A Pilot Comparison of Multispectral Fluorescence to Indocyanine Green Videoangiography and Other Modalities for Intraoperative Assessment in Vascular Neurosurgery

BACKGROUND: Digital subtraction angiography (DSA) is the gold standard for vascular imaging, but is not easily integrated into a continuous microsurgical environment. Other available modalities for intraoperative vascular assessment have their own limitations.

OBJECTIVE: To investigate multispectral fluorescence (MFL), a new technology based on indocyanine green (ICG) fluorescence, which may provide advantages over current intraoperative imaging modalities.

METHODS: Cadaveric intracranial aneurysm models and turkey wing bypasses were created and tested with white light and micro-Doppler ultrasound, indocyanine green videoangiography (ICG-VA), MFL, and DSA in conditions mimicking surgery. Assessments with these modalities were scored by 7 neurosurgeons.

RESULTS: DSA was significantly better than other modalities in evaluating the vasculature (P < .0001), but was significantly less ergonomic and efficient (P < .0001). MFL and ICG-VA were not significantly different from each other. Both were significantly better than white light/micro-Doppler ultrasound in assessing occlusion and patency (P ≤ .011), and both were better than DSA in ergonomics and efficiency (P < .0001).

CONCLUSION: MFL performs similarly to ICG-VA in a laboratory setting. Further study will be required to determine whether it compares favorably in the operating room. While DSA is the standard for cerebrovascular visualization, MFL and ICG are significantly more ergonomic and efficient.

KEY WORDS: Vascular, Microscope, ICG, MFL, Intraoperative angiogram, Doppler, Indocyanine green, Multispectral fluorescence

Fast, accurate assessment of vascular anatomy and blood flow is critical during open cerebrovascular surgery. The safety and effectiveness of procedures involving the clipping of aneurysms, extirpation of arteriovenous malformations, and bypass depend upon this information. Information regarding flow and stenosis is critical to avoid hemorrhagic and ischemic complications. Accurate visualization is not only necessary during the normal procedure (eg, to ensure occlusion of aneurysms or patency of the parent vessel or anastomoses), but also during complications and surgical crises where swift action is required. However, all current intraoperative visualization modalities for vascular structures have limitations and drawbacks.

Digital subtraction angiography (DSA) is considered the gold standard for vascular visualization, but is not easily integrated into continuous microsurgery. More portable, less invasive modalities—such as micro-Doppler ultrasonic probes (micro-Doppler ultrasound (MDUS)) and indocyanine green videoangiography (ICG-VA)—provide more immediate, useful assessments of the vasculature. ICG-VA entered the cranial neurovascular arena in 2005.
DSAs and ultrasound (US) have been utilized in cerebrovascular procedures since 1956 and 1979, respectively. Each of these modalities has its associated limitations. DSA requires travel from the operating room (OR) to the angiography suite, use of C-arm florescopy equipment, or a hybrid OR-angiography suite, all of which have disadvantages. Doppler US provides limited information and can even yield inaccurate Doppler signal from transmitted pulsations in an occluded vessel. ICG-VA requires a direct line of sight and necessitates a pause in surgery to view the recorded images.

Some studies comparing ICG-VA with DSA show a concordance rate of 90% or higher. Others indicate that, in clinical usage, ICG-VA is not nearly as reliable. MDUS-to-DSA comparisons—while fewer—also show high concordance rates. This shows that these imaging modalities under ideal circumstances may be nearly as accurate as the gold standard. The ideal tool in intraoperative vascular neurosurgery is an efficient imaging modality that gives accurate information, is safe, and does not delay the surgical task at hand. Multispectral fluorescence (MFL) is a technology that may allow for vascular imaging, overlaid real-time onto the surgeon’s live view of the vascular anatomy. Used first in surgical oncology, it is based on the ICG-VA-marking process and requires the same line of sight for fluorescence. It differs from conventional ICG-VA in that the traditional white light of the surgical field can be used simultaneously.

