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Manuscript # SNI_369_2024

Original Article (Neuro-oncology)

3D Ultrasound-augmented image-guidance for surgery of high grade gliomas – A quantitative analysis focused on the extent of resection

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Abstract

Background. We have retrospectively reviewed our series of brain tumour patients operated using 3D IntraOperative UltraSound (IOUS) to report technical advantages and areas of improvement.
Methods. Clinical and radiological data of patients with diagnosis of High-Grade Glioma IV (HGG-IV) operated with and without IOUS were retrieved and analysed.
Results. We have found 391 patients operated using IOUS coupled with neuronavigation and 257 using neuronavigation stand-alone. We have selected a pool of 60 patients with diagnosis of GB, comparing two equally sized groups operated with and without IOUS, respectively. Average Extent of Resection (EOR) in the IOUS group was 93%, while in the control group it was 80%. IOUS was significantly associated with improved EOR ($p < 0.0004$), even when accounting for other factors affecting EOR. The average overall survival (OS) was 13.4 months, and the average progression-free survival (PFS) was 7.4 months. Cox Proportional hazard model showed an advantage

in OS on patients operated using the IOUS. No statistically significant effect was observed on PFS.
Conclusion. Intraoperative ultrasound coupled with image guidance is associated with an improved EOR and possibly an improved OS. While we are aware of several limitations related to the present analysis, these data support routine use of IOUS as a safe and reliable technology. Larger, prospective series with updated IOUS technology are desirable to verify the accuracy of these results.

Keywords

intra-operative ultrasound, 3D ultrasound, high grade glioma, neuro-oncology, ultrasound

Financial Support and Sponsorship:

Nil

Conflict of Interest

There are no Conflict of Interest

Patient Consent Declaration

Patient's consent not required as there are no patients in this study.

Ethical Approval:

The research/study approved by the Institutional Review Board at Research Authority – Bloomsbury Research Committee, number 19/LO/1763, dated 17/10/2019.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation

The author(s) confirms that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using the AI.

Manuscript Document Properties:(*Extracted from the manucsript file*)

Word Count	**6,408**

Character Count	**216,927**
Aproximate Number of Pages (excluding figures)	**15**

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Introduction

The research on new intra-operative devices to improve brain tumour resection is an ever-developing field in neurosurgery. Traditional neuro-navigation systems use preoperative Computer Tomography (CT) or Magnetic Resonance Imaging (MRI) data to plan surgical procedures and guide surgeons to tumour margins, allowing a greater degree of precision, safer surgery, and a more complete tumour resection that correlates with clinical outcome [1, 7, 13, 28, 32, 34]. They are, however, limited by their failure to compensate for the anatomical changes of the brain during surgery.

IntraOperative UltraSonography (IOUS) is an appealing solution, offering a cheaper, faster, and more accessible alternative to intraoperative MRI. However, it is still not considered the imaging modality of choice by many surgeons. In the past IOUS has been criticised for its poor spatial resolution, and the loss of image quality to surgical artefacts (air, blood, and instruments) detracts from its usefulness in tumour resection surgery [14]. Furthermore, most commercial ultrasound scanners only provide a 2-D cross-sectional view, often in the oblique orientation, which is difficult to interpret.

The image fusion of IOUS and preoperative MRI has improved the quality of tumour resection and the clinical outcome of surgery [12, 18] and preliminary work has shown that the introduction of IOUS has coincided with an improvement in survival following glioblastoma resection in Trondheim (Norway)[29]. Moreover, the more defined resolution and the development of new software allowing the surgeon to perform a 3D reconstruction have substantially improved the performance of current IOUS devices [16].

The present study is a retrospective review f our experience using intraoperative 3D-IOUS. We have performed a subgroup analysis on a cohort of patients affected by High-Grade Gliomas Grade IV (HGG-IV) who underwent IOUS-aided surgical resection and compared them with a control group operated using a standard, stand-alone neuro-navigation system.

Aims

This study retrospectively examines data from a series of HGG-IV surgeries. We investigated whether combining 3D Intraoperative Ultrasound (3D IOUS) with neuronavigation improves the Extent of Resection (EOR) compared to standard neuronavigation alone. Additionally, we sought to discern any survival benefits in terms of Overall Survival (OS) and Progression Free Survival (PFS).

Materials and methods

Neuronavigation systems – technical features

This study utilised the data from surgeries assisted by two primary neuronavigation systems. Ethical clearance for data usage was granted by the Health and Research Authority – Bloomsbury Research Committee, London. REC reference: 19/LO/1763, IRAS project ID 265404.

As mentioned, two specific devices were considered for this purpose.

1. **Sonowand©**: a combined 3D-IOUS neuro-navigation device – Sonowand©. Lindseth F et al. detailed technical specifications[18]. The device is an infrared-based neuro-navigation system combined with two differently sized ultrasound probes. It operates independently or in tandem with 3D ultrasound. The optical neuro-navigation tracker links the 3D ultrasound via a specialised tracking mechanism (**Figure 1**). After the standard preparatory steps and the application of the Mayfield clamp, fiducial landmarks are pre-set on the patient and registered based on the pre-operative volumetric MRI scan. Neuro-navigation is utilized during the operation. Post-craniotomy, the ultrasound probe, encased in a sterile cover, is synchronised with the tracking system using a dedicated tracking device coupled to the IOUS (see **Figure 1**). The device then captures 3D intra-operative ultrasound images and aligns them with the volumetric MRI registration. This capture focuses on a single spatial plane, and the system automatically generates images for the other two planes, thus providing axial, sagittal, and coronal projections (hence the "3D" definition). The surgeon can use the IOUS either use the IOUS either freehand or perform multiple IOUS registrations during tumour removal to monitor the resection progress in real-time.

2. **Medtronic© Neuronavigation**. A globally recognised neuronavigation system known for its accuracy and reliability. Unlike Sonowand©, it does not incorporate IOUS, making it an apt contrast for this study. The device utilizes an infrared tracking system and offers registration through fiducial or anatomical landmarks. It also features a surface tracer option (**Figure 2**)

The process of image registration for both devices is similar, but aside from the obvious difference of the IOUS coupling or lack thereof, the Sonowand system allows for multiple intra-operative reads and acquisitions, thus providing a real-time image at any point of surgical resection and partially adjusting for imaging shift (**Figure 3**). All cases coming from the present series were operated using either of these two neuro-navigation devices.

Data collection

Population samples have been selected from the data stored on three neuro-navigation devices. Collected cases were divided into two categories:

1. **Exposure group**. Patients operated using the 3D-IOUS-Neuronavigation integrated system. Two identical and interchangeable 3D-IOUS-stealth coupled devices (marked 51 and 55) were used to extract data.
2. **Control group**. Patients operated using the stand-alone neuro-navigation system (Medtronic© Neuronavigation).

