# The diagnostic properties of intraoperative ultrasound in glioma surgery and factors associated with gross total tumor resection


## Author information

- **Bodil Karoline Ravn Munkvold** (Medical Student with Research Programme) [1]
    - Postal address: NTNU, Faculty of Medicine and Health Sciences, N-7491 Trondheim, Norway. E-mail: bodilkm@stud.ntnu.no.
- **Asgeir Store Jakola** (MD, PhD) [2,3,4]
    - Postal address: Blå stråket 5, plan 3, 41345 Göteborg. Telephone number: +46 31-342 27 63. E-mail address: jakola.asgeir@gu.se.
- **Ingerid Reinertsen** (MSc, PhD) [5,6]
    - Postal address: SINTEF Technology and Society
      P.O. Box 4760 Torgarden, NO-7465, Trondheim, Norway. Telephone number: +47 90212159. E-mail address: Ingerid.Reinertsen@sintef.no.
- **Lisa Millgård Sagberg** (MSc) [2,5,7]
    - Postal address: Edvard Griegs gate 8, Nevro øst * 121.03.032. Telephone number: +47 72575697. E-mail address: lisa.millgard.sagberg@ntnu.no.
- **Geirmund Unsgård** (MD, PhD) [2,5,7]
    - Postal address: Edvard Griegs gate 8, Nevro øst. Telephone number: +47 72575274. E-mail address: geirmund.unsgard@ntnu.no.
- **Ole Solheim** (MD, PhD) [2,5,7]
    - Postal address: Edvard Griegs gate 8, Nevro øst. Telephone number: +47 73 59 20 20. E-mail address: ole.solheim@ntnu.no.

1. **The Faculty of Medicine,** Norwegian University of Science and Technology, Trondheim, Norway
2. **Department of Neurosurgery**, St. Olav's University Hospital, Trondheim, Norway
3. **Department of Neurosurgery,** Sahlgrenska University Hospital, Gothenburg, Sweden
4. **Institute of Neuroscience and Physiology,** Sahlgrenska Academy, Gothenburg, Sweden
5. **Norwegian National Advisory Unit for Ultrasound and Image Guided Therapy**, St. Olav's University Hospital, Trondheim, Norway
6. **SINTEF,** Department of Medical Technology, Trondheim, Norway

7. **Department of Neuroscience**, Norwegian University of Science and Technology, Trondheim, Norway

**Corresponding author information:**

**Bodil Karoline Ravn Munkvold**, Medical research student, Faculty of Medicine, Norwegian University of Science and Technology, Post Box 8905, N-7491 Trondheim, Norway (bodilkm@stud.ntnu.no).

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# Abstract

## Objective

In glioma operations, we sought to analyze sensitivity, specificity and predictive values of intraoperative 3D ultrasound (US) for detecting residual tumor compared to early postoperative MR imaging. Factors possibly associated with radiological complete resection were also explored.

## Methods

144 operations for diffuse supratentorial gliomas were included prospectively in an unselected, population-based single institution series. Operating surgeons filled out a questionnaire immediately after surgery, stating if residual tumor was seen with US at the end of resection and rated US image quality (good, medium, poor). Extent of surgical resection was estimated from pre- and postoperative MRI images.

## Results

Overall specificity was 85% for "no tumor remnant" seen in US images at the end of resection as compared to postoperative MRI findings. Sensitivity was 46%, but tumor remnants seen on MRI were usually small (median 1.05 ml) in operations with false negative US findings. Specificity was highest in low-grade glioma operations (94%), and lowest in patients who had previously undergone radiotherapy (50%). Smaller tumor volume and superficial location were factors significantly associated with gross total resection in a multivariable logistic regression analysis, while good ultrasound image quality did not reach statistical significance ($p = 0.061$).

## Conclusion

The specificity of intraoperative US is rather good, but sensitivity for detecting the last milliliter is low compared to postoperative MRI. Tumor volume and tumor depth are the predictors of achieving gross total resection, while ultrasound image quality was not.


