Ultrasonic evaluation of pathological brain perfusion in acute stroke using second harmonic imaging

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Abstract

Objective—To evaluate the use of transient response second harmonic imaging (HI) by means of ultrasound to assess abnormalities of cerebral echo contrast agent enhancement in patients with acute stroke.

Methods—The study comprised 25 patients with acute onset of hemispheric stroke (<24 h) with sufficient insonation conditions and 14 control subjects without cerebrovascular disease. All stroke patients had HI, extracranial and transcranial colour coded duplex examinations of the arteries supplying the brain, and clinical examinations (European stroke scale) performed in the acute phase, on day 2, and within 1 week. Acute CT was repeated within 1 week and facultatively accompanied by angiography. Examinations using HI were performed in an axial diencephalic plane of section using the transtemporal acoustic bone window. After bolus application of galactose based microbubbles, 61 ultrasound images with a cardiac cycling triggering frequency of once every 2 seconds were recorded and evaluated off line. Focal perfusion deficit was identified if no contrast enhancement was visualised in a circumscribed region of interest and insufficient temporal bone window was excluded. In cases of reappearance of contrast enhancement reperfusion was assessed.

Results—Adequate cerebral contrast enhancement could be seen in 21 subjects. In seven, a large hemispheric deficit of contrast enhancement affecting the entire middle cerebral artery (MCA) territory was detectable; the lentiform nucleus was affected in three subjects. Assessment of cerebral contrast abnormalities was possible in two patients with superficial MCA infarctions but in none of the patients with lacunar ischaemias. None of the control persons had focal deficits of cerebral echo contrast enhancement. In all patients with complete MCA infarction and striatocapsular infarction, presumed ischaemic areas in HI examinations correlated with final CT findings. Overall sensitivity and specificity of HI examinations for predicting the final infarct size and clinical prognosis was determined in three patients, in two patients circulatory arrest due to malignant brain oedema with missing contrast enhancement in the entire cerebral hemisphere could be seen. Extent of contrast enhancement deficits significantly correlated with the clinical status on admission and after 1 week (p<0.01).

Conclusions—Second harmonic imaging is the first ultrasonic technique that enables visualisation of pathological cerebral echo contrast enhancement. Because this method identifies deficits of focal contrast enhancement in patients with acute stroke and allows estimation of the final infarct size and clinical prognosis, it may help to select and monitor patients for invasive therapies.

Keywords: transcranial sonography; contrast media; harmonic imaging

There is considerable variability in the extent and localisation of infarction in patients with middle cerebral artery (MCA) territory stroke, but the accurate clinical discrimination between different patterns of MCA stroke is difficult. Because perfusion imaging may detect ischaemic lesions earlier than CT and distinguish the stroke subtype and severity of cerebral ischaemia, there is growing interest in perfusion imaging for predicting recovery, differentiating stroke pathogenesis, and monitoring therapy. The information on flow is measured directly in the target organ and therefore includes alterations in perfusion caused by collateral pathways. Validated proportional indicators of cerebral blood flow and potential diagnostic tools in stroke are TC-HMPSO-SPECT, PET, and perfusion and diffusion weighted MRI. The main disadvantages of these methods are that they are time consuming, require the use of radioactive tracers, are expensive, or are intolerable for critically ill or restless patients. Non-invasive and easily available perfusion studies are clearly needed—for example, in future thrombolytic stroke trials. In this regard, second harmonic imaging (HI) may represent a useful bedside tool for a reliable measurement of brain perfusion. This technique is based on the nonlinear emission of harmonics by resonant microbubbles pulsating in an ultrasound field. The emission at twice the driving frequency, termed the second harmonic, can be detected and separated from the fundamental frequency. The advantage of the harmonic over the fundamental frequency is that contrast agent microbubbles resonate with harmonic frequencies, whereas adjacent tissues do so.
very little. In this way, HI may enhance the signal to noise ratio and the ability of B mode scanners to differentiate bubbles in the tissue vascular space from the echogenic surrounding avascular tissue. Physiological and pathological myocardial perfusion could be assessed in previous studies. More recently, three studies on HI in neurosonology have shown that this technique allows identification of physiological parenchymal cerebral echo contrast enhancement in different brain areas and is related to brain perfusion. To evaluate the diagnostic potential of HI in the assessment of perfusion abnormalities in patients with acute stroke, we performed a pilot study in 25 patients with acute hemispheric infarction.

