FLUORESCENCE GUIDED VERSUS CONVENTIONAL SURGICAL RESECTION OF HIGH GRADE GLIOMA: A SINGLE CENTRE 7 YEAR COMPARATIVE EFFECTIVENESS STUDY

By

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LIST OF ABBREVIATIONS

Abbreviation	Description
AA	Anaplastic astrocytoma
AO	Anaplastic oligodendroglioma
AOA	Anaplastic oligoastrocytoma
5-ALA	5-aminolevulinic acid
CNS	Central nervous system
CI	Confidence interval
CT	Computed tomography
EOR	Extent of resection
FDA	Food and Drug administration
FG	Fluorescence guided
FGA	Friedlein A
FGB	Friedlein B
GBM	Glioblastoma multiforme
GTR	Gross total resection
Gy	Gray
HGG	High grade glioma
ICU	Intensive care unit
IQR	Interquartile range
KPS	Karnofsky performance scale
MRI	Magnetic resonance imaging

NCCN	National Comprehensive Cancer Network
NCR	National Cancer Registry
NTR	Near total resection
OT	Operation theatre
PFS	Progression free survival
PpIX	Protoporphyrin IX
PR	Partial resection
RT	Radiotherapy
SD	Standard deviation
SPSS	Statistical package for Social Science
STR	Subtotal resection
TMD	Tumor maximal diameter
TMZ	Temozolamide
vs	Versus
WHO	World Health Organization
XRT	External beam radian treatment

ABSTRAK (BAHASA MALAYSIA)

Topik :

PERBANDINGAN PEMBEDAHAN BERPANDUKAN “FLOURESCENCE” DENGAN PEMBEDAHAN KONVENSIONAL DALAM MERAWAT “GLIOMA” PERINGKAT TINGGI : KAJIAN PERBANDINGAN KEBERKESANAN PUSAT UTAMA DALAM TEMPOH 7 TAHUN

Latar Belakang:

Gred tinggi “glioma” adalah ketumbuhan otak jenis invasif dan agresif yang membawa “prognosis” teruk. Meskipun strategi therapeutik yang mengubati gred tinggi “glioma” telah berkembang dengan laju, Malaysia masih gagal mencapai rawatan yang standard berdasarkan panduan “National Comprehensive Cancer Network”, iaitu “resection” ketumbuhan yang maksimum serta rawatan kemoterapi dan radioterapi. Walaupun “gross total resection” berjaya memanjangkan jangka hayat pesakit, tetapi ianya kekal sebagai cabaran yang serius bagi pakar bedah otak disebabkan oleh kesukaran untuk membezakan sempadan ketumbuhan daripada tissue otak yang biasa. Hospital Sungai Buloh adalah hospital kerajaan di Malaysia yang pertama sekali dalam melaksanakan pembedahan Flourscence berpandu dalam rawatan gred tinggi “glioma” semenjak tahun 2010.

Objektif

Tujuan penyelidikan ini adalah untuk menganalisa kesan rawatan ketumbuhan otak dalam hasil ikhtiar hidup dan taraf kerja pesakit melalui pembedahan berpandukan “Flourescence”

berbanding dengan pembedahan konvensional. Kami akan mengkaji factor-factor penting yang mempengaruhi ikhtiar hidup semua pesakit yang mempunyai gred tinggi “glioma”. Di samping itu, kami juga mengenal-pasti kesan tahap “resection” terhadap taraf kerja dan ikhtiar hidup pesakit.

Kaedah kajian

Ini adalah kajian permerhatian retrospektif. Sejumlah 74 pesakit gred tinggi “glioma” baru yang menyertai kajian ini adalah mereka yang menjalani pembedahan dari 1st Januari 2008 sehingga 31st Disember 2014. 37 pesakit yang menjalani pembedahan berpandukan “Flourescence” dari Januari 2010 sehingga 31st Disember 2014 dan memenuhi “inclusion” kriteria yang ditentukan akan menyertai dalam kajian ini. 37 pesakit dari kumpulan perbandingan adalah mereka yang menjalani pembedahan konvensional dari Januari 2008 sehingga 31st Disember 2014. Pesakit akan diberi tarikh temujanji di klinik kepakaran saraf otak sebelum balik rumah (kebanyakan adalah 6 miggu dan 6 bulan dari tarikh pembedahan). Pesakit yang tidak layak terlibat dalam kajian ini adalah umur kurang daripada 18 tahun atau melebihi 65 tahun, wanita hamil, status fungsi kehidupann “KPS” sebelum pembedahan < 70, lebih dari satu ketumbuhan di otak, kedudukan ketumbuhan yang dalam and susah dibuangkan, ketumbuhan di bahagian tubuh badan yang lain sebelum ini dan pernah menerima rawatan. Semua maklumat pesakit yang diperolehi melalui rekod perubatan elektronik akan dianalisa dengan menggunakan “Statistical Package for Social Sciences” versi 22.0. Min dan tahap ralat dikira bagi nilai berterusan, frekuensi dan peratusan bagi nilar kategori. Ujian “Pearson Chi-square” digunakan untuk nilai kategori bandingan antara dua kumpulan. Sekiranya nilai jangkaan frekuensi kurang dari lima bagi

dua puluh peratus sel, maka Ujian Fisher Exact akan digunakan. Jangka hidup pesakit yang dikira dari tarikh pesakit menjalani pembedahan sehingga pesakit meninggal atau tempoh tamat penyelidikan akan dianalisa dengan kaedah “Kaplan Meir”. Faktor-faktor peramal hidup pesakit akan ditentukan dengan menggunakan model “Cox Proportional Hazards Regression”. Nilai nyata ditentukan pada nilai *p* kurang dari 0.05.

Keputusan

Seramai tujuh puluh empat pesakit gred tinggi “glioma” telah menjalani pebedahan di Hospital Sungai Buloh antara Januari 2008 dan 21st Disember 2014. pesakit yang menjalani pembedahan berpandukan “Flourescence” hidup lebih lama berbanding dengn pesakit yang menjalani pembedahan konvensional, iaitu 12 bulan berbanding dengan 8 bulan ($p < 0.02$). Tanpa rawatan kemoterapi dan radioterapi, pesakit dari kumpulan “Flourescence” hidup selama 8 bulan berbanding dengn pesakit kumpulan konvensional yang cuma hidup selama 3 bulan ($p = 0.006$). Tiada perbezaan dari segi taraf kerja antara dua kumpulan setelah 6 minggu dan 6 bulan pembedahan dijalanan. Anallisa berkaitan dengan faktor-faktor yang nyata secara statistik yang mempngaruhi hasil ikhtiar hidup pesakit termasuk status fungsi kehidupan sebelum permbedahan yang melebihi 80 ($p = 0.01$), kaedah pembedahan ($p = 0.001$), gred “glioma” ($p = 0.001$) dan rawatan kemoterapi dan radioterapi ($p = 0.001$). Analisa berkaitan dengan faktor-faktor yang nyata secara statistik yang mempngaruhi hasil ikhtiar hidup pesakit melalui “multiple logistic regression” termasuk ketumbuhan di lokasi “ non-eloquent/ near eloquent” ($p=0.092$), kaedah pembedahan ($p = 00.087$) dan rawatan kemoterapi dan radioterapi ($p = 0.094$). Walaupun, kesan tahap “resection” tidak mencapai statistikk yang nyata, pesakit yang

mempunyai tahap “resection” ketumbuhan yang melebihi 90% hidup selama 10 bulan, iaitu 2 bulan lebih panjang berbanding dengan pesakit yang cuma mencapai tahap “resection” yang kurang dari 90%.