**Keywords**: Glioma; Image-guided surgery; Magnetic Resonance Imaging; Neuronavigation; Ultrasound

# The diagnostic properties of intraoperative ultrasound in glioma surgery and factors associated with gross total tumor resection

## Introduction

The diffuse infiltrative growth pattern of grade II-IV gliomas makes a radical surgical removal virtually impossible. [1] Even though distant spread is always present and acknowledged, for practical and safety reasons surgery is usually targeting the radiological tumor, as defined from MRI. Survival improves with increasing extents of resection as defined from pre- and postoperative MRI in both low-grade (LGG)[2–4] and high-grade gliomas (HGG). [5–9] However, an accurate intraoperative delineation of the radiological defined tumor can be challenging and small remnants may consequently be left behind.

Intraoperative ultrasound (US) was first described as a potential tool for guiding resection of intracranial tumors in 1980 (Rubin et al. and Voorhies and Patterson ). [10–12] It is still widely used as it enables fast, inexpensive and real-time intraoperative imaging. Integration in a neuronavigation system enables simultaneous navigation in preoperative MRI volumes and intraoperative 3D US [13] US imaging in real-time, or neuronavigation based on 3D US, can potentially increase extents of tumor resection, [14–16] and has been in routine use in our department since 1997 as the sole technical aid in glioma surgery. We have earlier reported favorable clinical outcomes in both patients with LGG, [17] and HGG[18] operated with this technique. Good US image quality is found to be significantly associated with extent of resection in HGG. [19] However, US image quality, i.e. how easy tumor borders are delineated, is known to vary between operations and to diminish during surgery, especially towards the end when the surgical cavity and resection plane often cause image artifacts. [20]

In this prospective study, we sought to analyze sensitivity, specificity and predictive values of intraoperative US for determining residual tumor compared to early postoperative MR imaging in an unselected, population-based series of patients with diffuse gliomas. We also sought to explore if US image quality was associated with radiological complete resection in unselected glioma patients.

# Methods

The Department of Neurosurgery at St. Olavs University Hospital serves a defined geographical catchment region with a population of approximately 720 000. The study sample is therefore practically an unselected population-based series. In the study period from September 2011 to December 2015, there were in total 289 operations, including 60 biopsies only of supratentorial diffuse grade II-IV gliomas originally classified according to the WHO 2007 classification. [21] We retrospectively attempted to reclassify low-grade tumors according to the WHO 2016 Classification System[22] as we wished to examine diagnostic properties of US in subgroups of WHO II gliomas separately. However, molecular markers were unavailable in 3 cases, and these were therefore classified as LGG Not Otherwise specified (LGG-NOS). The inclusion criteria were informed consent, surgical resection (as opposed to biopsy only) of a supratentorial diffuse grade II-IV glioma, surgeon-reported data available on presumed residual tumor after US guided resection, and postoperative MRI available. As seen in figure 1 outlining the inclusion process, 144 US guided operations met the inclusion criteria. 10 different surgeons performed the operations, and data was collected prospectively.

All operations were done in general anesthesia, and guided by SonoWand Invite neuronavigation system. Fluorescence guided surgery or awake surgery was not used in the study period. For expected HGGs where diagnostic MRI demonstrated edema, high-dose corticosteroids were used from radiological diagnose until surgery. For suspected low-grade lesions without significant edema, corticosteroids were not prescribed.

The primary surgeon filled out a questionnaire immediately after surgery, before early postoperative MRI was carried out. The following questions were analyzed in the present study: 1) use of neuronavigation (y/n), 2) neuronavigation modality (3D MRI, 3D US, or 2D US), 3) ultrasound image quality evaluated at the beginning (first acquisition), during, and at end of surgery (poor, medium or good) (figure 2), 4) number of US image recordings during the operation, and 5) residual tumor seen with US at the end of surgery (yes/no) (i.e. reporting whether tumor remnant was visible or not using ultrasound only, regardless of whether surgeon was aware or suspected a residual tumor). Ultrasound image quality was classified as poor when

impossible to visualize tumor borders, as medium when difficult but possible, and as good when visualization of tumor boundaries was clear, like done earlier[19] and by others. [23,24]