Methods

Patients

This study enrolled 25 patients with stroke (15 men and 10 women ranging in age from 35 to 86 years with a mean age of 68.2 years) and 14 control subjects (10 men, four women; mean age 64.3 years) without cerebrovascular disease. Inclusion criteria were adequate trans-temporal insonation conditions in fundamental B mode imaging with the depiction of the third ventricle and adjacent parenchymal structures. For patients with stroke, an acute onset of neurological symptoms consistent with hemispheric infarction in the territory of the middle cerebral artery less than 24 hours before admission was required. Stroke onset was defined as the time the patient was last known to be without neurological deficit. Acute HI studies were done within 24 hours of stroke onset, on day 2 after admission, and within 1 week. Examinations were accompanied by extracranial and transcranial colour coded sonography (TCCS). Clinical evaluation, CT, and angiography (CT or digitally subtracted angiography) were routinely performed. Time points were chosen to image acute stages, early phases of possible spontaneous reperfusion, and to compare the final extent of infarction in follow up CT with the HI examinations. Exclusion criteria were evidence of cerebral haemorrhage on the admission CT, a history of pre-existing neurological disease, prior stroke, or transient ischaemic attack, age under 18 years, known galactosaemia or pregnancy, and unstable neurological deficit. All patients had a full neurological assessment by one of us at admission. The European stroke scale (ESS) score was measured at the same time as each of the HI examinations. All subjects or their relatives gave informed consent for ultrasound examinations.

Ultrasound Studies

We performed all ultrasound studies using a Hewlett Packard SONOS 5500 duplex device in connection with an extracranial 7.5 MHz linear scanner and a transcranial 2 MHz sector transducer capable of fundamental and harmonic imaging with a transmitting frequency of 1.8 MHz and a receiving frequency of 3.6 MHz.

Fundamental Sonography

We performed extracranial and transcranial colour coded examinations of the arteries supplying the brain. For the diagnosis of an occlusion or a high grade stenosis of the internal carotid artery, or an occlusion or branch occlusion of the middle cerebral artery, identical criteria were used as in previously published studies.

Harmonic Imaging Examination

All patients were examined using the transtemporal approach. The axial dienecphalic plane of section was found by tilting the probe 10 degrees from a horizontal mesencephalic plane towards the third ventricle. This structure can be easily identified by the hyperechogenic margins, the anechogenic lumen, and the adjacent hypointense thalami. The ultrasound frame rate was switched to once every 2 seconds. A galactose based microbubble suspension (4 g; Levovist, Schering AG Berlin, Germany) in a concentration of 400 mg/ml was manually injected as a bolus into the antecebular vein cannulated with an 18 gauge catheter, followed by a rapid saline flush. From the start of the injection, the HI examination was stored on magnetic optic disk over a period of 2 minutes (61 images) and evaluated offline. Semiquantitative measurements of digital data were performed by visual assessment of B mode images and by derivation of time intensity curves in defined regions of interest. A normal cerebral perfusion was assumed if grey scale intensities in B mode images increased after echo contrast application and if time intensity curves showed three characteristic phases: (1) a baseline period of 10–20 seconds; (2) a sudden increase of acoustic intensity to a maximum within a few seconds; and (3) a slow decrease of acoustic intensities until the end of the examination. Two conditions were required to confirm an ischaemic area in HI examinations:

(1) Missing contrast enhancement in a focal parenchymal brain area in harmonic grey scale images and absence of characteristic time intensity curves.

(2) Adequate contrast enhancement in at least one region in the identical scanning line as the suspected ischaemic area (exclusion of insufficient bone window).

Images were manually aligned during the evaluation of time intensity curves to correct for patient and ultrasound probe motions. The size and localisation of the detected ischaemic area in HI examinations was classified into the following subgroups:

(1) Complete MCA territory: missing contrast enhancement in the region of the lentiform nucleus and the entire adjacent white matter, thalamus spared.

(2) Partial; (a) lenticulostriatal region: missing contrast enhancement in projection to the lentiform nucleus sparing the entire white matter and the thalamus; (b) superficial: missing contrast enhancement in a circumscribed area of the white matter, normal enhancement in projection to other parts of the white matter, the lentiform nucleus and the thalamus.
Circulatory collapse of the affected brain hemisphere was assumed if contrast enhancement was not detectable in the entire brain hemisphere in follow up examinations after normal contrast enhancement in at least one region in a previous HI examination. Definition of reperfusion was reappearance of contrast in a region with missing contrast enhancement in a previous examination. All HI studies were evaluated independently by two experienced transcranial B mode sonographers (JF, TP). The presence of an ischaemic brain area was established when both ultrasound investigators had a consenting opinion. In cases of disagreement no perfusion abnormality was assumed.

