TEE group

At inclusion there was no difference between age groups or between the 'TEE-group' and the 'non-TEE-group' regarding prescribed ACEI, anticoagulants or insulin. Use of aspirin, beta-blockers and statins were more prevalent among older patients. So were also calcium inhibitors and sodium nitrate. Diuretics and statins were more frequently used in the 'non-TEE-group'. In the 'TEE-group' younger patients also had lower CRP, blood glucose and cholesterol levels.

There was a higher incidence of patients with LV akinetic segments in the older age groups, but no significant difference in mean ejection fraction. Only 13 patients had an EF ≤35%, 9 of these in the 'non-TEE-group', all 13 being ≥50 years. A LV thrombus was found in two patients. There were 9 ASDs, 7 of which were found in the 'TEE-group' and 8 where ≥50 years.

On carotid duplex, some degree of plaque formation within the carotid bulb internal carotid artery (ICA) was reported in 18.2 % of patients < 50 years and in 68.3% of the patients ≥50 years. High grade stenosis of ≥70% was present in 3% of patients <50 years and in 9% of the older part of the population.

In the 'TEE-group' of 453 patients, relevant information was obtained in 199 (43.9%) patients. The most frequent observations were complex plaques in the proximal aorta found in 100 patients (22.1%) and PFO in 82 patients (18.1%), Fig. 5.
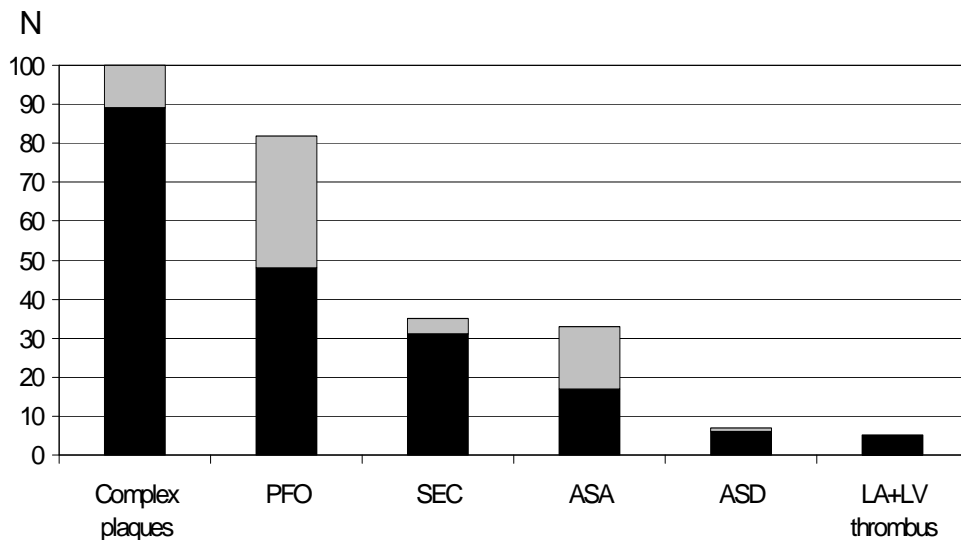
Fig. 5. Number of relevant TEE findings in totally 453 patients, bars divided by age, ≥ 50 years black parts and < 50 years grey parts.
Abbreviations: PFO, patent foramen ovale; SEC, spontaneous echocardiographic contrast; ASA, atrial septal aneurysm; ASD, atrial septal defect; LA, left atrial; LV, left ventricular.

In the 'non-TEE-group' there were only 25 patients with relevant TTE findings (6%), whereof 23 had one or more akinetic segments in the left ventricle and 2 patients had an ASD. Of the 25 patients with relevant TTE findings, 19 had a history of ischemic heart disease (IHD) and in the 6 remaining patients (1.4%), 4 had a pathologic electrocardiogram (ECG) suggesting IHD.

In patients <50 years of age, there were few relevant findings besides PFO. This was especially true for patients without previously known IHD, Fig. 6. In the TEE group < 50 years and a final diagnosis of cerebral ischemia, 12 patients with complex plaques were found. All of these had a medical history, carotid duplex findings of atherosclerosis or laboratory results indicating severe risk thereof.
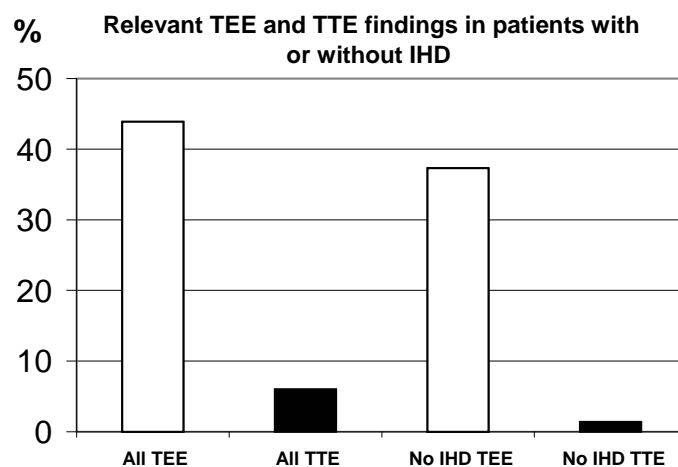