Regarding the inclusion criteria, we have collected all HGG-IV cases where the surgeon was planning to achieve a gross total resection - GTR (100%) or at least a near total resection – NTR (>90%) of the tumour, as per the operative note record entry, and excluded all cases where a partial debulking or a biopsy was intentionally performed. Cases were selected by matching the intra-operative stealth and IOUS scan details, and those into the operative notes where a surgeon was clearly documented that, in his/her opinion, GTR or NTR was achieved. Patients undergoing surgery without the use of any neuro-navigation devices were also excluded. Cases were selected based on a homogeneous timeframe, meaning all patients included were operated during the same period. Six senior surgeons were equally involved in the treatment of patients from the two groups. Out of this group of consultants, three anonymised surgeons (C1, C2 and C3) were dedicated neuro-oncology surgeons and most of the cases of the present series were

treated under their care. All selected patients had a pre-operative and an early post-operative (<48 hours) volumetric MRI scan T1-weighted (T1w) with Gadolinium (Gd). Patients without volumetric images were omitted. Patients undergoing post-op CT scan as a radiological method to check for the extent of resection were also excluded. The selection of cases was randomly performed by a research fellow (author GA), who operated on a random selection of all patients with a diagnosis of HGG-IV and with the appropriate inclusion criteria. Specifically, once the inclusion criteria screening was performed, the cases were randomly selected from the available pool by removing all identifiable data, assigning a random number using a randomisation function on the dedicated database, and subsequently extracting an equal number of cases from the exposure and control group for transfer to the software for statistical analysis (see below). All the senior surgeons were kept blind to the results.

The following clinical data have been collected from both groups: age at diagnosis, sex, comorbidities, consultant neurosurgeon responsible for the procedure, adjuvant treatment, OS, PFS, and performance status (PS). Pre- and post-operative radiological data in the form of pre- and post-op volumetric MRI scans have been collected for each patient to establish tumour volume and the presence of postoperative residual. Pre- and post-operative volumetric calculations on tumour volumes and post-op residuals (when present) have been performed on the above-mentioned sequences by a dedicated neuroradiology team. GTR was defined as the absence of enhancing residual on an early (< 48 hours) post-operative volumetric T1w with a Gd MRI scan. Blind imaging data were provided to the radiology team (authors IS, OC and AW) so that they were not aware of which group the patient was extracted from (exposure vs control) to limit confirmation bias. The location of the tumours in terms of depth and proximity to cortical and subcortical eloquent areas has also been considered in our analysis.

The primary outcome considered for the present study was the EOR in the two groups, in terms of both volume reduction and the presence/absence of the residual. The secondary outcomes were the OS and PFS in the two groups.

Statistical analysis

Statistical analysis was performed using RStudio (ver. 2021.09.2) running R (ver. 4.1.2). A linear regression model was built to test the effects of each variable on the EOR in R. The following variables were considered: use of IOUS, sex, age at diagnosis, depth of the most superficial and deepest point of the tumour, tumour size, proximity to an eloquent area, surgeon operating, and whether the case was a recurrent HGG-IV or not. Regarding the secondary outcomes (OS and PFS), a Cox proportional hazard model was built to weigh different factors' roles. In addition to the previously mentioned fields, the following ones were considered: peri-operative performance status, percentage of volume resected, MGMT and IDH status, post-operative chemo-radiotherapy (CRT) ,and the presence of peri-operative complications. Kaplan–Meier curves were built to compare OS and PFS for the following variables: use of IOUS (Y vs N), peri-operative complications (Y vs N) and post-operative treatment (no CRT, palliative CRT, or radical CRT). Plots were generated in GraphPad Prism (ver. 9.3.1). Statistical significance was determined using the Mann-Whitney U test (box-and-whisker plots) or log-rank (Mantel-Cox) test for Kaplan-Meier curves.

Results

We have retrieved 391 tumour patients operated using either of our two 3D-IOUS-stealth coupled machines (numbered 55 and 51), and 257 operated using stealth neuronavigation. The remaining patients were operated using different neuro-navigation devices or without neuro-navigation.

Thirty HGG-IV cases operated using IOUS-stealth coupled devices, and thirty operated using Medtronic were randomly selected from the pool of cases fitting the inclusion criteria. All cases included in the analysis had surgery between the 1st of January 2014 and the 31st of December 2017. Those cases' clinical and radiological features are summarised in Table 1 – a more detailed summary, including a more precise location of the tumours, is listed in **Supplementary material**. The mean age of the patients at diagnosis was 55 years (± 15); 21 patients were females, and 39 were males (M:F ratio = 1.8).

The average tumour volume for the whole pool of cases was 91.16 cm^3 (± 72.69). Tumour volume was higher in the IOUS group (mean 118 cm^3, ± 86 cm^3) compared to the control group (mean 64.14 cm^3, ± 42 cm^3. **Fig. 4a, p** =

0.007). Depth points of the tumours were comparable between the two groups: the average most superficial point was 1 cm in both groups (min 0 cm, max 3, SD ± 0.8), while the average deepest point was 5 cm deep (± 1.29 and ±1.44 in the IOUS group and in the control group, respectively). Six and seven cases were in proximity of eloquent areas in the 3D-IOUS group and the control group, respectively. All cases located near an eloquent area were operated using neurophysiological monitoring, awake surgery, or a combination of the two, depending on the indications.

Five patients overall were operating using 5-AminoLevulinic Acid (5-ALA), 2 in the 3D-IOUS group and 3 in the control group. The routine use of 5-ALA in HGG-IV surgery was introduced as standard in clinical practice in the UK only in 2018. This was not part of our routine practice before that date and given the exiguous number of patients treated using 5-ALA, this factor was not included in our final analysis.

As expected, EOR was found not to be normally distributed, with two clear peaks seen in the density distribution, separating a large population with optimal resection (n = 51, mean 92% ± 8%) and a small population with sub-optimal resection (n = 9, mean 55% ± 6%). The average EOR in the IOUS group was 93% (± 10%), while in the control group it was 80% (± 17%). There was a significant increase in EOR in the IOUS group compared to the control group, independent of other variables (**Fig. 4b**, $p = 0.0004$) known to affect resection, such as tumour depth and location near an eloquent area. Superficial access to the tumour was also linked to an improved EOR ($p < 0.03$). Interestingly, a weak association between consultants not normally performing neuro-oncological surgery and an improved EOR has also been found ($p = 0.05$).

The IDH1 gene has been reported as wildtype in 44 cases, mutant in 9, and was not analysed in the remaining 7 cases. The MGMT gene was unmethylated in 33 cases, methylated in 12 cases, and was not analysed in the remaining 15 cases.

The pre-operative performance status (PS) was 0 in 42 patients, 1 in 17 patients, and 2 in one patient. Immediate post-operative performance status was 0 in 40 patients, 1 in 11 patients, 2 in 2 patients, 3 in 5 patients and 4 in 2 patients. Four patients experienced significant post-operative complications: two in the form of severe post-operative cognitive and neurological impairment (PS 4), two developed acute hydrocephalus post-op, treated with ventriculo-peritoneal shunting. One of the patients with hydrocephalus also developed aspiration pneumonia, which was

successfully treated with antibiotics. We did not observe post-operative ischemic complications in either of the groups. Where observed, the worsening post-op status was in all cases related to postoperative oedema.

The average overall survival (OS) was 13.4 months (±8), and the average progression-free survival was 7.4 months (±7).