Rumusan

Penyelidikan in menunjukan pesakit mendapat manfaat lebih dari segi hasil ikhtiar hidup sekiranya menjalani pembedahan berpandukan “Flourescence” berbanding pembedahan konveksi tanpa menyebabkan kesan buruk terhadap taraf kerja pesakit. 4 faktor nyata yang mempengaruhi hasil ikhtiar hidup pesakit telah dikenapasti. Tetapi, cuma 3 factor nyta yang mempengaruhi hasil ikhtiar hidup pesakit telah dikenapasti melalui “multiple logistic regression” analisa. Kesan tahap “resection” tidak mencapai statistik yang nyata disebabkan oleh cuma segolongan kecil pesakit dari kumpulan “Flourescence” yang dapat menjalani pengimejan “MRI” kepala setelah pembedahan. Kita berharap hasil dari penyelidikan boleh dijadikan rujukan dan mencetuskan minat penyelidik untuk menjalani penyelidikan yang melibatkan lebih banyak pesakit terutama “multi-centered randomized trials” bagi tujuan mempekukuhkan kesimpulan penyelidikan ini . Kita juga menyaran supaya pusat neurosurgical yang lain di Malaysia juga melaksanakan penggunaan “adjunct: 5-ALA” dalam merawat pesakit yang mempunyai ketumbuhan otak gred tinggi dan memanfaatkan lebih banyak pesakit.

ABSTRACT (ENGLISH)

Title

FLUORESCENCE GUIDED VERSUS CONVENTOINAL SURGICAL RESECTION OF HIGH GRADE GLIOMA: A SINGLE CENTRE 7 YEAR COMPARATIVE EFFECTIVENESS STUDY

Background

High grade gliomas (HGGs) are locally invasive and aggressive brain tumors that carry dismal prognosis. Despite the rapidly evolving therapeutic strategies to treat HGGs, Malaysia still failed to demonstrate the expected standard HGGs management. According to National Comprehensive Cancer Network (NCCN) Guideline, the current standard management of HGGs includes maximum safe microsurgery resection followed by chemotherapy and radiotherapy. Complete resection, though increases median survival, is often hindered by the challenge of demonstrating the tumor border reliably. Department of Neurosurgery Hospital Sungai Buloh therefore has adopted fluorescence guided (FG) surgery using 5-aminolevulinic acid (5-ALA) as the first public hospital in Malaysia to overcome this shortcoming since 2010.

Objectives

The aim of this research was to evaluate the effects of fluorescence guided (FG) surgery on overall survival and functional outcome as compared to conventional surgery. Besides, the study also aimed to find out the independent predictors that are associated with survival in

high grade gliomas (HGGs) patients. Lastly, the effect of the extent of surgical resection on overall survival and functional outcome in FG patients was also evaluated.

Methodology

This study was a retrospective observational study. 74 patients with histologically proven high grade gliomas (HGGs) were recruited between January 2008 and December 2014. 37 HGG patients that underwent FG surgery from January 2010 until 31st December 2014 and fulfilled the inclusion criteria were recruited. Another 37 patients from conventional group was recruited consecutively from January 2008 until 31st December 2014 as the comparison group. The follow-up periods were done according to the scheduled appointment date (mostly at 6 weeks and 6 months) from the date of operation, which ended on 30rd June 2015. Patients, who were below 18 years old and above 65 years old, preoperative KPS <70, recurrent multicentric, midline deep seated tumor, history of malignant tumor at other body site, known and suspected pregnancy were excluded in the study. Data entry and analysis was done using Statistical Package for Social Sciences (SPSS) version 22.0. Mean and standard deviation were calculated for continuous variables, and frequency and percentage for categorical variables. Pearson Chi-square were identified, however if expected frequency of less than five were more than twenty percent of cells, Fischer's Exact Test was applied. Patient's cumulative life span following the date of surgery at our institution were analyzed using Kaplan Meier method and the evaluation of difference survival existence between groups through log rank test The prognostic factors for predictor of survival in high grade glioma patients were determine using Cox Proportional Hazards Regression Model. The significant value was set at *p* value less than 0.05.

Results

Seventy four High grade glioma patients treated in Hospital Sungai Buloh between January 2008 and 31st December 2014 were studied. Significant survival advantage was observed in fluorescence guided group compared with conventional group (12 versus 8 months), $p<0.02$. Even without adjuvant therapy, HGG patients from FG group survived longer than those from conventional group with a documented median survival of 8.0 months versus 3 months ($p = 0.006$). No significant differences of postoperative Karnofsky performance scale (KPS) between groups. At 6 week and 6 months postoperative, FG patients in the study did not succumb into worsening of functional outcome as compared to conventional group. Patient in both group also showed better KPS scores in the short term and long term follow up period. Multivariate analysis identified four independent predictors of survival: KPS>80 ($p=0.01$), histology ($p<0.001$), surgical method ($p<0.001$) and adjuvant therapy ($p<0.001$). Multiple logistic regression analysis showed only three predictors of survival: tumor located in non-eloquent or near eloquent area ($p=0.092$), surgical method (0.087) and adjuvant therapy (0.094). Although FG patients did not demonstrate significant influence of extent of surgical resection on patients' survival and functional outcome, patients with GTR/ NTR appeared to survive longer than STR (10.0 months vs 8.0 months).

Conclusions

This study showed a significant clinical benefit for HGG patients in terms of overall survival using FG surgery without resulting in worsening of postoperative function outcome when compared with conventional surgical method. Besides, four independent predictors of survival were also identified using multivariate analysis in the present study:

Karnofsky performance scale (KPS), tumor grade, surgical method and adjuvant therapy. Whereas, the predictor of survival in multiple logistic regression analysis only include tumor located in non-eloquent area, surgical method and adjuvant radio-chemotherapy. Even though, we were unable to establish a statistically significant finding in term of the effect of extent of surgical resection, the results obtained could be due to a limited number of patients that had early postoperative magnetic resonance imaging (MRI). This we hope will generate interest among future researchers to carry out multi-centered randomized trials which can analyze larger amounts of patients to further draw a concrete conclusion to support our claim. We hope that this surgical method can be introduced to other public hospital in Malaysia thus improve the clinical outcome of high grade glioma (HGG) patients.

