Laser speckle imaging identification of increases in cortical microcirculatory blood flow induced by motor activity during awake craniotomy

Clinical article

EVA KLIJN, M.D.,1 HESTER C. HULSCHER, M.D.,2 RUTGER K. BALVERS, M.Sc.,2 WIM P. J. HOLLAND, M.Sc.,1 JAN BAKKER, M.D., Ph.D.,1 ARNAUD J. P. E. VINCENT, M.D., Ph.D.,2 CLEMENS M. F. DIRVEN, M.D., Ph.D.,2 AND CAN INCE, Ph.D.1

Departments of 1Intensive Care and 2Neurosurgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands

Object. The goal of awake neurosurgery is to maximize resection of brain lesions with minimal injury to functional brain areas. Laser speckle imaging (LSI) is a noninvasive macroscopic technique with high spatial and temporal resolution used to monitor changes in capillary perfusion. In this study, the authors hypothesized that LSI can be useful as a noncontact method of functional brain mapping during awake craniotomy for tumor removal. Such a modality would be an advance in this type of neurosurgery since current practice involves the application of invasive intraoperative single-point electrocortical (electrode) stimulation and measurements.

Methods. After opening the dura mater, patients were woken up, and LSI was set up to image the exposed brain area. Patients were instructed to follow a rest-activation-rest protocol in which activation consisted of the hand-clenching motor task. Subsequently, exposed brain areas were mapped for functional motor areas by using standard electrocortical stimulation (ECS). Changes in the LSI signal were analyzed offline and compared with the results of ECS.

Results. In functional motor areas of the hand mapped with ECS, cortical blood flow measured using LSI significantly increased from 2052 ± 818 AU to 2471 ± 675 AU during hand clenching, whereas capillary blood flow did not change in the control regions (areas mapped using ECS with no functional activity).

Conclusions. The main finding of this study was that changes in laser speckle perfusion as a measure of cortical microvascular blood flow when performing a motor task with the hand relate well to the ECS map. The authors have shown the feasibility of using LSI for direct visualization of cortical microcirculatory blood flow changes during neurosurgery.

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KEY WORDS • functional neurosurgery • microcirculation • cerebral blood flow • optical imaging • cortical mapping

Abbreviations used in this paper: CCD = charge-coupled device; ECS = electrocortical stimulation; fMRI = functional MRI; LSI = laser speckle imaging; ROI = region of interest.
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It is an optical modality that uses a low-power laser diode for illumination of organ surfaces and a grayscale CCD camera for imaging the area of interest at a normal video acquisition rate (for example, 25 Hz). Laser speckle contrast analysis exploits the fact that a random speckle pattern is generated when tissue is illuminated by laser light. The pattern is dependent linearly on the movement of red blood cell flow. The contrast imaging is processed to produce a color-coded live image that correlates with perfusion in the tissue. It is specifically sensitive to microcirculatory flow, and we validated this fact in humans by comparison with microcirculatory flow measurements obtained by direct observation of the nailfold microcirculation. Laser speckle imaging has been extensively tested in animals and humans to measure flow in different organs and on surfaces, such as skin, retina, and mucosal surfaces. In these settings, LSI has proven to be a simple, safe, and reliable method for continuous online measurement of microcirculatory blood perfusion. To our knowledge, only 2 studies have utilized this imaging technique during neurosurgery, but the technique has yet to be explored for use during awake craniotomy for functional brain mapping. The technique is especially suitable for clinical scenarios in which surface contact is undesirable and microcirculatory perfusion in large areas requires online monitoring.

Our aim in the present study was to investigate whether LSI is suitable for mapping functional brain areas during neurosurgical procedures. For this purpose, LSI measurements were performed on brain tissue in patients undergoing awake neurosurgery. To determine the ability of LSI to detect local changes in cortical microcirculatory perfusion, patients were asked to perform a motor task, and the results were compared with ECS mapping data.

Methods

Patient Population

This study was a prospective, single-center observational study. The medical ethics committee of the Erasmus Medical Center, Rotterdam, approved the protocol. All procedures were performed in accordance with the Helsinki declaration. Written informed consent was obtained from all patients.

All patients with an indication for an awake craniotomy procedure were eligible for inclusion in the study. The indication for an awake procedure at our center is suspicion of a low-grade glioma in or near eloquent brain areas, such as motor or language areas.