Fig. 6. The two left bars show proportion of relevant findings in TEE (white) compared to TTE (black) with no regard to medical history. The two bars to the right shows the result in patients free from ischemic heart disease.

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The proportion of performed TEE decreased with increasing age, while the proportion of relevant TEE findings increased with age, Fig. 7.
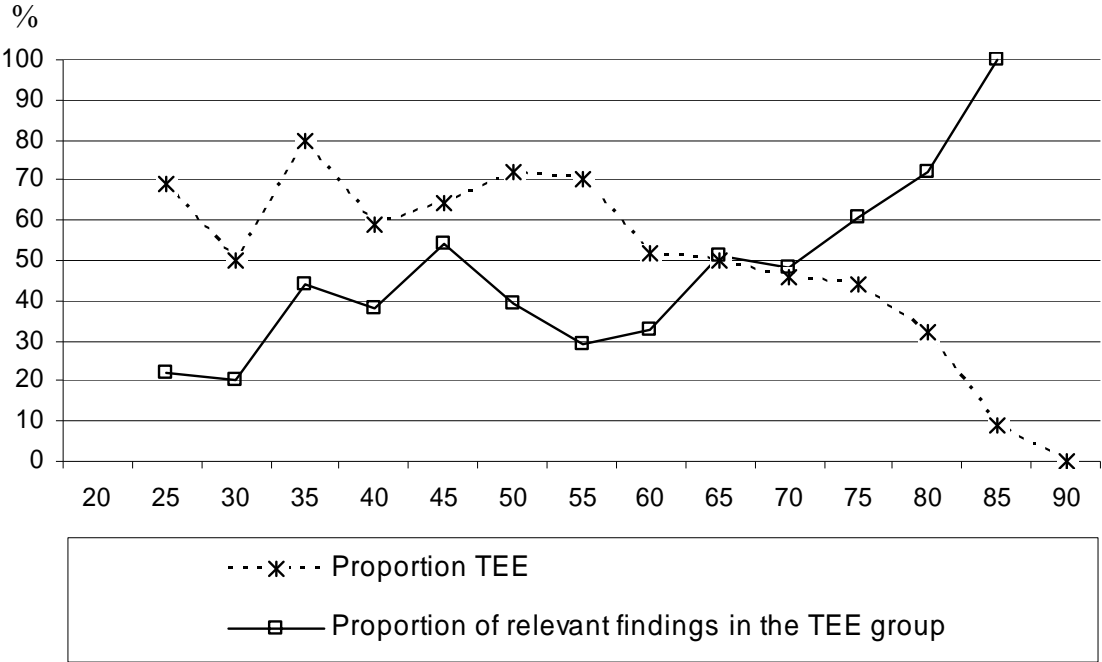

Fig. 7. The proportion of relevant findings in relation to the proportion of performed TEE investigations at different age of the patients.

To evaluate the possibility to predict relevant findings other than PFO by the risk factor pattern, we studied the number of risk markers for each patient. More than 90% of patients above 50 years had at least one of the following risk markers; current or previous smoking, history of hypertension, diabetes, coronary disease, claudicatio, carotid plaques or cholesterol >6 mmol/l. The combination of 2 risk markers was present in 89% of patients above 50 years. In patients above 50 years and with less than 2 risk markers, relevant findings other than PFO still were made in 17%.

If absence of atherosclerosis in the neck arteries had been applied in this population as a reason to avoid TEE, we would have been unable to diagnose 52 protruding aortic arch atheromas of which 16 protruded ≥ 4 mm and 2 were mobile.

Evidence of stroke risk or ongoing embolization from carotid arteries by transcranial Doppler

Presence and influencing factors of MES (Paper II)
The number of MES detected within 30 minute TCD monitoring ranged from one to 24. Spontaneous MES were registered in 63/197 (32.0%) cases on the symptomatic side and in 8/178 (4.5%) cases on the asymptomatic side. Registered MES were never accompanied by onset of new neurological symptoms.

The incidence of MES did not change in subgroups of blood flow velocities above the cut-off value of $\geq$70% stenosis on the symptomatic side.