FLUORESCENCE GUIDED VERSUS CONVENTIONAL SURGICAL RESECTION OF HIGH GRADE GLIOMA: A SINGLE CENTRE 7 YEAR COMPARATIVE EFFECTIVENESS STUDY

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Introduction: High grade gliomas (HGGs) are locally invasive brain tumors that carry dismal prognosis. According to National Comprehensive Cancer Network (NCCN) Guideline, the current standard management of HGGs includes maximum safe microsurgery resection followed by chemotherapy and radiotherapy. Although complete resection increases median survival, difficulty to demonstrate the tumor border reliably intraoperatively is a norm. Department of Neurosurgery Hospital Sungai Buloh has adopted fluorescence guided (FG) surgery using 5-aminolevulinic acid (5-ALA) as the first public hospital in Malaysia to overcome this shortcoming.

Objectives: The aims of this study were to evaluate the effect of FG surgery on overall survival and functional outcome as compared to conventional surgery. It also aimed to identify the significant independent predictors of survival in HGGs patients. Lastly, the effect of the extent of surgical resection on overall survival and functional outcome in FG patients was also evaluated.

Patients and Methods: This study was a retrospective observational study. 74 patients with histologically proven high grade gliomas (HGGs) were recruited between January 2008 and December 2014. 37 HGG patients that underwent FG surgery from January 2010 until 31st December 2014 and fulfilled the inclusion criteria were recruited. Another 37 patients from conventional group was recruited consecutively from January 2008 until 31st December 2014 as

the comparison group. The follow-up periods were done according to the scheduled appointment date (mostly at 6 weeks and 6 months) from the date of operation, which ended on 30rd June 2015. Statistical Package for Social Sciences (SPSS) software version 22.0 was used in the statistical analysis.

Results: Significant longer survivals were observed in FG group compared with conventional group (12 months versus 8 months), $p<0.02$. Even without adjuvant therapies, HGG patients from FG group survived longer than those from conventional group (8.0 months versus 3 months), $p = 0.006$. No significant differences of postoperative Karnofsky performance scale (KPS) between groups at 6 weeks and 6 months after surgery. Multivariate analysis identified four independent predictors of survival: KPS>80 ($p=0.01$), histology ($p<0.001$), surgical method ($p<0.001$) and adjuvant therapy ($p<0.001$). Multiple logistic regression analysis showed only three predictors of survival: tumor located in non-eloquent or near eloquent area ($p=0.092$), surgical method (0.087) and adjuvant therapy (0.094). Although FG patients did not demonstrate significant influence of extent of surgical resection on patients' survival and functional outcome, patients with GTR/ NTR appeared to survive longer than STR (10.0 months vs 8.0 months).

Conclusion: This study showed a significant clinical benefit for HGG patients in terms of overall survival using FG surgery without resulting in worsening of postoperative function outcome when compared with conventional surgical method. However, the results might not be conclusive as this is a single centre study with small sample size. We advocate a further multi-centered, randomized control trial to support these findings before FG surgery being implemented as a standard surgical treatment for all HGG patients in local practice.

Dr Azmin Kass Rosman: Supervisor
Dr Marymol Koshy: Co-supervisor

1. INTRODUCTION

High grade gliomas (HGGs) are the most common and deadly adult primary intrinsic brain tumors that account about for about 2% of all cancers (Louis *et al.*, 2007; Kohler *et al.*, 2011). In the US, the annual incidence is about 5/100,000 individuals with a peak incidence being in the fifth and sixth decades of life (Dolecek *et al.*, 2012). Estimated number of new cases using the world standard population of primary malignant central nervous system tumor in 2008, was 3.8 per 100,000 in males and 3.1 per 100,000 in females (Ferlay *et al.*, 2010).

National Cancer Registry (NCR) recorded 471 cases of brain, nervous system cancer in Peninsular Malaysia in 2007. The age standardized incidence rate (ASR) was 2.3 and 1.9 per 100,000 male and female respectively, based on the National Cancer Registry of Malaysia data (Zainal Ariffin and Nor Saleha, 2011). HGGs were the most common histological type of primary brain tumor accounted for 34.6 % (262) of all nervous system tumors over the period between 2003 and 2005 (Chye *et al.*, 2008).

HGGs are more common among men compared to women (Wen and Kesari, 2008). The median age of patients at the time of diagnosis is 64 years in the case of glioblastoma multiforme (GBM) and 45years in the case of anaplastic gliomas (Dolecek *et al.*, 2012). These tumors are characterized by their rapid proliferation, marked infiltration and dismal prognosis (Iacob and Dinca, 2009). They are associated with tremendous morbidity and eventually nearly all the patients will experience recurrence and die of disease progression.

HGGs account for about 60-75% of all gliomas. According to World Health Organization (WHO), HGG tumors are grade III and IV tumors. Grade III tumors are diffusely infiltrate neoplasms with marked proliferative potential, high cellularity, nuclear atypia and increased mitotic activity. The grade III tumors consist of anaplastic astrocytoma (AA), anaplastic oligodedroglioma (AO) and mixed anaplastic oligoastrocytoma (AOA). Besides nuclear atypia and high mitotic activity, the pathognomonic features of Grade IV GBM are microvascular proliferation and/ or necrosis (Louis *et al.*, 2007).

Currently, they are no prevention strategies for HGGs. The risk factors that may predispose to development of brain tumors include chemical carcinogens, infection, radiation or virus (Ohgaki and Kleihues, 2005). However, only ionizing radiation exposure had been established to be the causal among the studied risk factors (Baritchii *et al.*, 2015). Genetic syndrome, such as Li-Fraumi is also frequently associated with the development of HGG (Ohgaki and Kleihues, 2005).

Clinical manifestations of HGGs are determined by several factors, including tumor size, rate of growth and location. Tumoral mass tends to compress the neural pathways and cause distortion of brain structures (Pang *et al.*, 2007). Patients can present with focal neurological symptoms that reflects the location of tumor within brain parenchymal or just symptoms of raised intracranial pressure (Yuile *et al.*, 2006). In the late phase of one cohort of HGG patients, drowsiness, dysphagia, progressive neurological deficit and seizure are the most common symptoms. In nearly half of the patients, seizures occurred in their end-of-life phase and more specifically in the week before dying in one-third of the patients (Sizoo *et al.*, 2010).