The Cox Proportional hazard model showed a significant advantage in OS on patients operated using the 3D-IOUS, with a Hazard Ratio (HR) = 0.196 and p = 0.017. As expected, administration of radical CRT was also associated with improved OS (HR = 0.199, p = 0.006). The presence of postoperative complications significantly affected survival (HR = 15.612, p = 0.002). We also observed differences in OS based on the presence of the tumour near an eloquent area (HR = 0.349, p = 0.013), the tumour volume (HR = 1.007, p = 0.047), and the surgeons performing the operations, both the "other" group (HR = 11.729, p = 0.004) and surgeon C3 (HR = 7.703, p = 0.013). However, only the peri-operative complications and application of CRT had independent effects on survival times (**Fig. 5a**). Only the administration of radical CRT was significantly associated with increased PFS (HR = 0.242, p = 0.013), and this association was seen as independent of other factors (**Fig. 5b**).

Discussion

Historical background and 3D IOUS development

The first ever reported application of the UltraSound (US) in neurosurgery was on a post-mortem case of a 54-year-old woman[10]. Since then, several intra-operative imaging devices have been developed to help neurosurgeons to localise the tumour before surgery. CT and MRI scans, introduced in clinical practice during the '70s and in the '80s, respectively, were two of the major technological advancements that allowed neurosurgeons to localize the target lesion more accurately inside the brain. Neuro-navigation frameless devices were introduced during the '90s, and they were the first tools allowing intra-operative localisation of the tumour [9], although they did rely on pre-operative images rather than real-time acquisition. IOUS appeared to solve the problem of real-time imaging technology. The preliminary reports showed successful intra-operative tumour identification [7]. Interestingly, in several cases, the

signal obtained from IOUS was different when compared to that obtained using traditional imaging technologies (CT or MRI scan), thus suggesting that the IOUS could be used not only as an intra-operative aid but also as a complementary tool for those lesions of unclear nature or margins [7]. Moreover, coupling the IOUS with neuro-navigation has greatly improved the possibility of midline shift adjustment during surgery[17, 23, 27, 35], and has been proven useful to improve tumour demarcation when close to eloquent areas [26]. In more recent years, further technological advancement has led to the development of 3D IOUS [30, 31]. The 3D reconstruction is automatically generated by the neuro-navigation software after an intra-operative acquisition through a single spatial plane. The images can be integrated with Doppler angiography when required, so that vessel encasement by an intracranial mass or aneurysm can also be detected [30, 31]. Recent research has also focused on the possibility of integrating IOUS with contrast[1] or, more importantly, planning surgical resection based on IOUS[23]. As discussed in the following sections, the system we have used is also partially oriented towards the same goal, which is to provide a cost-contained and more reliable and real-time option to improve EOR.

An extensive systematic review and meta-analysis focused on IOUS has shown an average GTR of 77%, an 82% concordance rate between IOUS and post-op MRI scan, and high sensitivity/specificity at the beginning of surgical resection (>90%) [19]. The same study failed to find a significant correlation between the use of IOUS and a significant impact on survival, which is the reason why we have attempted a quantitative analysis on a subgroup of relatively homogeneous patients.

Our results

Our study delves into the utility of 3D-IOUS in HGG-IV surgeries. Generally, HGG-IV and anaplastic gliomas grade III presented diverse IOUS results. While anaplastic astrocytomas and oligodendrogliomas were hard to discern due to mixed boundaries with surrounding oedema, necrotic and cystic areas were typically discernible. For this study, HGG-IV were chosen due to their prevalence and because there is consensus on defining the EOR by looking at the enhancing component. On IOUS, HGG-IVs mirrored MRI features: the enhancing capsule was hyper-echogenic, whereas the necrotic core was hypo-echogenic. Recurrent HGG-IV and recurrent anaplastic grade III are more challenging tumours to visualise on 3D-IOUS, in our experience (**Figure 6**). As highlighted by other authors [19], the

intra-operative imaging quality varies based on the stage of surgical resection. Superficial, small-sized lesions were typically very well visualised by the acquisition and during resection. However, even a modest to moderate amount of bleeding cause a visible artefact that can hamper surgical view beyond the limits of resection (**Figure 7**).

We have included two comparable groups of HGG-IVs, operated by a heterogeneous pool of surgeons. This selection aimed at choosing a homogeneous group of patients, tumours, and treatment groups to address how much the EOR would change with or without the IOUS and whether there could be an impact on OS and PFS. The tumours included had different locations, sizes, and proximity to the surface. However, the number of tumours located near eloquent areas was similar between the exposure and the control group (6 vs 7, respectively). Moreover, the average depths of the tumours were comparable between the two groups, in terms of the most superficial and the deepest portions. On the other hand, pre-operative tumour volumes were higher in the 3D-IOUS group than in the control group, which was presumably a stochastic effect. Despite the average larger tumour volume pointing towards a potentially more challenging surgical resection, the EOR was still more generous in the 3D-IOUS group, thus suggesting that IOUS makes a difference in the quality of EOR regardless of tumour size. Our results show that, when all possible factors are considered, the EOR is increased when IOUS is used. This result aligns with those from the international literature[21, 25, 31], and the evidence, although not consensual, seems to point towards the direction of a clear advantage in using IOUS to improve EOR[2]. In that regard, some authors stressed the fact that IOUS is an operator-dependent technology, needing a learning curve and without a standardised training pathway[6]. Such limiting circumstances could easily explain the lack of homogeneous results and point towards the necessity of further studies and a more defined educational and professional pathway for the application of IOUS in different centres. Interestingly, in our series the use of IOUS also resulted in a borderline statistically significant improvement of OS, although the advantage was not massive. This finding seems consistent with what some other groups have recently reported [4, 15, 21, 22, 33]. While most of these series are retrospective, and not rarely mix different histological subtypes[33], one relatively small randomised controlled trial also confirmed these results[15].In our series, the small number of patients included and the numerous confounding variables suggest that this outcome should be taken cautiously. A potential prospective study enrolling a more conspicuous pool of patients, with stratification based on tumour location, size, and depth, might be helpful to verify this finding. A more recent systematic review compared IOUS with other intra-