Successful bilateral TCD monitoring of 178 patients with unilateral symptoms revealed MES within the symptomatic side in 33.7% subjects while only in 4.5% within the asymptomatic side ($p<0.0001$). Furthermore, comparing the occurrence of MES in the presence of bilateral $\geq$ 70% ICA stenosis, the symptomatic side was five times more embologenic (15/42, 35.7%) than the asymptomatic one (3/42, 7.1%) ($p<0.0001$).

In two cases, MES (1 MES/30min and 6 MES/30min respectively) were registered on the asymptomatic side with ICA stenosis of <70% and with signs of intracranial ICA siphon stenosis. In another three cases PMD-TCD proved paradoxical MES through the anterior communicating artery from the symptomatic side to the contralateral side with asymptomatic ICA occlusion.

The stroke risk factors in patients with and without MES were not significantly different in age, gender, current smoking, hypertension, hyperlipidemia, grade of symptomatic side ICA stenosis or type of last ischemic event before TCD monitoring.

The results suggest an inverse correlation between the time since the last symptoms and the number of MES within 30 minute monitoring (Spearman's rank correlation coefficient -0.189, $p=0.009$). Furthermore, the median (IQR) time since the last ischemic event symptoms was shorter in the patient group with MES [+] than in MES [-] (19 (33.5) vs. 38 (55.5) days; Mann-Whitney U test, $p=0.013$).

In 7/197 symptomatic vessels (3.6%), MES were elicited by proximal comp-CCA. But in six out of these seven, spontaneous MES were recorded within 30 minute monitoring. No MES appeared on the asymptomatic side during comp-CCA. Comp-CCA showed poor intracranial collateral compensation on the symptomatic side in another six patients, in whom MCA flow velocities almost disappeared during ipsilateral comp-CCA. Two of these six patients experienced mild hypoesthesia in the contralateral hand but it resolved immediately after stopping the test, and micro-embolic signals were not detected.

There was no difference in intrastenotic maximum blood flow velocities between MES [+] and MES [–] ICA stenoses.

The ultrasound plaque characteristics of the symptomatic side were evaluated in all 197 patients. In 11 cases plaques were not classified and attributed to any particular type in Gray-Weale Scale. They were defined as 'unidentified type' in the study.

We correlated the occurrence of MES with plaque types excluding all shadowing and unclassified plaques. The embologenic activity showed a tendency to be more prevalent in patients with plaque type 1-2 (predominantly echolucent) rather than 3-4 (predominantly echogenic) respectively, ($p=0.07$), table 2.

Table 2. Plaque subtypes in MES [+] and MES [–] patients with symptomatic ICA disease

Plaque subtype	MES [+] N=53	MES [-] N=114
Predominantly echolucent (Type 1+2), N /Total (%)	31/80 (39%)	49/80 (61%)
Predominantly echogenic (Type 3+4), N/Total (%)	22/87 (25%)	65/87 (75%)

MES [+] = patients with detected microemboli; MES [-] = patients without detected microemboli;

Flow characteristics in the aortic arch allowing retrograde embolization to the brain (Paper III)

Baseline characteristics and plaque location

The 25 patients with aortic plaque were 69 yrs ± 8 years of age on average (age range 46 to 82 years). Among these 25 patients (10 women), 39 regions (of 75; n=25 x regions=3) contained detectable plaques.

Plaques were most commonly detected in the patients with a suspected ischemic stroke, 43 % (13/30) of whom had aortic plaques of ≥ 4 mm, mainly in the aortic arch. The presence of plaques increased significantly with age (p<0.0001). The locations of the 39 plaques among the 25 patients with aortic plaques were as follows: 4 in the ascending aorta, 20 in the arch and 15 in the descending aorta. Eight patients had plaques at more than one region (6 at two regions, and 2 at three regions). There was no significant difference between the arch and the descending aorta in terms of plaque prevalence. A total of 21 regions in 17 patients contained plaques of ≥ 4 mm.

VTI measurements
Some degree of diastolic retrograde flow within the aortic arch was observed in all 56 patients. Patients with plaques at any location ≥ 4 mm had a higher VTI ratio compared to those without such plaques (0.60±0.23 vs. 0.45±0.21, p<0.05). However, there was a difference depending on plaque location. In patients with any plaques within the aortic arch, the VTI ratios were significantly higher than among those with no plaque or plaques only in the descending aorta.

Measurements of aortic stiffness
Aortic strain was significantly lower at age > 50 years than below (p<0.01), which indicates a stiffer aorta. Pulse pressure also increased significantly with age (p=0.002). Independently of age, patients with plaques in the aortic arch tended to have lower strain values in the descending aorta (p=0.06), in the direction of a stiffer aorta. Increasing systolic and diastolic aortic dimensions were significantly related to increasing blood pressure (p<0.005).