To date, no US Food and Drug Administration-approved technology exists for real-time visualization of vasculature that provides accurate, high-definition, color imaging of surgical anatomy augmented with information on flow and patency. In this study we sought to compare 4 visualization technologies, using anatomic models that have proved beneficial in teaching cerebrovascular neurosurgery: (1) white light with Doppler US; (2) ICG-VA; (3) MFL; and 4) DSA. To the authors’ knowledge, this is the only study of its kind attempting to quantify whether an emerging technology (MFL) provides better visualization without the limitations associated with other technologies. Our hypothesis is that MFL is noninferior to ICG-VA and offers advantages over the other modalities tested in this study. This is a pilot study meant to assess the viability of this new technology, which would need to be further validated with clinical studies in the future.

METHODS

Testing was done on cadaveric heads and turkey wings. Institutional review board approval was therefore not necessary.

Materials

Cadavers

Three cadaveric heads were obtained and prepared so that the vascular tree could be actively perfused. This technique requires cannulation of the bilateral carotid arteries, vertebral arteries, and jugular veins and has been previously described. The heads are preserved without embalming solutions. Briefly, the method allows pulsatile flow through the cadaveric arteries, simulating an intraoperative scenario.

In the cadaver heads thus prepared, 3 kinds of saccular aneurysms were created in locations commonly encountered in the clinical setting: the middle cerebral artery bifurcation, the paraclinoid internal carotid artery; and the posterior communicating artery origin. The methodology for their creation is detailed elsewhere. All aneurysms were created by one of the authors (E.A.), and were later clipped by other individual surgeons prior to assessment with the imaging modalities as detailed below. The aneurysms were clipped under reperfusion in a standard surgical setting employing microsurgical instruments and the operating microscope. Clipping was performed by 3 of the authors.

When assessing the aneurysms with white light and MDUS, the aortic pump was allowed to continue to perfuse the cadaveric vasculature with saline. To assess ICG fluorescence and MFL (Figure 1A-C), a 3-way stopcock was used to inject our indocyanine green (ICG) solution into the line. The ICG solution was made with 10 mg ICG diluted in 500 mL of water, with 10 mL of cow blood as a protein binder. For DSA, the same stopcock was used to inject Omnipaque into the line (Figure 1D).

Turkey Wings

Three turkey wings were obtained for performing microvascular anastomoses during this study. These were used for the brachial artery and vein so that an end-to-side anastomosis could be sewn in a method that is described in more detail elsewhere. Two end-to-side anastomoses were sewn by one of the authors (C.N.), and the third was sewn by a senior vascular neurosurgeon who was not a part of the study (M.L.). Of note, this third bypass model was sewn a week prior to the rest of the lab and was refrigerated and kept moist until the time of the lab.

For this study, the proximal end of the brachial artery was transected and dye injection was performed as described previously. Briefly, the turkey wing anastomoses were perfused with a 4-Fr catheter inserted into the cut end of the artery. This was then attached to a syringe filled with the ICG solution or plain saline. The syringe was used to push the liquid through the vessel anastomosis in a pulsatile fashion. Plain saline was used when evaluating the anastomosis using white light and Doppler; the ICG solution was used when testing ICG-VA or MFL, and Omnipaque was used when assessing DSA (Figure 2).

Lab Setup and Technologies Used

While the aneurysm models were prepared ahead of time, all procedures and evaluations were conducted at a research institute when the study began. Angiograms were performed using a Zeego (Siemens, Munich, Germany) single-plane angiography suite at the Medical Education Research Institute, Memphis, Tennessee.

For the white light and MDUS modality, vessels were assessed with a 20-MHz Doppler transceiver and disposable 2-mm micro-Doppler probe (Mizuho America, Union City, California) and viewed under magnification with the microscope using plain white light.

Two M530 OH6 surgical microscopes (Leica Microsystems, Wetzlar, Germany), with digital cameras integrated into the microscope, were used for evaluating ICG-VA and MFL. In both microscopes, filters and their respective imaging software could be activated via a button on the handgrip—FL800 for ICG and MFL800 for MFL. In both cases, the Leica Image Processing Unit allowed images to be viewed directly in the microscope’s eyepiece or on the monitor mounted on the scope. One microscope was mounted with the standard camera and fluorescence accessory for ICG, and the other with a camera and fluorescence...
accessory for MFL. On the ICG scope, resolution on its integrated camera was $752 \times 582$ pixels with a 1/2" (charge-coupled device) sensor and a frame rate of 50 fps. The MFL microscope was fitted with 1/1.2" CMOS sensor with a resolution of $1920 \times 1200$ pixels and a frame rate of 60 fps.