**COMPUTED TOMOGRAPHY STUDIES**

Non-contrast brain CT was performed immediately after admission with a Somatom Plus 4A Scanner (Siemens, Erlangen, Germany). Slice thickness (axial planes parallel to the orbitomeatal line) was 4 mm from the sella region up to the cella media. The initial scans were visually assessed for early infarction signs such as obscuration of the lentiform nucleus, sulcal effacement, hyperdense MCA sign, or visible hypodensity in the MCA territory. A CT angiography or digitally subtracted angiography was added if deemed important for therapeutic decision. Follow up CT was done within 1 week after ictus. The final extent of the stroke related brain damage was classified according to other studies as:

- Complete MCA infarction affecting both the area supplied by the lenticulostriate branches and superficial branches of the MCA
- Superficial MCA infarction affecting areas supplied by pial branches of the MCA (with or without involvement of the adjacent white matter)
- Lenticulostriatal infarction affecting the area supplied by the lenticulostriate branches of the MCA
- Subcortical lacunar infarction
- Internal border zone infarction
- No infarction.

Sensitivity, specificity, and positive and negative predictive value of HI examinations for predicting an MCA infarction on follow up CT were calculated. Statistics were performed employing Fisher’s exact test and non-parametric analysis of variance (ANOVA).

**Results**

**CLINICAL AND CT FINDINGS**

Nine of 34 consecutive patients with stroke had to be excluded due to temporal hyperostosis, allowing neither assessment of intracranial arteries in conventional TCCS examinations nor performance of HI studies. From our sample of 25 patients with stroke fulfilling the inclusion criteria, HI could not be performed in four due to inadequate contrast enhancement despite a sufficient temporal bone window in fundamental imaging. Clinical and demographic data of the remaining 21 patients (14 men, seven women) ranging in age from 35 to 86 years (mean age 65.4 SD (12.2) years) are presented in the table. Two of these patients (6 and 7) were previously described in an initial report on HI in acute stroke. The mean interval from the onset of stroke to the first HI examination was 8.8 (SD (8.5) hours, range 1 to 23 hours). In one patient with MCA main stem occlusion, local thrombolysis with streptokinase infused into the occluded artery through a catheter was performed during the
initial angiography (patient 6). One patient (11) was treated with rt-PA intravenously according to the protocol of the NINDS trial. The other patients were treated with aspirin or, in cases of atrial fibrillation, with intravenous anticoagulation. On admission, five patients were severely affected (ESS 1–40), seven had moderate stroke symptoms (ESS 41–70). Two patients died due to malignant brain oedema with cerebral herniation, two patients underwent decompressive surgery due to malignant brain infarction, one patient had surgery for secondary intracerebral haemorrhage. On admission, 11 patients had a normal CT, eight showed early CT changes, and two demarcation of an infarction. In follow up CT examinations, seven patients had complete MCA infarction, seven had superficial MCA infarctions, three had striatocapsular infarctions, and four had none or other types of infarction (one lacunar infarction, one patient with an infarction of the pons was included by mistake due to clinical findings).

VASCULAR PATHOLOGY IN FUNDAMENTAL ULTRASOUND AND ANGIOGRAPHIC EXAMINATIONS
Extracranial and transcranial sonography showed abnormal findings in 11/21 patients. Three patients had an occlusion of the internal carotid artery at admission. Six patients showed occlusions of the MCA main stem confirmed by angiography. Significant reduction of mean flow velocities of the MCA main stem indicating MCA branch occlusions were seen in two patients. Follow up ultrasound examinations were consistent with vessel recanalisation in 7/11 patients.

SECOND HARMONIC IMAGING EXAMINATIONS
Controls
Thirteen of 14 control subjects with adequate insonation conditions exhibited adequate cerebral contrast enhancement in HI examinations. In all these subjects, acoustic intensities of the thalamus, white matter, and lentiform nucleus increased after the application of the echo contrast agent. According to our criteria, none of the control subjects had focal areas of missing echo contrast enhancement consistent with ischaemia.