operative imaging techniques, including intra-operative MRI (iMRI), fluorescence, and tractography[2]: the authors were also cautious regarding the survival benefit of IOUS. While the compound evidence towards an improvement of EOR seems to be adequately supported, the effect on OS is still debatable and it needs to be clarified, accounting for several confounding factors. A promising, more extended randomised controlled trial is currently in the recruitment phase in the UK[24]. It is also worth stressing that the same finding did not apply to PFS in the present series, and it's not entirely clear why this should be the case Several authors pointed out that PFS and OS are not always linearly correlated in cancer series and PFS is actually an approximated metric, which might benefit from more refined and updated versions [3, 5]. Assuming the OS finding is significant in our series, PFS might not be as accurately represented, given that the follow-up scan time points were less homogeneous between different patients It is still possible, however, that the observed effect on OS is random, and the PFS results truly reflect the overall trend, implying that IOUS deployment might not have a real survival effect. The Cox proportional hazard model highlighted the impact of CRT on OS, an expected finding already known from the international literature[11, 20]. Post-op complications' impact seems significant on the log-rank test (**Figure 5a**, middle graph) and the Cox proportional hazard model ($p = 0.002$). On a closer analysis of the results, all these patients but one showed a poor PS post-op and were not offered a post-op radical CRT regimen. We observed no major complications significantly affecting OS or PFS related to the CRT regimen itself, despite Temozolomide (TMZ) having been interrupted in 3 cases due to blood toxicity. Another interesting finding concerns the stratification of patients according to the surgeon operating on them. We have purposely decided to include this variable in the analysis to consider the inevitable inter-operator variability: different surgeons have different approaches and techniques when resecting a tumour. As expected, most patients were operated by dedicated neuro-oncology consultants, with a few being operated by general neurosurgeons (grouped together and marked as "others"). EOR did not show significant differences among the three neuro-oncology surgeons operating, although the Confidence Interval (CI) was broader in C2 compared to C1 and C3. Differences in resection technique, patient PS, comorbidities, and proximity to eloquent areas could account for the discrepancy. However, even more interesting, the differences in EOR did not translate directly into differences in OS and PFS, with C1 being the consultant with the highest associated survival but EOR comparable with that of C3. Also, C1 has operated on a lower number of cases included, which might have skewed the analysis. Tumour depth has not significantly affected EOR, OS or PFS. Still, this effect is likely to be linked to pre-operative

case selection: deeply located lesions are unlikely to be selected for GTR to start with. This is also true for the performance status, MGMT and IDH1 status and their impact on OS and PFS. Finally, both groups demonstrated an equal distribution of complications and deteriorating post-operative neurological outcomes. Upon examining the subset of patients who experienced significant post-operative impairment, it was noted that only a single patient had a glioma situated in an eloquent region, specifically the occipital cortex. In the two post-operative hydrocephalus cases, the tumours were positioned in the hippocampal and trigonal regions, respectively. This suggests that the likelihood of post-operative hydrocephalus was anticipated. Notably, in this series, the influence of complications appeared to have statistical significance exclusively concerning overall survival (OS). However, there seemed to be no correlation with any other factor, barring the anticipated statistical variances.

Limitations

The present data collection and relative analysis have been conducted to the best of our knowledge without selection biases and limiting access to non-blind data to all the authors involved in the analysis. However, we are aware that the present study still has several limitations. First, this is a retrospective review based on data previously collected. We have, therefore, excluded a great number of cases due to incomplete or missing pools of data. For example, many patients have not been scanned within 48 hours post-op, and in many others, we found lack of a volumetric T1 with Gd scan, either pre- or post-op. We believe it was crucial for homogeneous data to retrieve only those cases where all the inclusion criteria were strictly followed. Still, this decision has inevitably reduced the number of cases we could include. Secondly, the dataset is quite heterogeneous, and some of the surgeons clearly preferred using the 3D-IOUS to start with because of the integration between neuronavigation and ultrasound. This is the main reason why we have included the operating surgeon as a possible confounding variable into the statistical analysis. Still, given the small pools of cases analysed, this factor might have impacted more than shown. Thirdly, the small sample included in the analysis did not allow for a more precise stratification of the data, meaning that tumours similar in locations and size could not be precisely compared. In our analysis, we have partially tried to solve this problem by accounting for tumour location in terms of eloquence proximity, pre-operative size, and depth. However, ideally, it would have been more adequate to compare outcomes from pools of tumours in similar locations. It is also worth noting that eloquent areas proximity is an outdated concept[8], and therefore a more precise stratification is desirable

for future studies. With this being a retrospective series, it was logistically challenging to stratify tumour locations more accurately. Fourthly, most of these patients were operated on an historical moment when the 5-ALA was not used as a standard of care in the UK, so unfortunately, we have a limited number of cases where this agent was used and a comparison analysis between that and the IOUS was not performed. It is important to stress this point, as 5-ALA has also been found to be superior to intra-operative MRI in one study[4]. However, we do not believe this has impacted significantly onto our results as most of the surgeons involved were senior and experienced enough to assume that the difference in their performance would have been negligible. Finally, we have relied on the clinical notes to include patients in the statistical analysis, meaning that we have assumed that the aim of the surgeon to achieve a GTR or NTR of the enhancing component was not biased. A relatively wide CI on EOR suggests that a degree of error was present in some cases. This is also the case when analysing the impact of the tumour molecular profiles in relationship with OS and PFS: MGMT and IDH statuses were missing in a significant minority of cases, because the present series dates to 2014 and molecular profiling was not fully included in the WHO classification yet, so their real impact was difficult to determine with the data available.

Conclusions

The present statistical analysis on HGG-IV cases shows an advantage in EOR using IOUS compared to the cases where IOUS is not used. This is in keeping with the more recent results from the international literature and highlights the possibility that an improved EOR is achieved when IOUS is deployed by an experienced team. Our analysis also suggests a potential advantage in OS in those cases where IOUS is used, and this holds true even when considering other factors known to affect survival, such as complications, administration of post-op radical CRT, PS, and tumour size. The OS finding needs further studies to be confirmed, as PFS seems to be unaffected by IOUS. Finally, IOUS and tumour resections remain heavily operator-dependent, suggesting that the results of the present analysis might have been affected by the above-mentioned limitations. A prospective analysis of a broader pool of patients, stratified according to a more precise tumour location, surgical technique, and pre-operative planning, is desirable to verify these findings.

Ethical statements

The authors do not have any conflict of interest to declare, financial or otherwise.

The present data collection is entirely anonymised, and it has received approval from the Health and Research Authority – Bloomsbury Research Committee, London. No funding was required for the present project. REC reference and project ID are as follows:

- REC (Research Ethics Committee) reference: 19/LO/1763
- IRAS (Integrated Research Application System) project ID: 265404

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Figures captions

Figure 1. 3D-IOUS intra-operative scanning. Left: intra-operative picture showing the dedicated tracking device coupled with the probe during and intra-operative IOUS acquisition, which links the intra-operative MRI scan with the IOUS images. Centre and right: a case showing two IOUS acquisitions, one performed after exposing the cortical surface (centre), the other at the end of the resection (right), showing the resection cavity.

Figure 2. A case (not included in the present series) showing a standard neuro-navigation deployment during surgery for an anaplastic astrocytoma. Left: intra-operative picture showing the probe. Centre: snapshot at the beginning of the case. Right: snapshot towards the end of the resection.

Figure 3. Flow-chart showing the different acquisition techniques. Aside from the obvious difference that the Sonowand© can be coupled with an IOUS probe, it allows for multiple acquisitions at any point during tumour resection, thus providing different views even during and at the end of the resection. IOUS probes can also be used free-hand, without coupling with neuronavigation.

Figure 4. a) Box-and-whisker plot of pre-operative volumes. Cases included in the 3D-IOUS group were found to have larger volumes compared to those included in the control group. b) Box-and-whisker plot showing the differences in EOR between the group where 3D-IOUS was used, and the one where it was not (Mann-Whitney U-test was used, $p < 0.0004$). This correlation was found to be independent from other variables.