One and two-year survival rates of high grade glioma patients are only 53.7% and 14.6% despite the understanding of several efforts on surgical technique, oncological therapies and molecular aspects of the tumor (Shinoda *et al.*, 2001). Median survival for GBM 12 months and 22 months in AA despite combination of surgery, chemoradiation (Ahmed *et al.*, 2014). Even there are ongoing trials, deaths can occur inevitably from either recurrent or the progression of disease.

Understand the factors that contribute to patients' prognosis is important to direct subsequent treatment strategies Specific tumor characteristic, clinical and therapeutic factors that influence survival of HGG patients has been studied. Studies consistently identified that age, functional status, tumor grade and extent of tumor resection are among the significant prognostic factors influencing survival (Buckner, 2003; Laws *et al.*, 2003). However, sex and tumor location were not an independent prognostic factor for progression-free survival (Ahmadloo *et al.*, 2013).

Among factors affecting patient's outcome, the extent of surgical removal has been proved to have a great impact (Sanai *et al.*, 2011; Stummer *et al.*, 2011b). A retrospective study showed that extended resection significantly prolonged both progression-free survival and overall survival (Keles *et al.*, 2006; De Bonis *et al.*, 2013). Increasing extent of resection was associated with improved survival independent of age, degree of disability, WHO grade or subsequent treatment modalities (McGirt *et al.*, 2009a).

In a multivariate analysis, resection greater than 98% in HGG patients was significantly associated with improved survival from 8.8 to 13.4 months (Lacroix *et al.*, 2001) . Sanai et

al demonstrated subtotal resection as low as 78% also correspond to a survival benefit and this trend continues even at the highest level of resection (Sanai *et al.*, 2011).

However, recently attention has also been paid to the influence of more extensive surgery on increase impairment of neurological function and reduces quality of life. The idea of completing excision without postoperative neurological deterioration remains a formidable challenge. McGirt et al found that extended resection heightens the risk of neurological deficit, which in turn leads to deterioration in quality of life (McGirt *et al.*, 2009b).

Several large series, however have demonstrated no difference in new postoperative deficit between patient undergoing gross total resection (GTR) vs subtotal resection (STR) (Stummer *et al.*, 2008; McGirt *et al.*, 2009a; Sanai *et al.*, 2011). Brown et al showed improvement of overall quality of life at 2- and 4-month follow up in patients with gross total glioma resection (Brown *et al.*, 2005). Ammirati et al also found that better neurological performance scores with an improvement of mean score of 6.8 in the completely resected group (Ammirati *et al.*, 1987).

Tumor recurrence typically occurs close to the resection bed, where there is an increased tumor cell density along the tumor margin (Pang *et al.*, 2007). Tumoral cells at the periphery of the tumor are more migratory and resistant to apoptosis, thus contributes to treatment resistance and relapse. Beyond the periphery, there is a sharp fall off in cell numbers as the distance from the resection cavity increases (Giese *et al.*, 2003). It is therefore hypothesized that local recurrence arises from resectable tumor left behind following an operation (Liang *et al.*, 1991). Aggressive resection thus would help prolong

progression free and overall survival by decreasing tumor burden and possibly increasing adjuvant therapy efficacy (Stummer *et al.*, 2011b).

Management of HGG remains very challenging and difficult as none are curative. Ongoing clinical trials include focusing on understanding the molecular mechanism and gene mutation aim to provide a promising individual approach. The best currently available multimodal treatment approaches of HGGs include surgical excision to the extent feasible, followed by external-beam radiation and concomitant temozolamide (TMZ) chemotherapy followed by an additional 6 cycle of TMZ administration (Stupp *et al.*, 2009).

A trial by the European Organization for Research and Treatment of Cancer (EORTC) showed that overall survival was 2 months higher with concomitant adjuvant radiochemotherapy followed by adjuvant temozolamide than with radiotherapy alone (Stupp *et al.*, 2005). More aggressive resection eliminates more tumor cells and thereby enhances the efficacy of subsequent adjuvant therapies. (Sanai and Berger, 2008; Stummer *et al.*, 2011b).

Based on National Comprehensive Cancer Network (NCCN) guidelines 2015, standard treatment of HGGs includes microsurgery resection followed by radio-chemotherapy (National Comprehensive Cancer Network) . Unfortunately, resection of HGGs is limited by the difficulty in discerning marginal, enhancing tumor intra-operatively. The invasive and infiltrative nature of HGGs often makes complete resection difficult (Jovcevska *et al.*, 2013). Achieving complete resection is challenging under conventional white light microsurgical techniques and only achieved in 23% to 36% of all HGG patients. Thus,

continuous refinements of surgical approaches and techniques, thus are investigated. Techniques that optimize intraoperative tumor identification and resection are needed.

Recently, 5-aminolevulinic acid (5-ALA), also known as Gliolan has emerged as a new surgical adjuvant used to identify glioma tissue under blue light. Introduction of this fluorescence-guided surgery in the mid 1990 provide an advanced method to visualize tumor at the cellular level. 5-ALA is a natural biochemical precursor that elicits synthesis and accumulation of fluorescent protoporphyrin in all epithelial and cancerous tissues. The concentration of fluorophore protoporphyrin IX (PPIX) is elevated in neoplastic cells, such as HGGs due to deficiency of ferrochelatase enzyme. Intra-operatively, under white light, no fluorescence is visible while shifting at violet-blue illumination tumor tissues appear red-light fluorescence (Puppa *et al.*, 2014).

The strongest evidence of extent of resection (EOR) improving outcome in HGGs comes from a randomized phase 3 trial of 5-ALA published in 2006 by the ALA-Glioma group study (Stummer *et al.*, 2006). The patient undergoes fluorescence-guided surgery had showed a 29% increase in the complete resection rate as opposed to the white light group. The 5-ALA group also had a higher 6-month progression free survival than the white light group (41% vs 21%) (Stummer *et al.*, 2006). Gross total resection was also achieved in 64% of the surgeries with preservation of all functional area and fiber tract (Feigl *et al.*, 2010).

Postoperative early Magnetic resonance imaging (MRI) is the only method of objectively assessing the extent of resection. It is recommended that MRI should be performed in the

first 3 days after surgery before non-neoplastic contrast enhancement due to surgical manipulation becomes radiologically apparent (Albert *et al.*, 1994). Determination of the amount of residual tumor is important to determine prognosis, direct further therapy and assess the response to therapy (Forsyth *et al.*, 1997). However, surgical outcome assessment via postoperative MRI is not a common practice procedure due to the limited MRI facility in Malaysia.