Experimental evaluation of aortic arch IMT - transthoracic ultrasound (Paper IV)
Anatomic validation of the ultrasound images
The exact location of a measurement site in the aortic arch at the level of the innominate artery was confirmed in all 10 animals during ocular inspections of needle positions after killing the animal and opening the chest with a puncture needle *in situ* and with pathohistology specimens showing the puncture channel, Fig. 8.
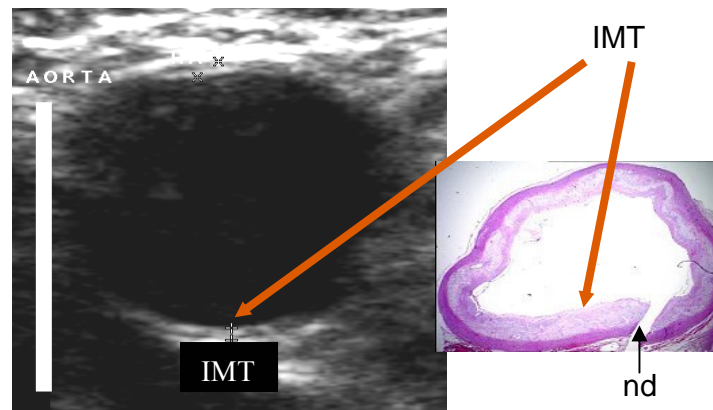

Fig. 8. Cross-sectional view of the ascending aorta. Corresponding histological section to the right, with a needle puncture channel visible through the arterial wall (nd). Shrinkage of the lumen area is clearly evident in the histology compared with the in vivo image.

Histological evaluation of ultrasound-assessed IMT measurements
When comparing the sonographic IMT measurements with the corresponding histological values (34 animals), we found correlation between the two methods (R squared = 0.8, $p<0.0001$), Fig. 9. The correction for vessel shrinkage due to the fixation of the aortic preparation did not improve this relationship between sonographic and histological outcome (R squared = 0.76, $p<0.0001$).
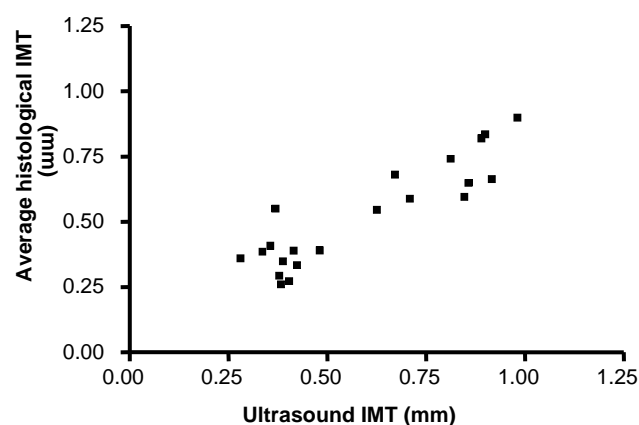

Fig. 9. Correlation between IMT values from histology corrected for shrinkage and ultrasound-measured IMT values.

The average ultrasound-measured IMT was 0.39 ± 0.01 and 0.81 ± 0.04 mm in the 36- and 60-week-old rabbits respectively. Because of the apparent effects of vessel lumen shrinkage in the histological preparations, IMT values from histology were systematically higher than the sonographic ones, with an average IMT_{hist} mean of 0.62 ± 0.02 and 1.14 ± 0.07 mm in the younger and older animals respectively. However, after correcting the histology values for the ultrasound-measured *in vivo* lumen area, the $IMT_{hist\ corr}$ values were 0.35 ± 0.02 and 0.7 ± 0.04 mm in the young and old group respectively and did not differ from the average IMT values directly assessed by ultrasound, i.e. systematic methodological errors by fixation procedures were excluded.

Assessment of progression of atherosclerosis
In the 13 animals in which repeated ultrasound examinations were performed at 21 and 36 weeks of age, an increase in IMT was confirmed in 12 of 13 animals, Fig. 10. The average IMT changed from 257.9 ± 13.9 to 380 ± 13.3 μm between 21 and 36 weeks of age (p=0.0002).
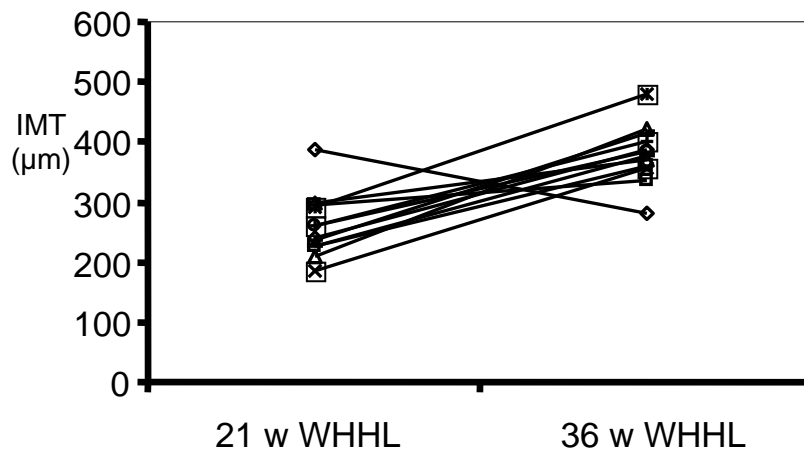