*Extent of surgical resection*

Tumor delineation in WHO grade IV glioma was estimated from the volume within the gadolinium enhancing areas on T1-weighted MR images. In WHO grade II and III gliomas tumor borders were defined based on the FLAIR sequence due to variable contrast enhancement. Resection grades were estimated from pre- and postoperative MR images. The volumes of ellipsoid-shaped lesions were calculated by applying the volume formula $V = \frac{4\pi r_1 r_2 r_3}{3}$, based on maximal tumor diameters, as done by others. [25] The volumes of cup-shaped residual tumors were calculated by subtracting the ellipsoid-shaped resection cavity from the volume of the tumor complex. More complex tumor configurations were manually segmented. [19] Gross total resection (GTR) was defined as no residual tumor remnant[25] (100%) seen with postoperative imaging. Near total resection was defined as ≥90% but <100% extent of resection, and subtotal resection was defined as < 90% extent of resection.

*Statistics*

Significance level was set at $p < 0.05$. All analyses were performed in SPSS version 21. Q-Q-plots were used to visually assess normal distribution. Pearson's Chi-square was used for significance testing in contingency tables. Mann Whitney U-test was used for examining differences between groups in skewed continuous data. Spearman's rho was used for assessing linear correlation. Binary multivariable logistic regression analysis was performed for exploring predictors for GTR. Hosmer and Leweshow test was used for determining goodness of fit of the logistic regression model. The diagnostic properties (i.e. sensitivity and specificity) of "residual tumor seen on US at the end of surgery" as judged by the surgeon immediately after surgery, was compared to extent of resection from pre- and postoperative MRI images. Sensitivity, specificity and predictive values were calculated from 2x2 tables.

*Ethics*

The study was approved by the Regional Committee for Medical and Health Research Ethics as part of a larger project (REC ref. 2011/974). Informed consent was obtained from all study participants, and the project is adherent to the Declaration of Helsinki. [26]

## Results

We included 87 (60%) primary operations and 57 (40%) re-operations. 47 (33%) were LGG resections (WHO grade II) and 97 (67%) were HGG resections (WHO grade III-IV). 3D ultrasound was used in 142 cases (99%), while 2 operations (1%) were guided by 2D US only. Neuronavigation based on preoperative 3D MRI was used in 142 (99%) operations.

Surgeon's report of residual tumor seen with US at the end of surgery (yes/no) was compared to residual tumor (yes/no) from the early postoperative MRIs (table 1). Figure 3 exemplifies three different glioma resections, comparing the last US recording to corresponding early postoperative MRI.

As seen in table 2, overall sensitivity was 46% and specificity was 85% for "no tumor remnant" seen in US images at the end of resection. In subgroups, specificity was highest in LGG operations, and the group that had not undergone previous radiotherapy (94%). Among LGGs, specificity was especially high in astrocytomas, increasing to 100%. Lowest specificity was found in patients who had previously undergone radiotherapy (50%). There was a better specificity in LGGs compared to HGGs, 94% and 77% respectively.

Specificity was 90% when image quality was good, as opposed to 79% when image quality at end of surgery was rated poor or medium. Positive predictive values ranged from 75-100%, with higher values in patients with LGGs (93%) (including the 3 cases in which molecular markers were not available, classified as LGG not otherwise specified (NOS)), reaching 100% in astrocytomas. Positive predictive value was 95% in the group who had not undergone radiotherapy. Negative predictive values ranged from 28-56%, with highest values in astrocytomas, and when image quality was rated good, and lowest when image quality was rated poor or medium.

In cases with false negative US findings at the end of surgery (i.e. no remnant seen with US), median extent of resection was 92.7% (range 12.0-99.8%) and remnant tumor volumes were median 1.0 ml (range 0.09-129.8 ml). In primary operations with false negative US findings at the end of surgery (i.e. no remnant seen with US), median extent of resection was 94.3% compared to 87.6% in reoperations (p =0.011).