Albert, et al demonstrated that the opinion of the surgeon at the time of surgery is remarkably unreliable, with only a 30% correlation with MRI(Albert *et al.*, 1994) . Therefore, MRI postoperatively is urgently needed to assess the extent ad success of surgery. Keles et al in 2006 found that the amount of residual tumor correlated with time to progression and overall survival with the most significant cutoff residual tumor volume was $10cm^3$ (Keles *et al.*, 2006). A great association between survival and recurrence with residual volume (RV) of $5cm^3$ is proven in a recent study (Chaichana *et al.*, 2013).

As shown in previous studies, the decrease in tumor mass is directly proportionate to the increase in effectiveness of combined adjuvant radio-chemotherapy (Stupp *et al.*, 2009). Evidence shows that increased radicality in tumor resection without incurring postoperative neurological deterioration has a positive impact on survival as well as maintaining a better quality of life (Vecht *et al.*, 1990; Stummer *et al.*, 2011a).

Despite the therapeutic strategies to treat HGGs are evolving rapidly, Malaysia still failed to demonstrate the expected standard HGGs management according to National Comprehensive Cancer Network Guideline. Department of Neurosurgery, Hospital Sungai

Buloh has adopted the new surgical adjunct, 5-ALA as the first public centre in Malaysia since 2010. This study investigated the effect of FG tumor resection using 5-ALA on patients overall survival and functional outcome as compared to conventional method. We also analyzed the significant predictors of survival in HGG patients. This study is done with the aim, to further establish the hypothesis that FG surgery does improve patient survival without causing worsening neurological deficit. We hope that this surgical method can be introduced to other public hospital in Malaysia and therefore improve patients' overall survival.

In Malaysia, due to the limitation of MRI facility, although postoperative plain computed tomography (CT) brain is not a standard imaging tool, it was routinely performed after surgery. The aim was to ensure no bleeding in the area of interest that warranted immediate intervention. The study highlights the importance of early postoperative MRI within 72 hours as a standard imaging tool as stated in NCCN guideline 2015. It helps to assess the extent of tumor resection, determine the prognosis of HGG patients and direct further therapy.

2. LITHERATURE REVIEW

2.1 CLASSIFICATION OF GLIOMA

Gliomas represent a group of low-grade and high grade brain tumors that originate from glia (from the Greek for 'glue'), the brain tissue that was traditionally viewed as providing support functions to neural cells such as nutrients, oxygen, mechanical support, guidance in development, immune functions and waste disposal (Van Meir *et al.*, 2010). The glia supporting tissue includes astrocytes, oligodendrocyte or ependymal cells (Louis *et al.*, 2007).

WHO classified the glial tumors into four prognostic grades based on the histological features: grade I (pilocystic astrocytoma), grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma) and grade IV (glioblastoma multiforme) (Louis *et al.*, 2007). Grade III and grade IV tumor are commonly referred to as high grade or malignant glioma.

They have an intrinsic tendency to differentiate along the astrocytic lineage, become more malignant over time and can be viewed as a continuum along an axis of increasing malignancy (Louis *et al.*, 2007) . Histological features that affect the prognosis include cellularity, nuclear atypia, mitotic activity, necrosis and microvascular proliferation. WHO grade II tumors have nuclear atypia alone. Grade III actrocytomas will display mitotic activity with nuclear atypia, whereas GBMs show nuclear atypia, mitoses, microvascular proliferation and/ or necrosis (Louis *et al.*, 2007).

Secondary GBM develops from grade II or III astrocytomas, whereas primary or de novo GBM occur without prior history of less malignant lesion. Amplification and / or over

expression of the epidermal growth factor receptor and murine double minute 2 (MDM2) gene are important in the development of primary GBM. Early mutation of TP53, over expression platelet derived growth factor leads to the development of secondary GBM (Van Meir *et al.*, 2010) .

2.2 GENDER

It is well known that men represent a higher proportion of HGG sufferers than women (Wen and Kesari, 2008; Ferlay *et al.*, 2010). Study done by Casaetelli et al showed that no association was found between gender and overall survival (Casartelli *et al.*, 2009). Chen et al in his study demonstrated that a significant correlation between female gender and overall survival on univariate analysis (Chen *et al.*, 2015). The longer survival in female patients had better survival compared to male may be due to the presence of hormones or tumor suppressor genes on the X chromosome (Seki *et al.*, 2002) .

2.3 PRESENTATION

HGGs produce symptoms by a combination of focal neurological deficits from compression and infiltration of the surrounding brain parenchymal and raised intracranial pressure. Thus, patients can present with a variety of symptoms, including headache, progressive sensorimotor neurologic deficits, cognitive changes or seizure (Wen and Kesari, 2008). Headache occurs in approximately 50% of HGG patients is usually diffuse but can be localized to the same side of the head (DeAngelis, 2001).

Unlike slow growing glioma, seizures affect approximately 25-60% of patients with HGGs (Chaichana *et al.*, 2009). Okumus et al found that seizure is a significant prognosis

factor for survival in uni- and multivariate analyses (Okumus *et al.*, 2012). Surgical removal of tumors improves intracranial pressure condition and often reduces epileptic seizures. Tumor-related epilepsy affects patient's quality of life significantly, causes cognitive deterioration and result in significant morbidity (Chaichana *et al.*, 2009).

2.4 FACTORS INFLUENCING OUTCOME

Several potential prognostic factors were analyzed to establish their effect on overall survival rate in high grade glioma patients. Table 2.1 showed the significant prognostic factors that influencing survival in the reported studies.

Table 2.1 Predictor factors of survival in patients with high grade glioma

Study	Patients	Patient variables							
		Age	KPS	Histology	Primary site	Tumor size	Extent of resection	Radio-therapy	Chemo-therapy
Ahmadloo et al. (2013)	233	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes
Ekici et al. (2013)	98	Yes	Yes	NA	NA	NA	NA	NA	Yes
Hilmani et al. (2014)	89	Yes	Yes	NA	NA	NA	NA	Yes	NA
Lacroix et al. (2001)	416	Yes	Yes	Yes	NA	NA	Yes	NA	NA
Li et al. (2009)	116	Yes	Yes	NA	Yes	Yes	Yes	NA	Yes
Salminen et al. (1996)	106	Yes	NA	Yes	NA	NA	NA	Yes	NA
Zinn et al. (2013)	21,783	Yes	NA	NA	NA	NA	Yes	Yes	NA

2.4.1 AGE

The median age of patients at the time of diagnosis is 64 years in the case of GBM and 45years in the case of anaplastic gliomas (Dolecek *et al.*, 2012). Age at diagnosis is

associated with survival in glioma patients (Lacroix *et al.*, 2001; Ekici *et al.*, 2013). Patient's age is inversely proportional to the period of overall survival. There is significant evidence between patient's younger age and longer survival times. (Casartelli *et al.*, 2009). Aggressive therapies administered to older patients are less effective, whereby they prolong survival time younger patients (Helseth *et al.*, 2010). Thus, mortality risk increases as the age increases.