Anesthesia Procedure

Patients received 1.5 mg of lorazepam on the evening before surgery. All patients were on a regimen of 8 mg of dexamethasone twice per day, while regular personal drug regimens were continued. Thirty minutes before inducing anesthesia, patients were given 7.5 mg of piracetam and 25 mg of promethazine. Piritramide was used to reduce pain perception during skull infiltration with 40 ml of bupivacaine 0.375% and epinephrine 1:300,000. Additionally, low doses of propofol were used for sedation and remifentanil for analgesia.

We also performed continuous monitoring of electrocardiographic activity, peripheral oxygen saturation, and invasive arterial pressure through the insertion of an indwelling catheter into the radial artery ipsilateral to the lesion.

Surgical and Stimulation Procedure

Craniotomy was performed with the patient’s head immobilized in a Mayfield headrest system (OMI, Inc.). A standard protocol was followed for all patients, which included preoperative fMRI for intraoperative neuravigation, bipolar cortical and subcortical motor mapping, and continuous clinical evaluation of motor responses. A cortical stimulator (Grass Technologies, Astro-Med, Inc.) was used to deliver square-wave pulses to induce depolarization of motor cortices. The intensity of the working current was increased from 6 to 12 mA (60 Hz frequency, 1-msec duration) until a motor response was evoked. Cortical stimulation was considered to be positive when the anesthesiologist and the patient observed a motor response. Areas with a positive response after cortical stimulation were labeled with numbers. When the exposed brain area was stimulated, a photograph of the exposed area was taken, with labels representing the total functional ECS map. This photograph was used for the offline laser speckle analysis.

Experimental Procedure

After the dura was opened, LSI and subsequent electrocortical mapping were performed while the patient was awake. After setting up the LSI system, patients were instructed to perform the following rest-activation-rest protocol for mapping the motor cortex. During baseline measurement, the patient and operating room staff were instructed to be completely silent for 1 minute. Subsequently, the patient was asked to start fiercely contracting his or her fist for a period of 1 minute, followed by a 1-minute period of complete rest and silence.

To correlate patient motor movement with cortical activity registered by the LSI device, we acquired simultaneous images using a synchronized security video camera focused on the patient’s hand.

Laser Speckle Imaging and Analysis

Laser speckle imaging was performed with a full-field laser perfusion imager (Moor Instruments, Ltd.), which was mounted in the craniotomy opening perpendicular to the brain at a distance of 30 cm. The LSI was set for low-resolution high-speed images, with a display rate of 25 Hz, time constant of 1 second, and camera exposure of 20 msec. The device used a Class 1 near-infrared laser source with a wavelength of 785 ± 10 nm. The CCD camera incorporated a band pass filter, which attenuates other wavelengths. The raw speckle images were used to compute the speckle contrast image. Software was used to calculate the speckle contrast for any given square of 5 × 5 pixels and assigned this value to the central pixel of the square. This process was then repeated across the image of 576 × 768 pixels to obtain the contrast map. For
each pixel in the speckle contrast map, the relative velocity of blood flow was obtained.

Although the LSI software provided a real-time flux time curve in specific ROIs, a customized program was written for postacquisition analyses for research purposes. The software was written in MATLAB (The MathWorks, Inc.) by our department (Erasmus Medical Center, Rotterdam, the Netherlands). The software was customized to analyze the changes in microcirculatory blood flow (flux) over time for any selected location of the laser speckle image. The raw images were loaded in the program, which enabled 4 sampling windows to be superimposed on the laser speckle images. The sampling windows could be varied in length and width. Sampling windows were placed on areas depicted as functional brain areas of the hand, as indicated on the photograph of the functional ECS map, which was made with ECS during surgery. For those 4 sampling windows, the program generated flux curves, depicted as line scans of the mean flux in the area of the sampling window over the time course of the total measurement. It also displayed a bar chart reflecting the mean LSI flux in a specified sampling window over a selected interval. Figure 1 features an example of the analysis procedure for the LSI signal.

Statistical Analysis

Values are reported as the means ± standard deviations. Each variable was assessed using analysis of repeated measurements (2-way ANOVA). When appropriate, post hoc analyses were performed using Dunn multiple comparison tests. A p < 0.05 was considered statistically significant. All analyses were performed using Prism (version 5.0, GraphPad Software, Inc.).