The study was financially supported by Leica, which provided the two microscopes used for the testing, and travel and lodging for 5 of the 7 neurosurgeons involved in the lab. No honoraria were given and the surgeons were not otherwise compensated for participating in the study.

Modality Evaluations

Seven vascular neurosurgeons, with varying experience levels from 1 to 31 yr postfellowship, independently judged the 4 different imaging modalities. These were white light/MDUS, ICG, MFL, and DSA. All surgeons were familiar with ICG in clinical practice, but none had significant experience with MFL. Two surgeons were dual trained open and endovascular neurosurgeons, and therefore had experience with angiography in their own clinical practice. Each imaging modality was judged on its ability to assess four different categories: occlusion, patency, ergonomics, and efficiency. The surgeons were told specifically not to judge the characteristics of the actual aneurysm clipping or bypass, but instead to judge the imaging modality's ability in assessing the aneurysm or bypass.

When looking at bypass specimens, occlusion refers to assessing leakage from the anastomosis, and patency refers to assessing patency of the bypass. When viewing specimens of aneurysm-clipping, occlusion refers to the assessment of aneurysm obliteration, and patency refers to the assessment of patency of parent vessels. In both cases, ergonomics and efficiency refer to the actual use of the imaging modality in judging that bypass. Ergonomics was used to score the physical convenience of the modality; efficiency was used to score the time taken to perform that modality.

Scores were on a scale from 1 to 10 with 1 being poor and 10 being excellent. For example, a severely inefficient imaging modality that was not all useful in juding the patency of the bypass would score a 1 in efficiency and patency. A modality that was very ergonomic, and made it completely clear whether the bypass was leaking, would score 10 in both ergonomics and occlusion. Surgeons were not given any further guidance on what a specific value in the scale should mean, or how to use the range of the scale.

The assessments of the 3 turkey wing anastomoses were presented to the 7 surgeons to be independently evaluated. For each bypass specimen, participating surgeons were shown 4 separate videos demonstrating white light/MDUS, ICG-VA, MFL, and DSA each in turn. The white light/MDUS video also included audio to capture the MDUS signal. Assessments of the 3 aneurysm models were done live with all surgeons watching through the microscope eyepiece and on the monitor, witnessing in order the white light/MDUS, ICG-VA, MFL, and then...
DSA assessments. All scores were independently recorded on paper score sheets and later digitized for analysis.

**Statistical Analysis**

Statistical analysis was performed using SAS software version 9.3 (SAS Institute, Cary, North Carolina). The means and amplitudes of the individual surgeons’ ratings were compared to ensure that scores could be combined. Imaging modalities were analyzed across the 4 categories of assessment using a one-way ANOVA with Tukey adjustment for multiple comparisons. The 3 assumptions were checked and met for applicability of the ANOVA test. Independence was guaranteed by the fact that each surgeon independently evaluated each modality with each model a single time. Normality of the residuals was checked and met. Homoscedasticity was met as verified with the Welsh test. For a given assessment criteria, imaging modalities were compared by the method of least squares, which uses the null hypothesis that the least squares mean for one modality equaled the least squares mean of the comparison modality. A P-value of <.05 was used as the threshold for statistical significance. Scores for each modality and category are reported as an average of all 7 surgeons’ scores (Figure 3).

**RESULTS**

**Occlusion and Patency**

Each modality’s occlusion score was similar to its patency score. WL/MDUS scored 6.5 and 5.9, respectively, for occlusion and patency. ICG-VA was significantly better than WL/MDUS, scoring 7.8 and 7.1, respectively, giving head-to-head comparison P-values of .0009 and .0058 for occlusion and patency when comparing ICG-VA with WL/MDUS. MFL scored 7.6 for occlusion and 7.0 for patency with no significant difference when compared with ICG-VA. Like ICG-VA, MFL was significantly better than WL/MDUS for assessing occlusion and patency. DSA, scored best for occlusion and patency at 9.3 and 9.5 with P-value of <.0001 when compared with any other modality for these 2 categories.