2.4.2 KPS

Patient's functional status is an overall accepted independent prognostic factor in HGG patients. Various scales for assessment of patient's performance have been developed and scoring system developed by Karnofsky is frequently used in the literature to assess patient's performance status (Schag *et al.*, 1984) . Karnofsky Performance Scale (KPS) is a well documented prognostic factor for survival and patient with an elevated KPS score have more favorable results (Lacroix *et al.*, 2001; Li *et al.*, 2009). Studies consistently demonstrated patients with a KPS score >70 have a better prognosis (Stark *et al.*, 2010b; Ahmadloo *et al.*, 2013)

The preoperative function class was assessed at admission before surgery, and the postoperative function class was the performance status approximately 4-6 weeks after discharged. In the series of Vecht et al, no significant difference was found in the incidence of worsening neurological function following GTR compared to limited resection (Vecht *et al.*, 1990). C. Abrudan et al however, found that a global improvement of neurological status was seen in >85% of the GTR patients (Abrudan *et al.*).

2.4.3 PRIMARY SITE

Gliomas commonly found in the cerebral hemispheres. Li et al reported that frontal lobe involvement is a statistically significant marker of delayed progression of postoperative disease (Li *et al.*, 2009). An early retrospective by Simpson, et al stated also the frontal lobe location offered a survival benefit (Simpson *et al.*, 1993). The effect of extensive resection on survival may also be partially affected by the tumor location. Deep seated tumors are often times less resection and therefore more prone to poorer outcomes.

2.4.4 FUNCTIONAL LOCALIZATION

Preoperative tumor functional localization and surgical accessibility are important in determining the feasibility of tumor resection (McGirt *et al.*, 2009b; Feigl *et al.*, 2010). A functional grading system (Friedlein grading A/B) was proposed (Friedlein *et al.*, 2015) . Friedlein A (FGA) was characterized by malignant glioma in a functionally silent area or near the functionally eloquent area (<8mm) whereas Friedlein B (FGB) was characterized by suspected malignant glioma in the functionally eloquent area.

Another high grade glioma grading system according functional localization was proposed by Sawaya, et al (Sawaya *et al.*, 1998). Sawaya I comprises a tumor that located in functionally silent areas of the brain (non-eloquent brain) such as frontal or temporal pole, right parieto-occipital lobe and cerebellar hemisphere. Sawaya II comprises a tumor that located adjacent to eloquent brain area such as near motor cortex, sensory cortex, calcarine fissure, speech center, corpus callosum, dentate nucleus and brainstem. Sawaya III comprises a tumor that located in functionally eloquent area such as motor or sensory

cortex, visual or speech center, internal capsule, basal ganglia, thalamus, hypothalamus, dentate nucleus and brainstem.

2.4.5 LATERALITY

Studies have shown the laterality of high grade glioma (dominant or non-dominant hemisphere) is not an independent predictor of survival or functional outcome (Polin *et al.*, 2005; Chen *et al.*, 2015).

2.4.6 HISTOLOGY

Histopathological diagnoses indicated HGGs according to WHO standards. A recursive partitioning analysis of three Radiation Therapy Oncology Group trials showed that among patients less than 50 years of age, astrocytoma with anaplastic and atypical foci was associated with significantly improved survival compared with glioblastoma. The tumor grade were consistently identified as an independent prognostic that influencing survival (Salminen *et al.*, 1996; Lacroix *et al.*, 2001). Presence of necrosis within the tumor is considered crucial, showing the aggressiveness of the cells and they outgrow the blood supply, regardless their angiogenic potential). According to the latest WHO classification, GBM can be divided into various subtypes. Primary GBMs, arise de novo and occur mostly in older age groups, with shorter survival time and more frequently compared to secondary GBMs that progress from astrocytoma of low grade (Louis *et al.*, 2007).

2.4.7 TUMOR VOLUME

Li et al revealed that tumor maximal diameter (TMD) was a significant factor related to overall survival in the univariate analysis (Li *et al.*, 2009; Ahmadloo *et al.*, 2013) . Amirjamshidi et al in his study demonstrated that patients with more voluminous tumor masses had the worst prognosis in univariate analysis ($p<0.007$), but lost its power in the final analysis. (Amirjamshidi *et al.*, 2010).

2.4.8 EXTENT OF RESECTION

Traditionally, surgical resection has been considered an option for relatively few patients with HGGs due to poorly defined borders of these infiltrative lesions. Besides achieving cytoreduction, tumor resection relieved symptoms of the patient and provides a conclusive pathologic diagnosis.

Recent studies have documented the importance of surgical resection as one of the important independent predictors of survival (McGirt *et al.*, 2009a; Stupp *et al.*, 2009). Thus, the aim of surgery is to maximize the extent of tumor resection while minimizing the surgical insult to the normal, viable brain parenchymal.

However, there is no consensus regarding the definition of complete tumor resection in the literature. Many author used the term of ‘complete’ (Stummer *et al.*, 2008), ‘gross total’(Hardesty and Sanai, 2012) or ‘more than 98% tumor’ resection (Lacroix *et al.*, 2001) according to post-surgical residual. Extent of excision was defined as gross total (>98% of the tumor removed), near total (>90% of the tumor removed), subtotal (<90% of the tumor removed) (Gulati *et al.*, 2011).

It is now level 2b (Oxford centre for evidence-based medicine) showing that gross total resection (GTR) provides a significant survival advantage as compared to incomplete resection of contrast-enhancing tumor (Stummer *et al.,* 2008). A complete resection of all gadolinium enhancing tumor significantly improves the survival effect of adjuvant therapy and chemotherapy (Stupp *et al.*, 2009). There is now class II evidence confirming the value of maximal cytoreductive surgery and synergistic effect between aggressive surgery and chemoradiation.

GTR also has been associated with several other benefits. By increasing the amount of tissue available for pathologic examination, GTR may help to improve diagnostic accuracy. It helps to improve seizure control and make it possible to administer local therapies like BCNU wafers. Patients are likely to require less steroid treatment, which reduces the risk of several steroid-related adverse events, including cushingoid habitus, steroid myopathy, peripheral edema and hyperglycemia (Olivi *et al.*, 2010).

2.4.9 RADIOTHERAPY

Postoperative adjuvant radiotherapy had been of key importance to the treatment of HGGs for decades. External beam radian treatment (XRT) is usually recommended to start within 2-4 weeks following surgical resection. The patients received a median dose of 54Gy (range 40-60) Gy, delivered 5 days per week in fractions of 1.8-2.0Gy with a curative intent (Ahmadloo *et al.*, 2013)

The addition of radiotherapy to surgery increases survival among patient with GBM ranged from 3 months to a range of 7-12 months (Stupp *et al.*, 2005). Besides, it was also obvious that radiotherapy without surgery resulted in consistently better survival than

those who were treated by surgery alone (Zinn *et al.*, 2013; Hilmani *et al.*, 2014). This great clinical significance, especially benefits the elderly or patient with surgical contraindication.