Figure 5. a) Whilst not independently significant, we have found a shift toward longer survival in the 3D-IOUS cohort. Peri-operative complications were found to have a highly significant impact: 3 patients showed markedly shorter OS. Radical CRT also significantly extends survival times, in keeping with the results of the known literature.

P values were determined by log-rank (Mantel-Cox) test. b) Overall, PFS is poor in all patients, and we found that the differences between groups are not substantially significant. 3D-IOUS does not appear to be significantly associated with significantly improved PFS. Radical CRT promotes longer PFS, as expected. P values determined by log-rank (Mantel-Cox) test.

Figure 6. a) A right frontal HGG as seen on the Sonowand neuronavigation sequence alone (left) and with the same sequence co-registered with 3D-IOUS. Visualisation of tumour boundaries is adequate, although the lesion often appears homogeneously hyperechogenic, with no clear distinction between the tumour capsule and the necrotic core. b) A case of left frontal recurrent glioblastoma. Apart from the size of the lesion requiring multiple acquisitions and therefore causing linear artifacts, the tumour appears challenging to be defined on IOUS due to the relatively homogeneous hyperechogenic signal.

Figure 7. Intra-operative picture of right posterior temporal glioblastoma before (left) and after resection (right). In this case, the necrotic core was well visualized. On the right side, note the presence of partial shift of the brain surface and a cone of hyperechogenic material due to the deposit of blood products at the bottom of the cavity.

Figure_1 Details

Print Resolution: **330 dpi**
Size (Px): W:2970 H:1237
Print Size: 9 * 3.75 Inches.

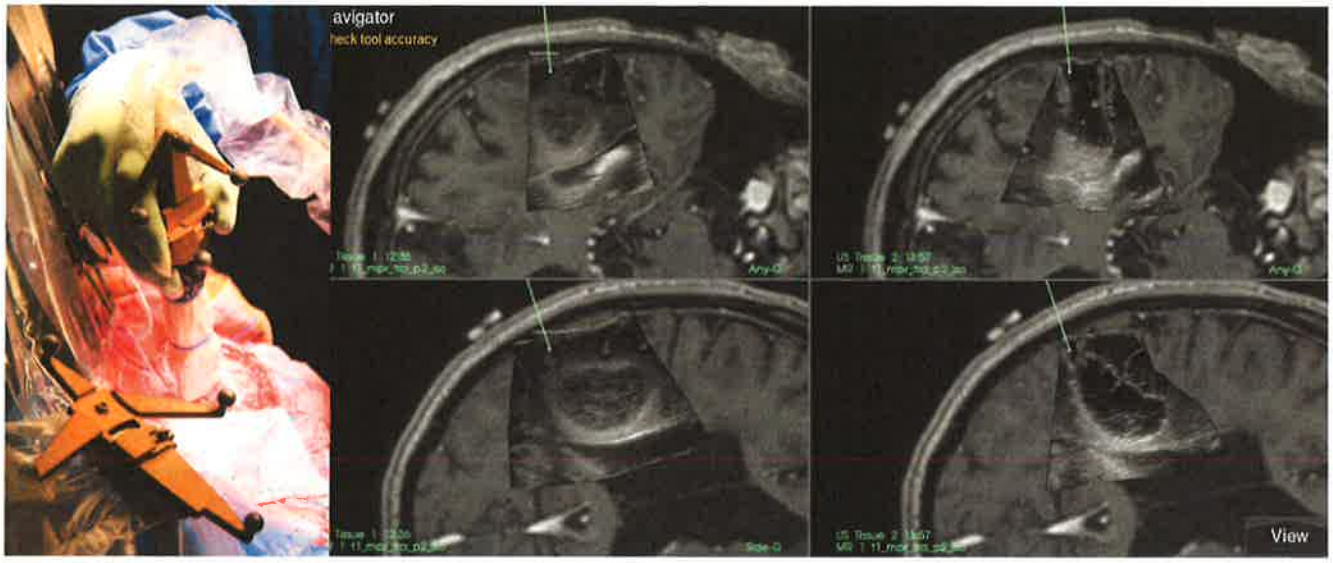

Figure_1 Legends

3D-IOUS intra-operative scanning. Left: intra-operative picture showing the dedicated tracking device coupled with the probe during and intra-operative IOUS acquisition, which links the intra-operative MRI scan with the IOUS images. Centre and right: a case showing two IOUS acquisitions, one performed after exposing the cortical surface (centre), the other at the end of the resection (right), showing the resection cavity.

Figure_2 Details

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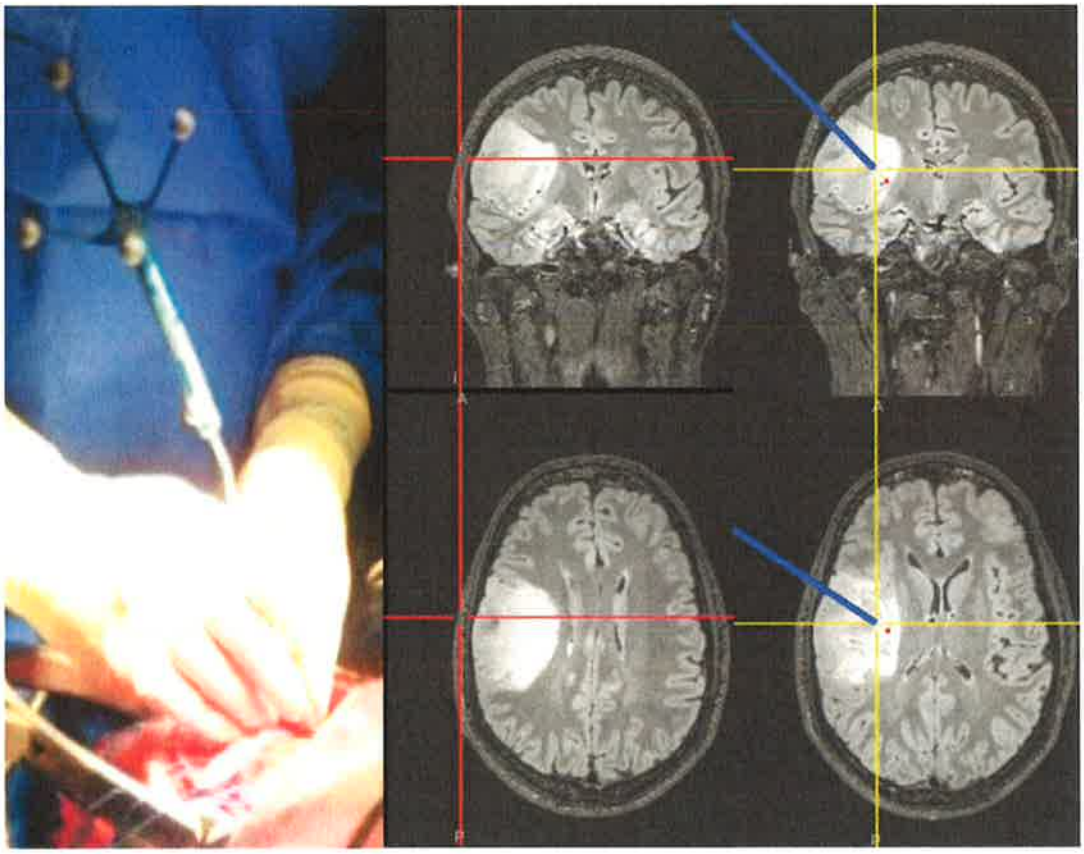

Figure_2 Legends

A case (not included in the present series) showing a standard neuro-navigation deployment during surgery for an anaplastic astrocytoma. Left: intra-operative picture showing the probe. Centre: snapshot at the beginning of the case. Right: snapshot towards the end of the resection.