Results

Eight patients were included in our study. Table 1 shows the baseline demographics and surgical covariates of the study group. In all of the patients, functional motor areas were mapped with ECS. Figure 2 shows an example of the results of ECS, with the white numbered labels representing the locations of mapped functional areas (speech and motor areas). Figure 1 shows the setup for offline analysis of the LSI signal. We used the ECS topography overview to choose the location of the ROIs and the control regions (with no functional activity during ECS mapping). Changes in cortical blood flow for the entire study population are depicted in the left panel of Fig. 3. In the cortical area involved in manual motor skills mapped by ECS, cortical blood flow increased from 2052 ± 818 AU to 2471 ± 675 AU (p < 0.001) during the stimulation protocol. In regions in which no functional motor areas were mapped during ECS (control regions), no changes in blood flow occurred. In the right panel of Fig. 3, changes in blood flow in the functional motor areas and control areas are represented for individual patients. In 6 of the 8 patients, a significant increase in cortical blood flow was seen during the stimulation protocol. In addition to the offline analyses, local changes in cortical blood flow in the functional motor areas of the hand could be visualized intraoperatively in the color-coded laser speckle images (Fig. 4). We did not observe any changes in mean arterial pressure, heart rate, or peripheral oxygen saturation during the experiments.

Discussion

In this study, for the first time, LSI was applied for

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**Fig. 1.** Laser speckle imaging identifies cortical microcirculatory blood flow increase following fist clenching during awake neurosurgery. A: Online black-and-white image seen using LSI. This image allows direct identification of the morphological location of flow changes. B: Processed online image in pseudo color showing areas of high flow (red). C: Photograph of operative setting. D: Offline analysis of sequences of images identified the ROI in panel B as being part of the cortex responsive to motor activity. Analysis of the changes in contrast in this area allows quantification of the response to fist clenching to be measured (each color is 1 subarea of the area of interest shown).
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TABLE 1: Baseline demographics of the study population*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Location</th>
<th>Pathology</th>
<th>Hb Concentration</th>
<th>ECS Confirmed</th>
<th>LSI Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29, M</td>
<td>parietooccipital rt</td>
<td>low-grade astrocytoma</td>
<td>8.9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>42, F</td>
<td>temporal rt</td>
<td>low-grade oligodendroglioma</td>
<td>8.6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>43, M</td>
<td>frontal rt</td>
<td>low-grade mixed oligoastrocytoma</td>
<td>7.8</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>34, F</td>
<td>frontoparietal lt</td>
<td>low-grade oligodendroglioma</td>
<td>7.2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>42, F</td>
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<td>low-grade astrocytoma</td>
<td>7.7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>38, F</td>
<td>temporal lt</td>
<td>glioblastoma multiforme</td>
<td>8.2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
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<td>frontal lt</td>
<td>low-grade astrocytoma</td>
<td>9.3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>25, F</td>
<td>frontoparietal lt</td>
<td>low-grade astrocytoma</td>
<td>7.7</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Hb = hemoglobin.

functional mapping of cortical perfusion in awake patients during resections of low-grade gliomas. The changes in laser speckle perfusion, as a measure of cortical microvascular blood flow when performing a motor task, correlated with the ECS mapping data. This correlation was demonstrated objectively by significant changes in laser perfusion blood flow in areas determined to be functional motor areas by ECS during hand clenching as compared with control regions (offline analyses). Furthermore, we saw visible changes in the color-coded laser speckle images when the motor task was performed during surgery. A specific advantage of LSI is the ability to superimpose morphological images of the cortex surface on functional perfusion images, allowing direct and detailed neurological identification of functional locations on the brain during surgery. In addition, it is a rapid-response imaging modality, which allows direct and instant assessment of cortical perfusion changes to motor or speech activity. Other functional imaging modalities that might be used during awake craniotomy for functional neurosurgery, such as infrared thermography and Doppler imaging, do not have such advantages.

To date, LSI has been used mainly in preclinical experimental research settings and rarely has been translated to human research. Most human studies utilizing LSI have assessed changes in skin perfusion in different pathological states. Only one of these studies has used LSI to study cerebral blood flow in humans. Hecht et al. studied 3 patients who underwent a cerebral revascularization procedure for aneurysm treatment. These authors concluded that LSI was a suitable technique for assessing bypass graft patency with high spatial resolution. Several experimental studies have already been focused on neurovascular coupling in the rat somatosensory cortex. Royl et al. performed LSI of the rat somatosensory cortex during a functional challenge via electrical forepaw stimulation. They found a correlation between LSI-measured cerebral blood flow and the underlying neuronal activity measured using the somatosensory evoked potentials recorded with epidural electroencephalography. These authors concluded that LSI is a reliable method of visualizing local changes in cerebral blood flow and suggested that it may be more accurate than oxygen-related methods, such as fMRI, in localizing neuronal activity. In several other experimental studies on cerebral blood flow, LSI has been shown to provide high-resolution images of the spatiotemporal dynamics of cerebral blood flow. However, none of these studies has addressed the question of whether LSI can be used to identify functional brain areas.