2.4.10 CHEMOTHERAPY

Chemotherapy is assuming an increasingly important role in the treatment of HGGs. Before 1999, nitrosourea-based agents (carmustine and lomustine) were the most commonly used adjuvant chemotherapeutic agent in HGG patients (Adamson *et al.*, 2009). However, patients did not show any improvement in term of response rate and overall survival.

Temozolamide (TMZ) is an orally available, imidazotetrazine-derived second-generation, methylating agent with simple, well tolerated and a favorable toxicity profile. It readily crosses blood-brain barrier to achieve a cerebrospinal fluid concentration that is approximately 40% of plasma. The most common adverse effect is hematologic toxicity (leucopenia and thrombocytopenia) (Mutter and Stupp, 2006).

This chemotherapeutic agent was administered concurrently with radiotherapy with a dose of 75mg/m^2 daily throughout the radiation course followed by 4-6 cycles of adjuvant temozolamide with a dose of 150-200mg/m^2 daily for 5 days, every 4 weeks (Ahmadloo *et al.*, 2013)

In a Phase III randomized study (EORTC 26981/22981-NCIC CE3), concomitant administration of temozolamide with radiotherapy increased median overall survival to 14.6 months compared with 12.1 months for radiotherapy alone (Stupp *et al.*, 2005). The

study also showed improvement in the 2-year survival rate from 10% to 26% (Stupp *et al.*, 2005). The overall survival in the TMZ arm was 9.8% in a 5-year analysis of the EORTC-NCIC trial that was published in 2009 (Stupp *et al.*, 2009). Therefore, TMZ administered concurrently during and after XRT has become a new standard-of-care treatment.

2.5 NEUROIMAGING

MRI with contrast is the most sensitive and specific study. CT may miss small tumors that are not enhanced with contrast agent. Anaplastic astrocytoma usually exhibit hypointense T1 and hyperintense T2-weighted signal abnormality that may include various degrees of contrast enhancement and edema. Whereas, GBM shows a typically heterogeneously irregular ring-nodular enhancing mass with central necrosis and surrounding vasogenic edema (Sathornsumetee *et al.*, 2007). Differential diagnoses for HGG include abscess, metastasis or primary CNS lymphoma (Behin *et al.*, 2003).

Contrast enhancement on MRI due to extravasation from tumor vasculature that lacks of endothelial tight junction of which is a well-known characteristic of HGGs (Scott *et al.*, 2002). Wong et al shows that higher maximum gadolinium uptake rate were associated with shorter survival in evaluating HGG (Wong *et al.*, 1998). GBM frequently has central necrosis and more extensive peritumoral edema than that associated with anaplastic astrocytoma. Absence of necrosis on imaging studies is an important prognostically favorable variable. A low density necrotic area within glioma is believed to indicate rapid growth and malignant behavior (Lacroix *et al.*, 2001).

CT immediate postoperative was obtained from each patient to diagnose asymptomatic complication that might be related to surgery. The extent of surgical resection was

determined by neuroradiologist through T1MRI images with contrast, which were obtained during the first 72hours postoperatively. Early postoperative MRI can determine the amount of residual tumor and also detect the surgical complication as early as possible. Besides, it is to avoid confounding the interpretation of images by postoperative inflammation and disruption of blood brain barrier (Albert *et al.*, 1994). Thereafter, MRI was performed at 6 months to look for evidence of radiological progression.

2.6 CONVENTIONAL RESECTION

Surgery has become the cornerstone in the initial management of HGGs. Besides establishing a histopathological diagnosis, the aim of surgery is to maximize tumor resection while sparing the normal functioning brain parenchymal. Conventional neurosurgical technologies for tumor resection involve the use to image guided system that provides an intraoperative guidance to the surgeon. The system was designed as such by using the pre-operative MR image, relates the location of surgical tools that tracked intraoperatively to the preoperative images and guides the surgeon throughout the patient's anatomy.

However, intraoperative brain shift and deformation after CSF drainage and bone removal had resulted in the inaccuracy of the image-guided system. The wisest approach for determining the completeness of HGG resection surgery is based entirely on the judgment of the surgeon. Clues such as tissue texture and discoloration, bleeding and vascularity of resection planes, and proximity to anatomic landmarks are used to judge the completeness of resection. The subjective nature of judging tumor margins based on gross cues yields a

highly inconsistent surgical outcome. Even in the experienced hands, the rate for complete resection of HGGs under white light vary between 13-20% (Albert *et al.*, 1994).

2.7 FLOURESCENCE-GUIDED SURGERY

2.7.1 General knowledge

5-aminolevulinic acid (5-ALA) is a natural biochemical precursor for the synthesis of heme in all mammalian cells (von Campe *et al.*, 2012). Both ALA and protoporphyrin IX (PpIX) are naturally present in the human body. As shown in Figure 2.1, ALA is formed in the mitochondrion from glycine and succinyl coenzyme A from Kreb cycle by the activity of enzyme ALA syhthase (ALAS). ALA then diffuses into cytosol and undergoes enzymatic modification through intermediate porphyrin before transported by adenosine nucleotide transporter back to the mitochondrion. The coproporphhyrinogen oxidase then act on the pprotoporphyrin towards heme production and inducing the synthesis of endogenous fluorescence molecule, protoporphyrin IX (PpIX) in the mitochondrion(Teng *et al.*, 2013). Ferrochelatase is responsible for converting the PpIX into non-fluorescent molecule, heme (Colditz *et al.*, 2012).