*Image quality and use of intraoperative ultrasound*

Just before starting the resections, surgeons performed 144 US image acquisitions. In 105 (73%) image quality was rated good, and in 39 acquisitions (27%) image quality was rated poor. During the operations 82/136 (60%) image acquisitions were classified to be of good quality, while 54/136 (40 %) were of poor quality (8 missing). At the end of surgery 55/144 (38%) image acquisitions were classified to be of good quality and 89/144 (62%) were of poor quality. Median number of US acquisitions during operations was 3 when image quality before resection was poor or medium vs. 7 when image quality was good (p< 0.001). Image quality before resection was rated significantly poorer in patients who had undergone radiotherapy, poor or medium in 47% vs. 22% (p=0.007).

*Predictors for gross total resection*

A multivariable logistic regression model was developed to explore if US image quality or number of US image recordings was associated with achieving GTR. Factors associated with a trend in the univariable analysis (p< 0.100) were included in the multivariable analysis. Non-eloquent tumor location (classified according to Sawaya[27] and /or as cases where functional MRI or diffusion tensor imaging was not used) was not associated with GTR (p = 0.184). Prior radiotherapy and number of US acquisitions were also not associated with GTR as univariables (p = 0.970 and p= 0.225). The other tested variables are presented in table 3.

The Hosmer and Leweshow test was not significant (p= 0.142), implicating that the regression model was a good fit. Predictive accuracy (i.e. how good the model is for predicting GTR) was 72%. As seen in table 3, depth of lesion, small tumor, good image quality, histopathology, and patient age were significant in the univariable analyses and were therefore included in the

multivariable logistic regression model. Only superficial location and small tumor size were significant predictors in the multivariable model.

## Discussion

In this prospective study assessing the diagnostic properties of intraoperative US in glioma surgery we report an overall low sensitivity of 46% but a rather high specificity of 85% for "no tumor remnant" seen in US images at the end of resection as compared to postoperative MRI findings. However, in operations with false negative US findings, tumor remnants seen on MRI were most often small (median 1.0 ml). In this tumor population, the positive predictive value was rather high (89%) while the negative predictive value was rather low (37%) for assessing "no tumor remnant" from intraoperative US. In subgroups, specificity was highest in the group that had not undergone previous radiotherapy and in LGG operations (94%), and lowest in patients who had previously undergone radiotherapy (50%). US image quality often diminished during surgery and image quality was associated with number of image acquisitions, indicating increased use when surgeon judged image quality to improve accuracy. Although good US image quality was a significant univariable with regard to achievement of GTR, it was not a significant predictor in the multivariable model in these unselected patients.

Maximal safe resection is the credo of glioma surgery. We would argue that maintaining specificity is more crucial than sensitivity for tools guiding glioma resection, because mistaking normal brain for tumor is more dangerous for our patients than mistaking tumor for normal brain. While the first could result in severe neurological deficits, the latter may result in suboptimal extents of resection. As seen, specificity of US findings toward the end of surgery can be lower in HGGs and particularly low in patients who have undergone radiotherapy. There is often tumor edema surrounding high-grade lesions, and they are often more heterogeneous with necrosis which again may produce image artifacts and affect ultrasound image quality. It is therefore not unexpected that specificity was found to be lower in HGG operations. Many surgeons may also use US less actively during HGG surgery because tumor tissue macroscopically looks more abnormal as opposed to LGG tissue. Additionally, fluorescence guided surgery is today increasingly used to improve intraoperative diagnostics in HGG. The study further confirms that

the diagnostic properties are quite good for LGG where intraoperative US is perhaps most frequently used for resection control today.