Fig. 10. The individual progression of IMT, as measured by transthoracic ultrasound, at 21 and 36 weeks of age in 13 WHHL rabbits.

Validation of reproducibility
Adequate images were obtained from all the study animals. The intra- and inter-observer variability for ultrasound IMT measurements was 7.2% and 8.5% respectively.

Discussion

<u>Prevalence of embolic sources in ischemic stroke (Paper I)</u>

In this analysis of consecutive patients referred for ultrasound investigation due to a suspected or proven ischemic stroke, we found that TEE gives a considerably higher proportion of positive findings compared to TTE, especially in patients without a history of ischemic heart disease. Furthermore, this study advocates the use of TEE also in patients above 50 years of age in contrast to some guidelines [52, 72, 73] suggesting TEE to be of greater value in younger individuals.

Comparison 'TEE-group' versus 'non-TEE-group'

The ´non-TEE-group´ was included to illustrate the complete consecutive cohort referred due to suspected ischemic stroke. Not surprisingly, we found more signs of IHD among subjects in the 'non-TEE-group', when a primary question was whether poor LV function or ventricular thrombus might explain possible embolization. Often, when there are clinical indications of cardiac disease, the clinician tends to regard this as a possible source of embolism and wants a TTE control of that particular disease entity. Certainly, mitral stenosis or LV aneurysm are putative embolic sources, while other valvular defects as aortic regurgitation might in itself not predispose for ischemic stroke. There is, to our knowledge, no study that demonstrates the justification of avoiding optimal search for cardiovascular source of embolization in patients having known cardiac disease, apart from atrial fibrillation [74, 75] and severe regional [76, 77] or global [78] LV dysfunction justifying anticoagulant treatment. Our data showed that carotid plaque and stenosis are more common in the 'non-TEE-group', most likely because the carotid finding was regarded to be the cause of cerebral ischemia. The use of other risk markers or combination of 2 risk markers did not identify all patients above 50 years with relevant findings on TEE. Thus, it is not possible to use the risk factor pattern as a tool to avoid unnecessary TEE investigations.

Interpretation of results considering age

Some previous studies have concluded that TEE is superior to TTE and should be the echocardiographic method of choice in the search of cardioembolic sources [79], especially in patients below 50 years of age [52, 72]. In contrast to such age policy, but concordant with findings by de Bruin et al, [80] our data shows that a higher yield of important findings are made in the part of the population above 50 years of age. In patients 50-55 years of age, a high proportion of TEE was performed, but with lower yield of relevant findings compared to older patients. Thus, aortic arch atheromas are predominantly found among older patients.

Although such atheromas can sometimes be visualized by TTE, [65, 81] they are often difficult to measure, while TEE is a reproducible technique for such measurement [64]. In our study few patients below 50 years of age, with a final diagnosis of cerebral ischemia, had complex aortic arch atheromas. Moreover, all of these had major risk factors for atherosclerotic disease or clinical proof of such. PFO is a common finding in this and other studies, [82] the prevalence of PFO was higher in younger patients which was also found by Hagen et al [83].

Carotid artery findings – correlation to aortic atherosclerosis

Carotid duplex ultrasound is an established technique with high accuracy to evaluate patients with an ischemic stroke [57, 58]. In younger patients it is used to find dissections and inflammatory vascular disease, while advanced atherosclerosis in this age group is rare. In older subjects, atherosclerotic plaques are prevalent and a likely cause of ischemic stroke, when causing advanced diameter reductions [84]. However, during the last decade, the awareness of atherosclerotic plaque in the thoracic aorta as a potential embolic source has grown considerably. Aortic plaques are more common in patients with carotid atherosclerosis [85], but an important finding in our study was that we cannot predict absence from aortic plaques by means of carotid investigation. Treatment by statins are initiated in most subjects with ischemic stroke, but patients with aortic arch plaque might also be considered for anticoagulant treatment [86, 87], which has not been proven beneficial in patients having carotid stenosis [88]. Aortic atherosclerosis is an important prognostic factor, possibly the only TEE finding to predict long-term prognosis in ischemic stroke after adjustment for all other clinical factors, [89] and the progression of aortic arch atheroma on TEE is associated with recurrent vascular events [90].