Figure_3 Details

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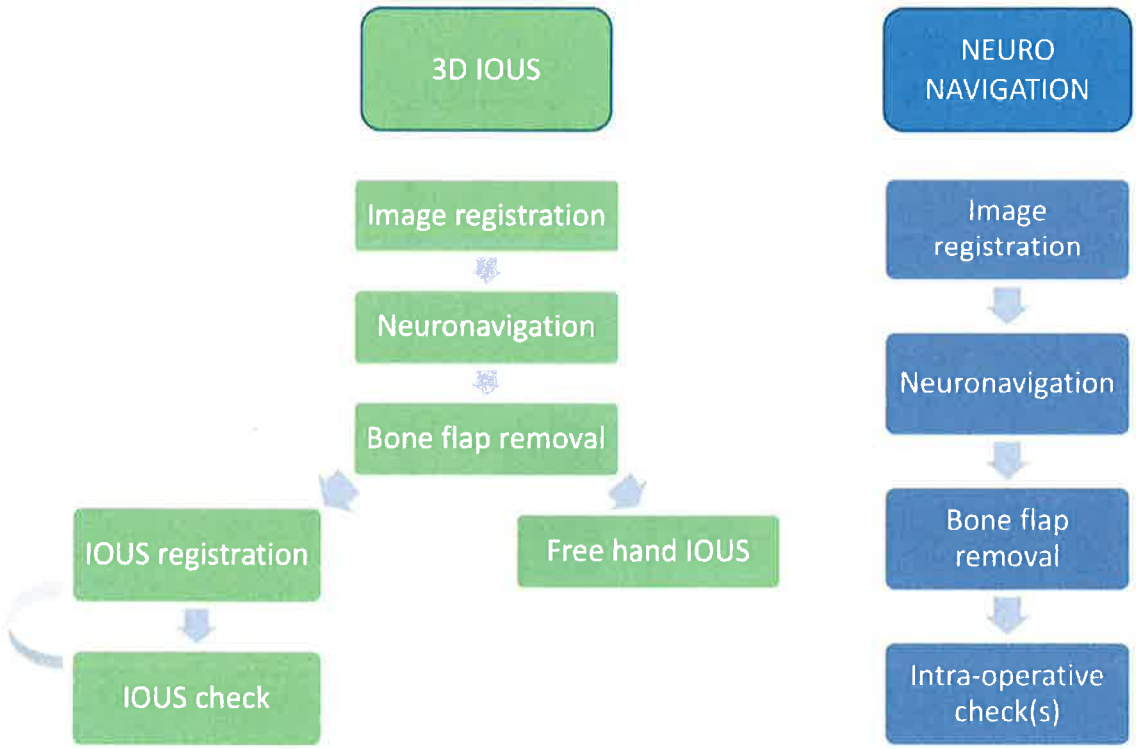

Figure_3 Legends

Flow-chart showing the different acquisition techniques. Aside from the obvious difference that the Sonowand? can be coupled with an IOUS probe, it allows for multiple acquisitions at any point during tumour resection, thus providing different views even during and at the end of the resection. IOUS probes can also be used free-hand, without coupling with neuronavigation.

Figure_4A Details

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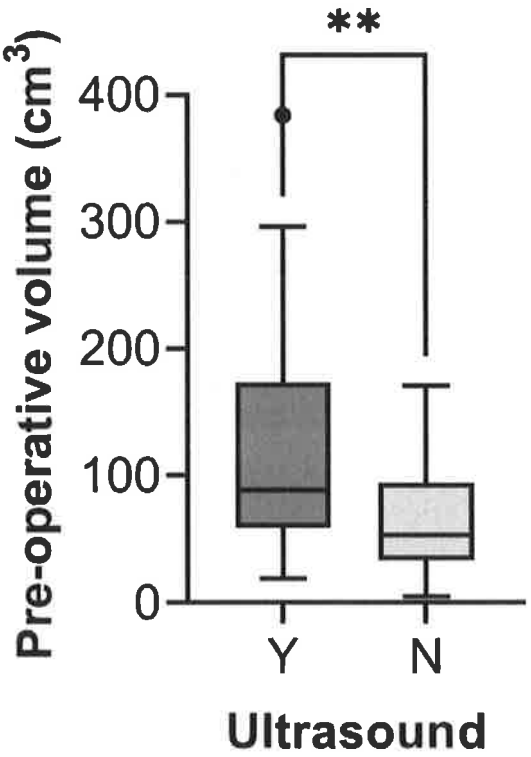

Figure_4A Legends

a) Box-and-whisker plot of pre-operative volumes. Cases included in the 3D-IOUS group were found to have larger volumes compared to those included in the control group.

Figure_4B Details

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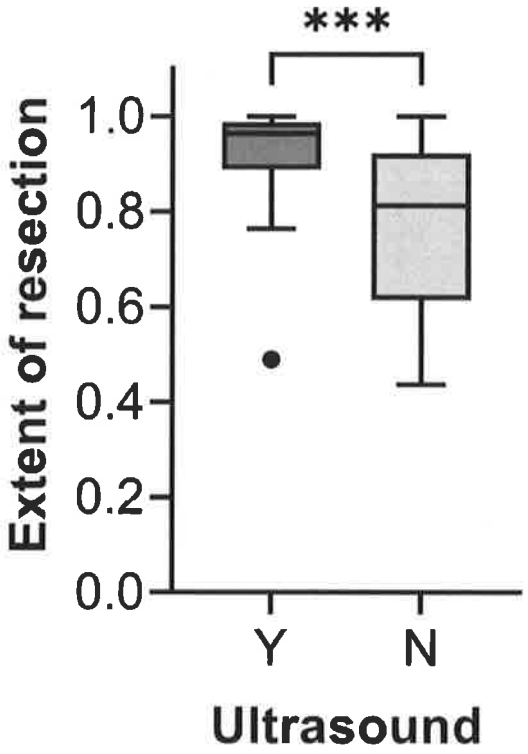

Figure_4B Legends

b) Box-and-whisker plot showing the differences in EOR between the group where 3D-IOUS was used. and the one where it was not (Mann-Whitney U-test was used, $p < 0.0004$). This correlation was found to be independent from other variables.