Because our study was designed as a feasibility study, it does not allow exact calculation of the spatial and temporal resolution in our setting; however, the spatial and temporal resolutions of LSI have been extensively studied elsewhere. Durduran et al. demonstrated that LSI can effectively measure, map, and characterize cerebral blood flow’s response to electric somatosensory forepaw and hindpaw stimulation with high temporal and spatial resolution. Their stimulus protocol was adapted to exactly calculate temporal resolution. They placed subdermal electrodes and used a mode of triggering that allowed an exact coregistration at the time of data acquisition and stimulation, permitting exact calculation of temporal resolution. In our setting this coregistration was much more difficult given the nature of the stimulation, that is, asking the patient to start fist clenching. Therefore, exact timing of the start of the motor task was not possible.
Using LSI in clinical research would overcome some limitations of other commonly used techniques for measuring cerebral blood flow and brain function. Fiber-based laser Doppler flowmetry enables reliable measurement of vascular perfusion but only in a small volume of tissue, requiring physical contact with the tissue surface. As a scanning modality, laser Doppler flowmetry does overcome this shortcoming but is a relatively slow technique and therefore cannot detect rapid perfusion changes. Thermography can generate tissue perfusion at a high frame rate but is based on changes in tissue temperature, and rapid changes in tissue perfusion do not immediately induce changes in temperature; thus, thermography is limited in its temporal resolution. Near-infrared spectroscopy measures microvascular oxygenation; therefore, it offers only limited information on actual flow alterations and can only provide measurements in a small volume of tissue. The newly developed full-field laser Doppler imaging is a promising technique but is still limited by small imaging areas, making it more laborious to visualize the total exposed brain surface, and it cannot yet provide real-time data. We have shown that LSI is capable of measuring microvascular tissue perfusion at rest and during different provocation tests and is related to capillary blood flow, as assessed by direct microscopic observation.

In clinical practice, intraoperative ECS mapping is routinely combined with preoperative fMRI to aid maximal resection of tumors located near functional brain areas. Although it is the gold standard, ECS is a time-consuming method of mapping different functional brain areas and carries the risk of afterdischarge activity, such as seizures. Alternatively, preoperative fMRI is noninvasive, but the potential loss of spatial validity from intraoperative displacement and deformation of the brain during surgery and the large-vessel effect extending the signal toward the draining vessels give it limited value in the precise intraoperative detection of functional cortex areas. Although LSI cannot be used for preoperative mapping, it does overcome most of the intraoperative drawbacks of ECS and fMRI. The technique has the advantage of providing visualization of video images combined with functional blood flow images, making it possible to accurately couple functional with anatomical images in a noncontact manner during surgery. In our setting we performed an offline analysis to determine the exact magnitude and location of increases in blood flow and to confirm our intraoperative observations. However, changes in local blood flow can be directly observed during surgery because the contrast imaging is processed to produce color-coded live images that correlate with perfusion in the tissue. This processing is done and can be visualized directly on screen using the software supplied by the manufacturer.

Despite these promising results, some limitations must be addressed. First, only 6 of our 8 patients showed changes in blood flow with LSI. The nonresponders can be explained by multiple factors, which must be ruled out in control experiments. One factor could be intersubject variability in task execution. A second factor is probably that electrophysiological activation to motor stimulus is more sensitive and has a higher resolution than blood flow changes to motor stimulation. Currently, LSI is not a sub-
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In this study, we demonstrated that LSI can be used to identify increases in cortical microcirculatory blood flow induced by motor activity during awake neurosurgical procedures. Using LSI as a macroscopic noncontact method to visualize cortical microcirculatory blood flow intraoperatively would overcome some of the limitations of ECS. Further research should be conducted to determine whether LSI could eventually replace ECS to map functional brain areas during surgery. It is anticipated that LSI will enter the realm of neurosurgery as a noninvasive online assessment of cerebral blood flow during neurosurgical procedures.

Conclusions

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Klijn, Hulscher, Balvers, Vincent, Dirven, Ince. Acquisition of data: Klijn, Hulscher, Balvers, Vincent. Analysis and interpretation of data: Klijn, Hulscher, Balvers, Vincent. Drafting the article: Klijn. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Klijn. Statistical analysis: Klijn. Administrative/technical/material support: Holland. Study supervision: Vincent, Dirven, Ince.

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Address correspondence to: E. Klijn, M.D., Department of Intensive Care, Erasmus MC University Medical Center, P.O. Box 2040, Room H 621, 3000 CA Rotterdam, The Netherlands. email: e.klijn@erasmusmc.nl.