The study demonstrates that the specificity of intraoperative US is often good, but sensitivity for detecting the last milliliter is low compared to postoperative MRI. Still, some patients had considerable tumor remnants that operating surgeons were not able to see using intraoperative US. To keep in mind, the surgeons were asked to judge if remnant tumor could be seen in the US images at the end of resection, not if they believed that the resection was radical. Interpretation of US images is a skill that will improve with experience. Our data reflect the results of everyday surgery and includes surgeons with variable experience in US image interpretation. Specificity was 90% when image quality at end of surgery was rated good as opposed to 79% when rated poor or medium. Image quality is subjective and may therefore not only depend on the given case, but also the experience or enthusiasm of the operator using intraoperative US. The surgeon utilized US more extensively when image quality was rated good compared to poor/ medium. Thus, less use of US as seen when US image quality was rated poor may be a self-fulfilling correlation, even in our center where intraoperative US is used routinely by all surgeons. If the tumor has unclear or irregular tumor borders as depicted with US at the beginning of surgery, the surgeon might perform fewer recordings. Irregular shape and diffuse tumor borders may also complicate the evaluation of residual tumor at end of surgery. There was a negative association between image quality before starting the resection and previous radiotherapy, with better specificity and higher positive predictive value in the group that had not undergone previous radiotherapy. Therapeutic cranial irradiation causes neuropathologic white matter changes including destruction of the blood- brain barrier, demyelination and occlusion of microvessels, which in turn deprive image quality. [28] Extents of resection were somewhat higher in cases with better image quality. Although we found statistically significant association between complete radiological resection and US image quality, there was only a trend after adjusting for other factors in the regression model. Small tumor and superficial location were the only explored factors that were significantly related to GTR in the multivariable logistic regression analysis. Thus, our previous finding that US image quality is associated with GTR in HGGs, [19] was not confirmed in the present unselected glioma series.

A study from 2016 including 11 LGG patients found that estimated percentage of residual tumor based on linear array intraoperative US alone was lower than the final residual tumor detected with intraoperative MRI (7.5% versus 14.5%), [29] perhaps indicating that tumor volumes as seen with US is smaller than the corresponding MRI volume. We recently did a study comparing LGG volumes segmented from preoperative MRI and intraoperative US, and found that the US volumes were smaller than the corresponding MRI volumes in 20 out of 23 patients. The median US tumor volumes equaled 74% of the corresponding MRI volumes, however increasing to 92% in astrocytomas. [30] Also in the present study, we find higher specificity in LGG astrocytomas than in LGGs with oligodendroglial components.

MRI is considered the gold standard with regard to defining tumor boundaries and defining GTR. However, biopsy studies have shown containment of tumor cells far beyond radiological tumor border on both MRI (T2-weighted and FLAIR)[31–33] and US. [34] It is important to acknowledge that the nature of the disease makes it impossible to remove all tumor cells without causing substantial morbidity because it is already widespread throughout the central nervous system at time of diagnosis. [1] A hypothetical 100% sensitive and 100% specific imaging modality for diffuse gliomas would therefore not necessarily be helpful, because maximizing tumor removal and thereby increasing survival must be balanced against risks of causing neurological deficits and decreased quality of life. An ideal system for image-guided surgery should preferably visualize tumor in a way to optimize the trade-off between survival gain and risk.

Several other tools are available for aiding decision-making in glioma surgery, including intraoperative MRI, fluorescence-guided imaging techniques (5-aminolevulinic acid (5-ALA) and fluorescein sodium (FNa)), and intraoperative stimulation brain mapping. [25,35–37] The usefulness of 5-ALA fluorescence guided surgery is examined in several studies presenting sensitivity, specificity and predictive values in patient groups diagnosed with HGGs. Sensitivities close to 90% are reported in most 5-ALA studies, while reported specificities vary between 53 and 96%. [38] False positive fluorescence is reported surrounding the resection cavity, [39–43] and in patients who have undergone adjuvant therapy, where reactive astrocytes seem to cause fluorescence and thereby mimic tumor. [44–46] Autofluorescence of normal brain tissue is also reported in some cases. [45] Thus, the strengths and weaknesses of US and 5-ALA in HGG surgery may be opposite,