Figure_5A Details

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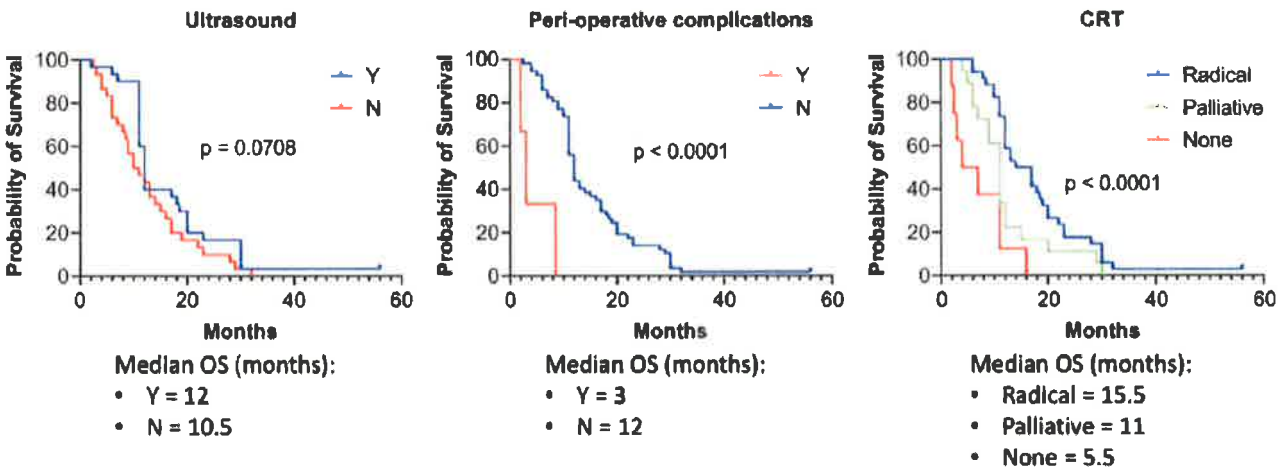

Figure_5A Legends

a) Whilst not independently significant, we have found a shift toward longer survival in the 3D-IOUS cohort. Peri-operative complications were found to have a highly significant impact: 3 patients showed markedly shorter OS. Radical CRT also significantly extends survival times, in keeping with the results of the known literature. P values were determined by log-rank (Mantel-Cox) test.

Figure_5B Details

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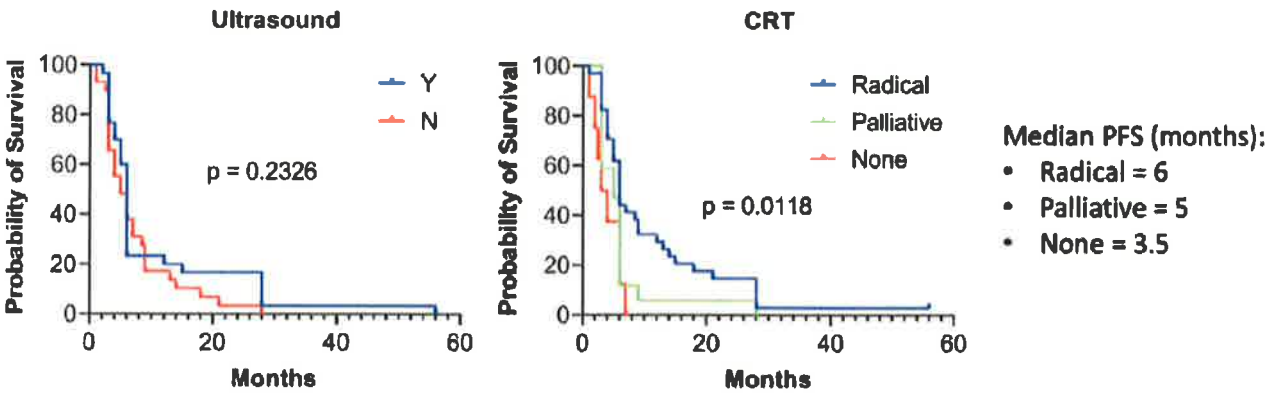

Figure_5B Legends

b) Overall, PFS is poor in all patients, and we found that the differences between groups are not substantially significant. 3D-IOUS does not appear to be significantly associated with significantly improved PFS. Radical CRT promotes longer PFS, as expected. P values determined by log-rank (Mantel-Cox) test.

Figure_6A Details

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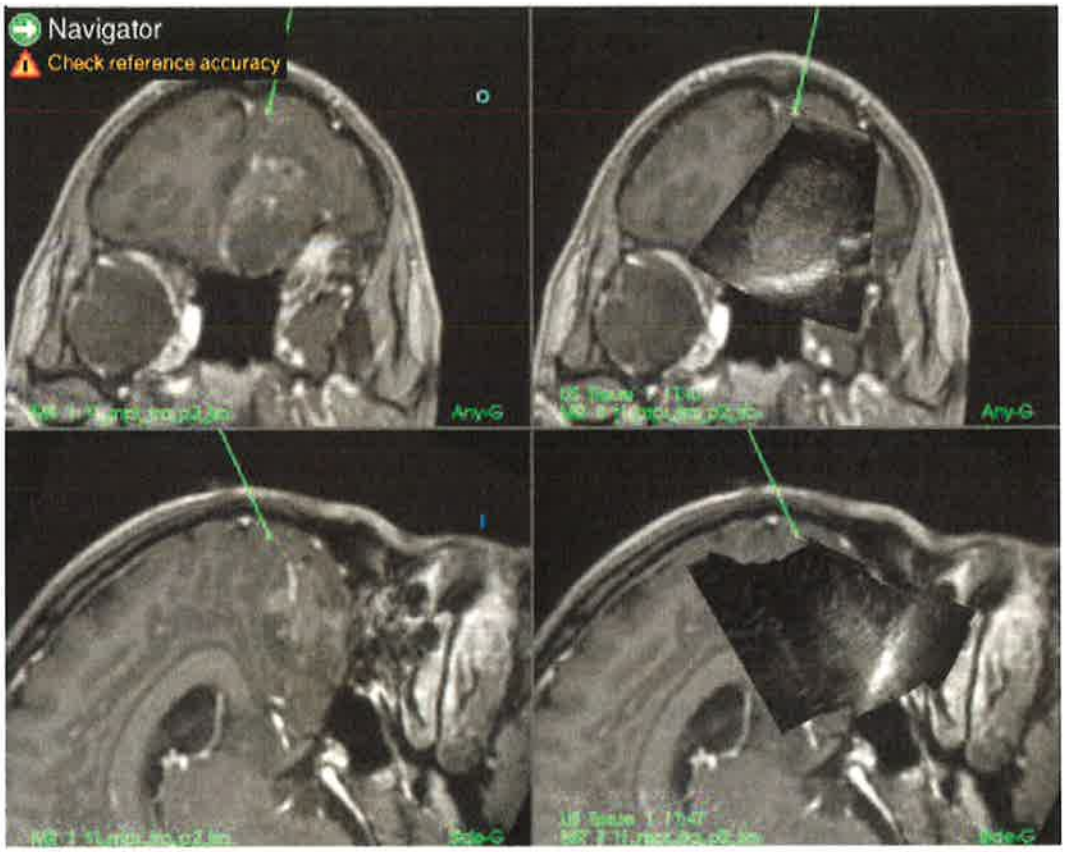

Figure_6A Legends

a) A right frontal HGG as seen on the Sonowand neuronavigation sequence alone (left) and with the same sequence co-registered with 3D-IOUS. Visualisation of tumour boundaries is adequate, although the lesion often appears homogeneously hyperechogenic, with no clear distinction between the tumour capsule and the necrotic core.