Patients with stroke
A total of 54 HI studies were performed. In 21 patients, clear cerebral contrast enhancement could be seen. Performance and evaluation of one HI examination lasted 5–10 minutes. All patients had studies in the acute phase of disease. Subacute or follow up studies could not be done in eight patients, due to early decompressive surgery (patient 14, subacute and follow up, patient 18 follow up), surgery of secondary intracranial haemorrhage (patient 19, follow up), massive agitation (patient 10 subacute study), death (patients 6 and 15, follow up), or other reasons (patient 17 and 20, follow up). In the acute studies, seven patients showed missing contrast enhancement in the complete MCA territory sparing the thalamus (see figures, patient 15). Two of those patients (6 and 15) showed missing contrast enhance-
ment in the entire brain hemisphere including the thalamus at the second examination and died due to malignant brain oedema after 3 days. Five patients had lacking contrast in projection to the lentiform nucleus or the superficial MCA territory; one and two of these showed reappearance of focal echo contrast agent distribution within 24 hours and 1 week, respectively. In follow up examinations of nine patients without depictable area of missing contrast enhancement in the acute examination, no changes were seen during follow up.

CORRELATION OF HARMONIC IMAGING EXAMINATIONS WITH COMPUTED TOMOGRAPHY, TCCS, AND CLINICAL RESULTS

All seven patients with missing contrast enhancement of the entire MCA territory developed complete MCA infarction. Both patients with missing contrast enhancement of the entire brain hemisphere in follow up examinations died due to malignant brain oedema with cerebral herniation.

Missing contrast enhancement in projection to the lentiform nucleus lasting longer than 24 hours was predictive of a striatocapsular infarction in all three patients. Extracranial and transcranial sonography showed MCA (n=2) and ICA (n=1) occlusion in these patients in the acute phase. In two patients with a superficial MCA infarction affecting cortical structures and the adjacent white matter, HI examinations could depict the absent contrast enhancement in projection to the white matter. Patients without a detectable area of missing contrast during the first and second HI examination developed superficial MCA infarction (n=5), lacunar (n=1), other infarctions (n=1), or no infarctions (n=2). In all patients of this subgroup, superficial MCA infarctions were in a cortical localisation and spared the adjacent white matter. Sensitivity and specificity for HI examinations for predicting MCA infarction in follow up CT were 75% and 100%, respectively. The positive predictive value was 100%, the negative predictive value 57.1%. In patients with low clinical scores (<60 points) on admission (p<0.01) and after 1 week (p<0.01), HI examinations significantly more often showed abnormalities of cerebral echo contrast enhancement (Fisher’s exact test). Different patterns of focal echo contrast enhancement deficits (complete, partial, normal) were
significantly associated with the clinical status on admission (non-parametric ANOVA, p<0.01). Within observer agreement for the identification of a complete or striatocapsular infarction was 100%. In two of five patients with superficial MCA infarctions, one observer considered harmonic imaging examinations to be abnormal, whereas the other did not diagnose perfusion abnormalities.

Discussion

It has been shown that HI produces accurate contrast enhancement in different parenchymal brain areas of healthy subjects after application of galactose-based microbubbles.19 The diagnostic potential of HI for the assessment of brain perfusion abnormalities has not been determined hitherto. In a preliminary study including two patients with acute stroke, we found abnormalities of echo contrast agent distribution that correlated with the final infarct size as well as the outcome of the patients.20 The major finding of the present prospective study in unselected patients with acute stroke is that HI is able to identify normal and pathological cerebral parenchymal echo contrast enhancement in most patients with stroke. Particularly large ischaemic areas affecting both the area of the lentiform nucleus supplied by the lenticulostrate arteries and the convex surface of the brain supplied by the superficial MCA could be easily identified and differentiated from isolated striatocapsular ischaemias or infarctions exclusively affecting areas supplied by the superficial branches of the MCA. Cortical infarctions in the territory of the superficial MCA could be identified if the adjacent white matter was affected as well. By contrast, lacunar infarctions could not be depicted. These findings are comparable with SPECT studies in acute stroke, although the number of patients in our series is relatively low. SPECT patterns of perfusion have been shown to have a high sensitivity and specificity within the first 48 hours for the localisation of large cerebral infarctions. It was stated that a normal SPECT during the first 6 hours of stroke implies minor or lacunar stroke with minor tissue damage, suggesting no need for decompressive surgery or intra-arterial thrombolysis.21 This practical approach to invasive treatment could be realised as well, based on the ultrasound examinations of our study. All patients with missing contrast enhancement in at most one region of interest had small infarctions on follow up CT. Large deficits of cerebral echo contrast enhancement affecting all parenchymal regions supplied by the MCA were associated with extensive brain lesions. Furthermore, the extent of echo contrast enhancement deficits correlated with short term outcome of the patients. In our series, 80% of the patients with perfusion deficits affecting the white matter and lentiform nucleus had a moderate and severe neurologica deficit after 1 week or needed decompressive surgery. Accordingly, quantitative analysis and visual patterns of brain perfusion seen on SPECT of patients with acute stroke were a useful predictor of stroke outcome, improving prognostic value of CT and clinical examinations.