MES occurrence in relation to symptomatic ICA stenosis, plaque morphology and intrastenotic flow velocities (Paper II)

Stroke risk in carotid stenosis

CEA significantly reduces the incidence of stroke in patients with symptomatic extracranial carotid artery stenosis, but in some cases this evidence may be of limited help to the clinician who has to make decisions for the individual patient. In patients with symptomatic 70-90% stenosis, the three-year overall risk of ischemic stroke could be reduced by about 50%. However, only 20% of these patients will have a major stroke on medical treatment alone. Surgery is therefore of no value in 80% of patients who, despite having severe symptomatic carotid stenosis, will remain stroke-free on medical treatment alone [91]. The percentage of unnecessary carotid interventions might even increase, adding statins or other new anti-hypertensive medications to the standard anti-platelet therapy [92, 93]. In both carotid endarterectomy trials (ECST and NASCET) [12, 13], the randomization of patients for CEA was exclusively based on the outcome of carotid angiography, disregarding the carotid plaque structure and its embologenic potential.

MES detection by TCD as risk stratification in carotid disease

The significant difference between MES incidence on the symptomatic compared to the asymptomatic side suggests possible association between the occurrence of ischemic events and micro-emboli. In almost 70% of high grade ICA stenosis no MES were present, this could partly be related to the brevity of our sampling period. Our findings support other reports, that clinically silent MES can be detected in 21-100% of cases when monitored with varying recording times from 20 minutes to four hours [94, 95]. The time delay between MES monitoring and the last ischemic event appears to be an important issue. Our study supports the findings of Markus HS et al. [96], showing that the sooner after the last event MES registrations are performed, the higher is the prevalence and frequency of micro-emboli.

Echolucent plaques have been associated with an increased risk of stroke, since MES were more frequently observed in an-echogenic/hypo-echogenic plaques than in iso-echogenic/hyper-echogenic carotid lesions [97-99]. However, Droste et al. found that the echogenicity of the plaque did not affect the number or the presence of MES [100]. Our study showed a tendency towards an association between plaque echolucency and the presence of MES (p=0.07). This trend suggests that the sample size and/or the time period for MES monitoring could be optimized in future studies.

High blood flow velocities including turbulence within the stenosis might mechanically provoke micro-emboli, but we did not found any correlation between high intra-stenotic blood flow velocities in ICA and the occurrence of MES. However, our data agrees with observations by Goertler et al. [101] that low post-stenotic blood flow velocities (<20 cm/s) behind >90% ICA stenosis reduces the appearance of embolic signals in recently symptomatic patients.

The fact that MES were mostly detected on the symptomatic side supports the hypothesis that bilateral TCD monitoring can facilitate the differentiation between cardiac and carotid embolic sources. We believe therefore, that the occurrence of MES is an indicator of plaque instability in symptomatic high-grade ICA stenosis .

Comp CCA helps to identify the intracranial vessels and to assess the collateral capacity within the circle of Willis. Its complication rate is very low when performed as proximally as possible [59] and as long as common carotid artery stenosis and carotid artery dissection is excluded by carotid duplex. During the last decade, we have routinely performed approximately 4,000 TCD investigations with comp-CCA without any complications. In this study, 6/197 patients had short-lasting symptoms of numbness in the contralateral hand during comp-CCA. These symptoms resolved completely immediately after stopping the test and are explained by insufficient collateral compensations during comp CCA. As a result, the surgeons were prepared for intra-operative shunting which was performed in four of these patients because of low carotid stump pressures. MES were provoked by comp-CCA in seven cases, six of these also displayed MES spontaneously during TCD monitoring.

The aortic arch as an embolic source in ischemic stroke (Paper III)

To our knowledge, the present study represents the first attempt of TEE-based Doppler quantification of the diastolic retrograde blood flow components within the aortic arch with various degrees of atherosclerotic vessel wall abnormalities. In all study subjects, we found a retrograde aortic arch blood flow, which varied with the presence and distribution of atherosclerotic lesions and their impact on aortic stiffness. Patients with plaques in the aortic arch, not only had the closeness of plaque to the cerebral artery orifices, but also a significantly increased diastolic flow reversal, and consequently a higher risk for retrograde cerebral embolization.

TEE has been suggested as the standard method to visualize the aortic arch [102-105]. TEE is a moderately invasive technique, and a transcutaneous approach has been applied for evaluation of plaque in the aortic arch [106]. Despite improvement in transthoracic ultrasound techniques [29], TEE still in most cases gives a more secure visualization of the aortic arch. It has a high spatial and temporal resolution, compares favorably to magnetic resonance evaluations [107], and is often more readily available.