**Ergonomics**

The scores in the ergonomics category were clustered for WL/MDUS, ICG-VA, and MFL. MFL scored best at 8.6, while WL/MDUS and ICG-VA were essentially tied at 8.1. There was no significant difference when comparing these 3 modalities on ergonomics. DSA scored significantly worse than the rest at 4.2 with P-values of <.0001 when compared with any of the other modalities.

**Efficiency**

Here again, WL/MDUS, ICG-VA, and MFL all scored similarly with averages of 8.5, 7.7, and 8.3, respectively. The difference between WL/MDUS and ICG-VA was technically significant with a P-value of .0394, but ICG-VA was not significantly worse than MFL (P = .1369). DSA scored significantly
FIGURE 3. Scores for the 4 imaging modalities averaged across all 7 surgeons and the 6 scenarios in which they were tested. The first 2 column groupings score the modalities’ ability to judge occlusion and patency and the second 2 column groupings score the ergonomics and the efficiency of the modalities themselves. An asterisk marks a score that is significantly different than other modalities in the same grouping.

worse in efficiency at 3.0 with $P$-value of <.0001 when compared with the other 3 modalities.

DISCUSSION

Intraoperative vascular imaging continues to improve as surgeons seek faster, more accurate, and more reliable methods to gain information about flow through the cerebral vasculature in the OR. The ideal method would not require interruption of the surgical procedure. Where early probes to measure flow were invasive and needed to be wrapped around a vessel, Doppler US provided a way to gain flow information in a less obtrusive way.\(^17\) It has been shown that ICG-VA is sensitive enough to be a clinically useful tool and is considered significantly easier than obtaining an intraoperative angiogram.\(^6\) Neither of these technologies allows the surgeon to continue working as he or she evaluates the flow in the vasculature or pathology.

MFL has been developed as a new method to allow an overlaid display, using the same dye as ICG (tricarbocyanine dye with a peak spectral absorption at 800 nm) to obtain vascular imaging. The surgeon can continue to work as the display reveals the fluorescence within the vasculature. The technical process for the surgeon is otherwise the same as with ICG-VA. The same dose of fluorescent dye can be used, and the same line-of-sight limitations exist. However, whereas ICG-VA can be viewed only on the monitor of the microscope in a retrospective fashion, MFL can be viewed through the ocular pieces of the microscope. This viewing method allows the surgeon to see the fluorescent signal laid over the surgical anatomy and the fluorescence while he or she is manipulating the tissue in the field. A video clip is created and can be reviewed on the microscope monitor in a method similar to ICG-VA, if needed. The results of this study indicated that MFL is similar to ICG-VA in the ability to assess aneurysm occlusion and parent vessel patency, in the ability to assess the patency and porosity of a bypass anastomosis, and in terms of ergonomics and efficiency.

In this study, both ICG-VA and MFL were found to be significantly superior to WL/MDUS in terms of the same vessel assessments. They were also found to be as easy to use and time efficient as the WL/MDUS. While Doppler US still has its place, ICG-VA and MFL have several clear advantages. The gold standard (DSA) still provides surgeons the best understanding of anatomy and patency/occlusion of vessels and aneurysms, but it is the most ergonomically disadvantaged, more expensive than ICG,\(^18,19\) and the least time-efficient modality.

Despite tremendous advances in endovascular neurosurgery, microsurgery remains an important and necessary option for many patients. For this reason, we must continue to evaluate and improve the tools that are used in cerebrovascular neurosurgery. Novel flow-measurement adjuncts, such as the ultrasonic flow probe developed by Fady Charbel, allow quantitative, real-time assessment of flow.\(^20\) These may offer more useful data to evaluate vessel characteristics than MDUS, and should be explored with a
further comparative study. We did not include a comparison here as the 4 modalities offered qualitative and not quantitative data. The results of this study show that the new technology of MFL may be as effective as ICG-VA, and compares favorably with the gold standard of DSA.