with high sensitivity in 5-ALA and perhaps higher specificity in US image data, supporting a possible usefulness of combined use. [47] However, the biggest advantages of intraoperative US is today guiding LGG resections. 5-ALA is currently not applicable in most LGGs because current microscope technologies are unable to detect protoporphyrin IX (PpIX) [48–50] in this patient group, a future possibility might be to use a fiber optic probe intraoperatively to perform a quantitative measure of PpIX also in LGGs. [51,52] The feasibility of confocal laser endomicroscopy (CLE) using FNa as a fluorophore to detect brain tumor tissue was examined by Martirosyan et al. in 2016. [36] Utility, specificity and sensitivity of CLE and frozen sections was compared with information from permanent histological sections (gold standard) in 66 tumors, including 21 gliomas. The sensitivity and specificity of CLE to diagnose gliomas and meningiomas were comparable to those of frozen section, using permanent histological sections as the standard. These data indicate that CLE both in vivo and ex vivo could allow identification of tumor areas during brain tumor resection. However, it was not possible to distinguish glioma tumor tissue and reactive tissue changes in all cases. In the present study surgeons used 3D US based on conventional B-mode imaging to delineate tumors during surgery. Other scanners, other probes and other settings might have given other results. For example, contrast-enhanced ultrasound (CEUS) may potentially improve delineation of brain tumors, adding additional biological information about vascularization, which can help the surgeon differentiate between tumor and edema. [53]

Despite use of fiducial markers, preoperative MRI gets less and less accurate as the surgical procedure proceeds due to brain shift. [54]US recordings provide updated real-time images not influenced by brain shift. However, using navigated US, preoperative MRI data is available for side-by side or "overlay" comparison to ease interpretation of US data. Due to brain shift direct comparison of MRI to US data is often less feasible at the end of surgery. Also, US imaging is prone to certain types of artifacts degrading image quality[55] and affecting interpretation and decision-making during surgery. Tumor resection may create US image noise when the resection cavity is filled with isotonic saline water. [56] The large discrepancy in attenuation between saline and brain tissue may affect images negatively by introducing bright artifacts below the resection cavity. High-intensity artifacts at the resection cavity wall may lead to both over- and underestimation of tumor volume by either mimicking tumor when there is none present, or mask

residual tumor. As seen from our results, the latter seems to be a bigger problem. As an alternative to saline in the resection cavities before US image acquisition, an acoustic coupling fluid has been developed. The fluid is assessed in an animal study, and appears safe under testing circumstances in both rats and pigs. [57] Clinical testing is currently done. This could possible improve US image quality, making the images easier to interpret, and thus further optimize risk-benefit ratio in glioma surgery guided by intraoperative US.

## Conclusion

In conclusion, the specificity of intraoperative US is high for predicting complete tumor resection at end of glioma surgery, especially in LGGs and in patients who have not previously undergone radiotherapy. However, sensitivity for detecting the last milliliter is low using US compared to postoperative MRI. US is prone to artifacts as surgery proceeds, but future developments may reduce artifacts, and in turn further improve the usefulness of US also at later stages in the surgical procedure.

## Disclosure

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# Figure legends

**Figure 1.** Flowchart showing included patients.

**Figure 2.** Ultrasound recordings obtained from two different glioma resections, exemplifying the difference in image quality. 1b is an example of very difficult tumor delineation due to poor US image quality. 2b is an example of good image quality, with clear delineation of tumor borders. The surgeon rated image quality medium when it was somewhere in-between good and poor, and tumor delineation was possible, although difficult. 1a and 2a are corresponding preoperative MR images.

**Figure 3.** 3-D ultrasound recordings obtained at end of glioma surgery (1b, 2b, 3b), compared to corresponding early postoperative MR images (1c, 2c, 3c) in three different cases:

1b Ultrasound: tumor remnant, 1c MRI: tumor remnant.

2b Ultrasound: complete resection, 2c MRI: tumor remnant.

3b Ultrasound: complete resection, 3c MRI: complete resection.

1a, 2a, and 3a are corresponding preoperative MR images.

**Table 1** 2x2 tables of residual tumor* seen with intraoperative ultrasound at end of surgery compared to the early postoperative MRI (gold standard).

**Table 2** Sensitivity, specificity and predictive values calculated from 2x2 tables.

**Table 3** Possible predictors for gross total resections were explored in a multivariable logistic regression analysis.