In cases of late reappearance of contrast enhancement in follow up HI examinations, tissue was irreversibly damaged. This finding correlates with experimental stroke studies and SPECT studies describing revival times of cerebral tissue after ischaemia of less than 6 hours. A missing echo contrast enhancement in the entire brain hemisphere during follow up was a specific finding for malignant MCA infarction with compromise of circulation due to increased pressure in the extravascular space. In agreement with this, Berrouschot et al22 reported activity deficits covering the complete MCA territory in most patients with neurological death due to malignant MCA infarction.

In this pilot study, we did not perform reference methods for the assessment of brain perfusion such as PET or SPECT. For this reason, the precise pathophysiological correlate of the area of missing echo contrast enhancement still has to be determined. Nevertheless, two indirect conclusions from the results of our CT examinations and previously published myocardial perfusion studies can be drawn to characterise the underlying pathophysiology of such regions and the reliability of HI. Firstly, in animal and human studies, risk area during coronary occlusion and infarct size after myocardial reperfusion could be quantified in HI examinations when using postmortem and radiographical examinations as reference methods, thus indicating the reliability of this technique to identify pathological perfusion.23,24 Secondly, as we did not find an increase in ischaemic volume lesion in follow up HI examinations, except for those with malignant hemispheric infarction, and the presumed ischaemic area did not exceed infarction size as measured on CT, the initial area of missing contrast enhancement most likely represents the maximum possible infarct size including the ischaemic core and the penumbra. For technical reasons, it was not possible to differentiate the core ischaemic area and the penumbra within this region. Ultrasound examinations were only able to differentiate between present and absent parenchymal contrast enhancement, because the signal to noise ratio of the reflected ultrasound signals was not appropriate to differentiate low flow (corresponding to the penumbra) from no flow (corresponding to the core infarction) within the ischaemic area.

In this study we refrained from the evaluation of quantitative HI indices derived from time intensity curves and used the semiquantitative criterion of missing or present contrast enhancement in a region of interest. The main problem of quantitative data derived from HI examinations is the non-linear relation between echo contrast concentrations and videointensity. Instability and variations in the size of microbubbles, depth dependent attenuation of the ultrasound beam, and signal processing of reflected ultrasound by the equipment software contribute to the complexity of quantification of HI investigations.25 At present, semiquanti-
ative analysis of contrast enhancement is the appropriate tool to evaluate physiological and, as shown by this study, pathological cerebral perfusion.

The major limitation of HI examinations in acute stroke is caused by anatomical peculiarities of the isonion through the intact temporal skull. Furthermore, 16% of those with adequate isonation conditions in conventional colour coded ultrasound examinations did not exhibit adequate contrast enhancement in our study. This finding can most likely be explained by the fact that the receiving frequency in our HI examinations (3.6 MHz) did not successfully penetrate through the temporal bone in some patients. Development of transducers with lower frequencies (for example, 1 MHz emitting and 2 MHz receiving frequency) may help to overcome this problem at the expense of anatomical resolution. In addition, an asymmetric temporal bone window may attenuate the ultrasound signal in circumscripted parts of the sector and has to be taken into consideration when evaluating perfusion abnormalities. A further disadvantage is that only sector shaped transducers can be used in transcranial ultrasound because of the narrow temporal bone window. The restricted contact surface of the probe on the head makes most cortical areas of the brain unassessable. By contrast, the white matter is easily and reliably depicts due to the favourable isonation depth, the median localization in the ultrasound sector, and the marked increase in optic intensity after echo contrast application.

In conclusion, our study describes a new technique for the assessment of cerebral perfusion abnormalities. Harmonic imaging provides a practical and cost effective bedside technique of locating cerebral ischaemia in acute situations. It is mainly restricted to large space occupying and striatocapsular MCA infarction, a normal echo contrast enhancement in victims of acute stroke may imply minor or lacunar stroke with minor tissue damage. Considering the widespread availability of duplex devices, this technique may represent a practical diagnostic alternative to MRI, SPECT, and PET examinations. Although our results are encouraging, larger trials are required to precisely establish predictive HI values with respect to the extent, severity, and short term outcome of hemispheric stroke. These studies should include a validation of HI examinations against other techniques for the assessment of brain perfusion such as perfusion or diffusion weighted MRI.

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