Both ICG-VA and MFL technologies utilize ICG dye, which has been in use in medicine since 1957, but only began to be used in cranial neurosurgery in 2005. MFL includes high-resolution (1920 × 1200 pixels) image injection technology along with a proprietary algorithm allowing surgeons visualization and field depth directly within the microscope. This provides simultaneous evaluation of blood flow within the anatomical background for visual orientation. By combining these elements, MFL negates the necessity for surgeons to compare a black-and-white image of the ICG dye with a white-light image. This has the potential to save time and improve patient safety. Choosing an intraoperative imaging modality involves trade-offs. If MFL demonstrates an improvement over ICG-VA without additional drawbacks, it may become a preferable intraoperative modality. The advantage of MFL is that the surgeon does not need to interrupt the procedure, and the results of this study indicate that its imaging fidelity is comparable to ICG-VA in a laboratory setting.

Limitations

This study has several significant limitations. This was an exploratory study that used simulated surgery to rate the visualization capabilities of 4 modalities during commonly encountered cerebrovascular procedures. First, while the “live cadavers” and turkey-wing models have a proven track record in approximating real-life conditions, no model can perfectly recreate the conditions present during actual microvascular surgery. Second, greater differences in ICG-VA vs MFL may have been obtained if a higher number of procedures had been performed or under different surgical scenarios. Third, the assessment tool for this study was investigator-developed subjectively and has not been validated in other work. As such, our results represent a summary of a small neurosurgical poll to assess adjunctive modalities in cerebrovascular neurosurgery. Finally, because this is an exploratory study, the results need to be validated with future studies.

While this study was conducted in an idealized laboratory setting, the results indicate that MFL is worth studying in a normal practice setting. Future investigations may include an evaluation of deeper and more complex vascular lesions, possibly aiming to discern the differences between the standard ICG-VA technology and MFL. Additionally, the ease of use of MFL and its sensitivity in the OR or simulated scenarios will need to be tested in a more objective manner.

CONCLUSION

MFL, as a new technology, performs similarly to ICG-VA in a laboratory setting and, therefore, may be expected to be as accurate as ICG-VA in the OR. It compares to DSA as ICG-VA does, having clear advantages over DSA in ergonomics and time efficiency, while DSA is still the gold standard in imaging sensitivity.

MFL may be a useful tool for cerebrovascular neurosurgeons. The extent to which the perceived benefits of MFL’s pseudocolor overlay are borne out in the OR is a topic that will require future investigation.

Disclosures

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**COMMENT**

This is an interesting pilot study looking at multispectral fluorescence (MFL), a new technology utilizing indocyanine green (ICG) fluorescence, as a means of intraoperative assessment of several different microvascular neurosurgical procedures. This new technology was compared to existing technologies of digital subtraction angiography (DSA), indocyanine green videoangiography (ICG-VA), and microdoppler ultrasonic probes (MDUS) using in vitro models utilizing cadaveric heads and turkey wings. Three different aneurysms were created and subsequently clipped microsurgically in 3 actively perfused cadaver heads. The other model utilized brachial artery and vein of 3 turkey wings to create an end to side microvascular anastomosis. Assessment of the potency and occlusion (leakage) of the anastomosis was accomplished via proximal injection of the artery. Seven vascular neurosurgeons independently evaluated each imaging modality utilizing a subjective 10-point scale to measure the ability to assess occlusion, patency, ergonomics, and efficiency. The responses were averaged and analyzed with the conclusion being that MFL performs similarly to ICG in the laboratory setting. DSA was found to be significantly better at assessing occlusion and potency but was judged less ergonomic and efficient. The authors frankly discuss some of the constraints of the study including the small numbers, the simulated nature of the surgical models and the subjective unvalidated assessment tool. The subjective nature of the assessment tool is particularly problematic when it comes to statistical analysis of the study. Significant P-value was calculated for difference of scores as small as 1.2. Although strictly speaking the difference in a patency score of 5.9 for WL/MDUS versus 7.1 for ICG-VA may be "statistically significant, there is now way to judge if that difference is relevant to performance or outcome in a clinical setting. Despite the limitations of this study, it should be of interest to cerebrovascular surgeons and provides worthwhile information regarding the new technology of multispectral fluorescence.

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