Retrograde diastolic flow in the proximal aorta and relation to aortic stiffness
Under normal conditions, retrograde aortic flow occurs in the ascending aorta in diastole as a support for the coronary artery blood flow. Increased blood flow reversal is seen in cases of aortic regurgitation [108], which in severe regurgitation may involve the descending aorta [109], In elderly patients without aortic valve regurgitation, but with stroke and systemic embolism, retrograde diastolic blood flow components have also been observed by means of the motion pattern of mobile aortic plaque formations [110].

We were able to demonstrate the existence and relative magnitude of retrograde diastolic blood flow at a critical location. In our patients, the antegrade and retrograde blood flow velocities, as well as the VTI, were calculated from the spectral Doppler signal. Retrograde blood flow components were shown in all patients, i.e. not only in stroke patients, but also in those with other diagnoses. This implies that cerebral embolism from plaques located distally to the origin of the cerebral arteries theoretically is possible in any subject.

We found increased proportion of flow reversal in patients with plaque formations in the aortic arch compared to those with plaque in the descending aorta, whose flow reversal did not deviate significantly from those with no plaques.

The relatively unstable flow pattern in the distal arch and proximal descending aorta seems to predispose for atherogenesis [111, 112]. This predilection site for plaques, in combination with an increased flow reversal among those with arch plaques such as we have shown, might cause a high risk situation for retrograde embolization. A crude odds ratio of 5.5 for ischemic stroke in case of plaque ≥ 4 mm situated in the distal arch (distal to the left subclavian artery) supports this presumption [1]. We therefore strongly suggest that complex plaques situated in the distal aortic arch are considered a potential source of cerebral emboli.

Transsthoracic evaluation of aortic arch atherosclerosis – experimental analysis of accuracy

The development of atherosclerotic plaques in WHHL rabbits is very similar to that observed in humans [113-115] and thereby provides an excellent tool for studies of plaque morphology [116], its progression [117, 118] and also its changes due to interventional treatment [119-122]. The large difference observed in IMT between individual rabbits in the current study clearly emphasizes the need for a technique that permits repeated examinations of the same animal over time. To enable repetition, the method of choice should be well tolerated (non-invasive), uncomplicated and relatively inexpensive. Although traditional histological techniques generate information down to cellular and sub-cellular level, repeated measurements to follow the progression of atherosclerosis are apparently impossible. Some of the alternative imaging modalities, e.g. magnetic resonance imaging (MRI), has been shown to be useful in vascular imaging. MRI can also be performed with an esophageal coil [123] and even intravascular MRI techniques have recently been introduced [124] to enhance image quality.

However, even though MRI is an established and important source of information in studies of atherosclerotic process, it is not well suited for serial investigations of WHHL rabbits because of the fairly complicated procedure and relatively high expense. Further, MRI with its limited spatial and temporal resolution usually requires mechanical ventilation of the animals, an approach that can be troublesome and may limit repeated examinations. Other methods such as intravascular ultrasound (IVUS) can provide high-quality images with good resolution at a high frame rate [125, 126]. However, most of the studies are terminal because of their invasive approach and do not therefore permit serial studies.

To some extent, histopathological specimens could be regarded as the "golden standard" and provide detailed information on wall structure down to cellular level. However, repeated studies are obviously impossible. Further, in spite of pressure fixation, the histopathological specimens in the current study were also still exposed to shrinkage, leading to incorrect IMT and lumen measurements.

IMT is a well-validated surrogate marker for the progression of atherosclerosis, as well as cardiovascular morbidity and mortality [127, 128]. Non-invasive sonographic IMT measurement is well tolerated, uncomplicated and has previously been shown to be highly reliable in human studies of carotid [129] and femoral arteries [130]. However, the common carotid arteries, often used for IMT measurements in humans, are usually free from measurable lesions even in old WHHL rabbits.

Instead, atherosclerotic vascular lesions typically begin at the minor side of the aortic arch and the ascending aorta. In addition, the anatomy of the aortic arch in WHHL is very similar to the human arch, which makes ultrasonic studies in this territory even more interesting.

Plaque formations in this aortic location are important findings in stroke patients, since they are associated with a high incidence of recurrent embolic stroke [34, 54]. In contrast with humans, due to the relatively thin chest wall of rabbits, the thoracic aorta can be readily visualized in detail with TTE using a high-frequency 15-Mhz linear ultrasound transducer with a frame rate of up to 300 frames/sec. This high-resolution probe provides images with an axial resolution of 120 μm and with an adequate penetration depth. The high temporal resolution of the ultrasound system facilitates real-time imaging, by preventing motion and respiratory artifacts.