Figure_6B Details

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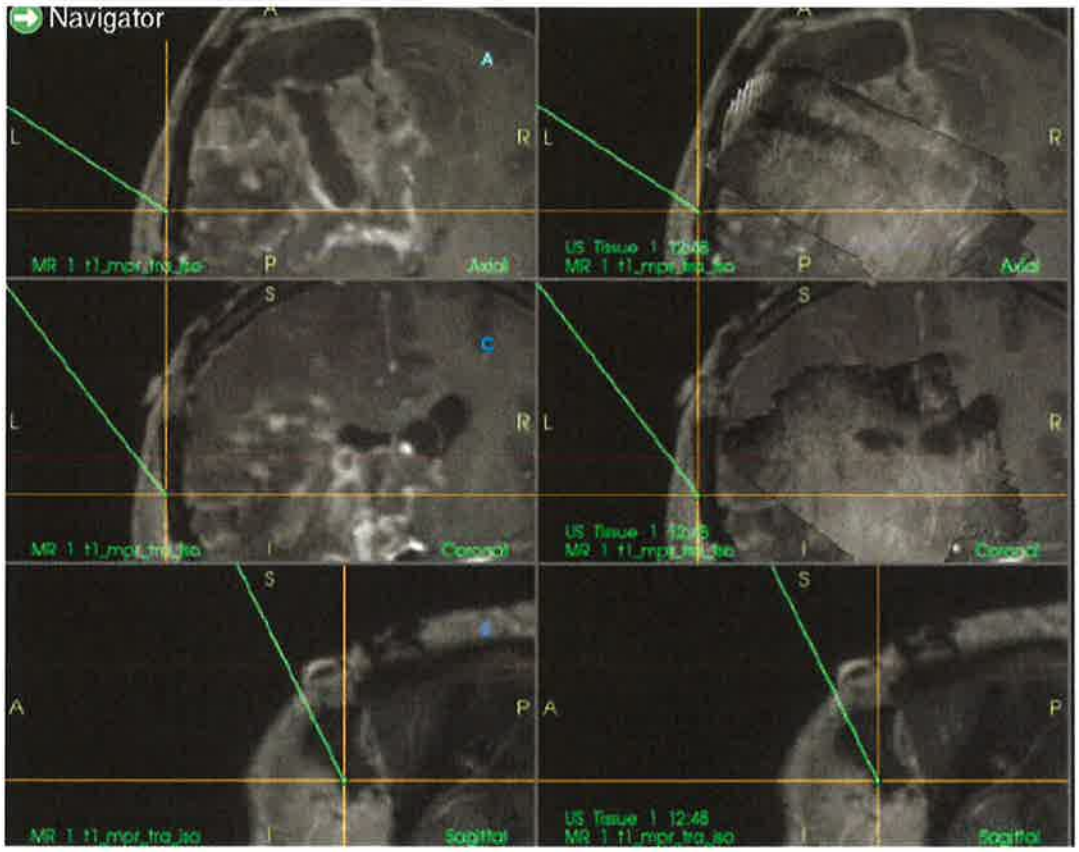

Figure_6B Legends

b) A case of left frontal recurrent glioblastoma. Apart from the size of the lesion requiring multiple acquisitions and therefore causing linear artifacts, the tumour appears challenging to be defined on IOUS due to the relatively homogeneous hyperechogenic signal.

Figure_7 Details

Print Resolution: **123 dpi**
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Print Size: 5.76 * 3.33 Inches.

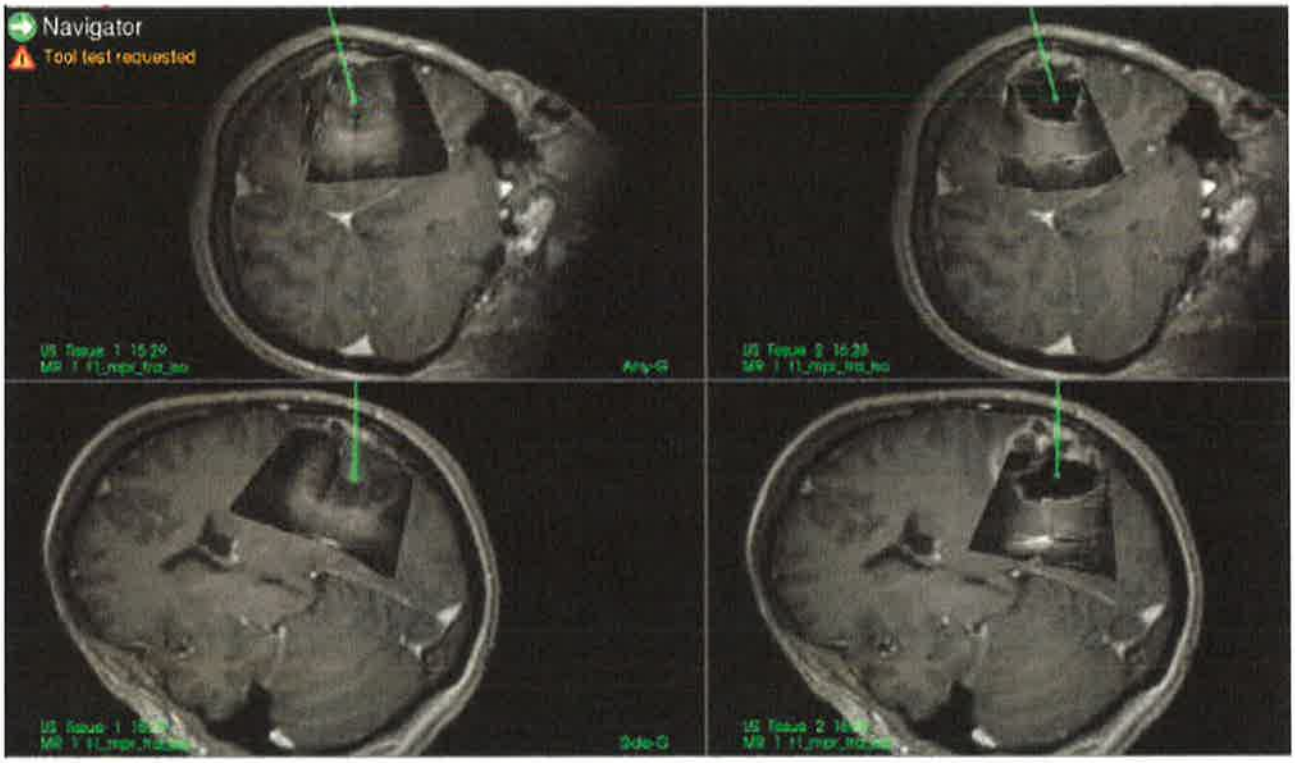

Figure_7 Legends

Intra-operative picture of right posterior temporal glioblastoma before (left) and after resection (right). In this case, the necrotic core was well visualized. On the right side, note the presence of partial shift of the brain surface and a cone of hyperechogenic material due to the deposit of blood products at the bottom of the cavity.