The innominate artery is an excellent anatomic landmark for the IMT measurement site in the ultrasound image. Using the systematic imaging protocol described before, IMT can be reproducibly measured in this specific vascular region. The choice of this specific vascular region also facilitates the histological identification of the measured vascular site. With skilled ultrasound operators and an unbiased offline image analysis approach, the inter- and intra-observer variability is excellent. The ultrasound-assessed IMT values correlate well with the histological measured average IMT values after correction for vessel shrinkage due to the histological preparation procedure. Further, ultrasound measurement accurately assessed IMT values also in the individual older WHHL rabbits, which showed a large inter-individual variability in IMT thickness. This method allows a high throughput, a skilled operator spends approximately 3 minutes on each rabbit to acquire all the necessary image data.

In the present study, we validated a non-invasive method for assessing IMT in the ascending aorta of WHHL rabbits. Using high-resolution ultrasound, IMT can be reproducibly measured in adult WHHL rabbits with an up to 100% success rate. This model, therefore, could serve to non-invasively monitor pathophysiologic processes and treatment effects. It also illustrates that, with good image quality, ultrasound evaluation of aortic IMT is accurate.

Treatment alternatives that warrant adequate diagnosis

This thesis deals with diagnosis of possible cardiovascular embolic sources, with an emphasis on atherosclerosis as an important part. Carotid duplex ultrasound is the most widespread tool for evaluation of the carotid arteries, although one should be aware of its limitations when deciding treatment [131]. In ischemic stroke, there is a need for rapid diagnosis which allows causative carotid stenosis to be treated within 2 weeks, or ideally probably within hours [132]. In patients with TIA or minor stroke, without an early intervention, there is no difference in treatment effect initiating either anticoagulation or antipletelet therapy within 6 months [88].

In patients without explaining cerebrovascular pathology, it has recently been demonstrated that treatment in patients undergoing TEE is largely influenced by the findings [133]. In our study (Paper I), possible etiology of stroke was detected in 43.9 % of patients undergoing TEE. The diagnostic yield was greater in patients above 50 years of age. The two major findings were PFO and complex aortic atheroma.

There is an ongoing discussion on the treatment of both these findings. In patients with PFO and no other source of embolism, the treatment options are closure or medical treatment, and the optimal strategy remains to be clarified. This is also the case concerning complex aortic arch atheroma. However, open studies indicate a beneficial effect of anticoagulation [86, 87, 134], and a randomized trial (ARCH; Aortic Arch Related Cerebral Hazard) is underway.

Stroke patients with plaques ≥4mm in the ascending aorta or proximal arch have a significantly increased risk of stroke recurrence. To allow treatment specifically of high risk aortic plaque, it is necessary to determine both their location and properties, so far in terms of size ≥4mm.

In the very early phase of ischemic stroke, thrombolysis is an emerging alternative. In this context, the future of TCD becomes even more exciting since the possibility of ultrasound enhanced thrombolysis [135] is now a reality and addition of microbubbles to further enhance clot lysis [136] and maybe even local delivery of thrombolytic drugs will become an option. Altogether, intensified treatment of stroke and increased understanding of the importance of early interventions puts an additional demand on the diagnostic accuracy and availability.

Conclusions

I: Normal findings on carotid duplex do not exclude the possibility of complex plaques in the aortic arch according to our study and a normal carotid duplex examination therefore does not obliviate the need for TEE in stroke patients. The highest diagnostic yield of TEE is found in patients above 50 years of age. The dominating relevant finding in this age group is complex aortic arch atheroma. Our findings indicate that TEE should not be limited to lower age groups.

II: Cerebral microembolization occurs in 30% of patients with symptomatic carotid artery disease and might therefore be a possible risk factor for stroke. MES are not dependent on intrastenotic blood flow velocities. Plaque characteristics, such as described by Gray-Weale classification only show a tendency for echolucency to correlate with MES emphasizing the need for new algorithms that identify plaque vulnerability with higher precision.

III: A diastolic retrograde flow component was present in the aortic arch of all the examined subjects. The reversed flow component might vary with aortic stiffness, and may be even more pronounced in patients with atherosclerosis causing a reduced Windkessel effect. This phenomenon could facilitate retrograde embolization to the brain from plaques located distally from the orifices of the carotid arteries.

IV: Transthoracic 2-D visualization of the aortic arch IMT, using high-resolution ultrasound, is an accurate and reproducible technique for the non-invasive follow-up of atherosclerotic disease in WHHL rabbits. The protocol facilitates a high-throughput approach and is well tolerated by the animals, which allows repeated examinations at identical vascular sites in the same animal.

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