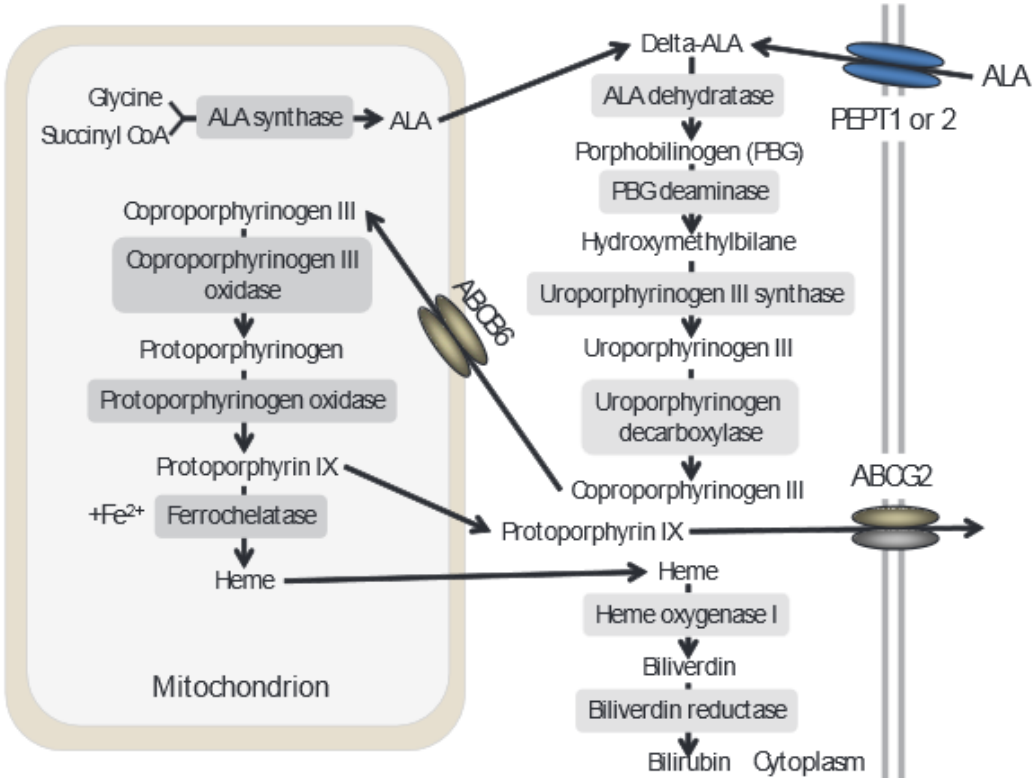

Figure 2.1: Porphyrin-heme biosynthetic pathway and putative mitochondrial transporters

There is a deficiency of ferrochelatase enzyme in neoplastic cells, such as those in HGGs. There is also association between high 5-ALA level and increased nitric oxide level via nitric oxide synthetase 2 upregulation. (Nokes *et al.*, 2013). Exogenous 5-ALA administration thus leads to increasing concentration of PpIX. Also the higher 5-ALA uptake by tumor cells can be due to disruption of blood brain barrier, increased neovascularization and overexpression of membrane transporter. (Hadjipanayis *et al.*, 2011). Accumulation of fluorophore PpIX in tumor cells will exhibit red-light fluorescence under blue light that looked relatively normal with white light.

2.7.2 Mechanism of action

5-ALA as drinking solution is rapidly and completely absorbed. After oral administration, PpIX plasma levels peaks at 2-6 hours, disappears by 12 hours and normal PpIX level is reached after 24hours (Teng *et al.,* 2013). It had a terminal half-life of 1-3 hours and excretion of PpIX is via hepatic metabolism and approximately 30% of administrated 5-ALA excreted unchanged in urine within 24 hours (Teng *et al.,* 2013). Thus, exposure of eye and skin to strong light sources (direct sunlight or brightly focused indoor light) should be avoided for 24 hours after administration of 5-ALA to prevent occurrence of photosensitivity (Colditz *et al.*, 2012).

2.7.3 Contraindication and side effect

Administrated of antacid, however should be avoided because the dye is easily decomposes in the presence of alkali. The drug should not be administered with other phototoxic substances such as tetracycline, sulfonamide or fluoroquinolones (Colditz *et al.*, 2012). The use of 5-ALA is also contraindicated in the case of porphyria, a genetic disease (Teng *et al.,* 2013). No trials have been performed in patients with clinically relevant hepatic or renal impairment and the safety and efficacy of 5-ALA in children have not yet been established. The most serious side effects include anemia, thrombocytopenia, leukocytosis, neurological disorders and thromboembolism especially when higher doses (>40g/kg body weight) are used (Panciani *et al.*, 2012).

2.7.4 5-ALA and clinical usage

The first paper detailing the use of 5-ALA for photodynamic diagnostic was in 1797(Malik and Djaldetti, 1979). Thereafter, clinical usage of 5-ALA was applied in

tumor originating in the epidermis, oral mucosa, bile ducts, urothelium, bronchi and others (D'HALLEWIN *et al.*, 2000; Guyon *et al.*, 2012). In 1998, it was approved by the Food and Drug administration (FDA) as a therapeutic drug for solar keratosis (Gold and Goldman, 2004).

2.7.5 5-ALA and Neurosurgery

Following the positive result of the phase III trial, 5-ALA has been granted by the European Medicines Evaluation Agency for the use in the European community in adult patients during surgery for HGG and named Gliolan. Patients were then treated according to the published protocol (Stummer *et al.*, 2006). The neurosurgeon is required to complete a certified FG surgery course before using Gliolan (Colditz *et al.*, 2012).

A 20mg/kg body weight of 5-ALA was administered orally in the presence of medical personnel 2-4 hours before induction of anesthesia. The solution was prepared by dissolving the content of one vial (1.5g) in 50ml of drinking water.

The patient was put on 4mg dexamethasone three times per day before the operation. All HGGs resections were done by consultant trained in the use of 5-ALA by using a microscope which enables switching from conventional white xenon illumination to violet-blue excitation light. PpIX will emit red light in the visible spectrum at 635 nm with a smaller peak at 704 nm to produce fluorescence after excited by blue light at a wavelength of 375 - 440 nm (Ishihara *et al.*, 2007). The operative microscope with a long pass filter only permits the passage of light greater than 440 nm. This then enable the surgeon to resect the red-violet tumor tissue in a gross total fashion.

Different fluorescent qualities were observed correlated to the tumor burden and WHO grades in HGG patients (Hefti *et al.*, 2010; Díez Valle *et al.*, 2011; Roberts *et al.*, 2011). Two types of fluorescence that were encountered during surgery include solid and vague fluorescence. According to Hefti et al, solid fluorescence indicates viable tumor tissue and thus appeared deep red, whereas vague fluorescence is the pink fluorescence that encountered between solid tumor and non-fluorescence blue brain tissue (Hefti *et al.*, 2010). Intraoperative fluorescence therefore is subjective and is mainly based on the judgment of the surgeon. Panciani et al found that the sensitivity of 5-ALA surgery was 91.1% with the documented specificity of 89.4% (Panciani *et al.*, 2012).

2.8 OVERALL SURVIVAL

Date of surgery was considered at the time of diagnosis. Overall survival was measured from the date of surgery to the date of death due to any cause, to the date of withdrawing from the study and to the final analysis. As an overall survival reflects a clinically meaningful benefit that can be objectively assessed, thus it is usually considered as the definitive end point for HGG patients.

A prospective clinical trial evaluating the utility of fluorescence-guided high grade glioma resection demonstrated a 4.9-month improvement in survival in patient undergoing complete excision compared to those with subtotal resection (Stummer *et al.*, 2011a). Whereas, the best adjuvant chemotherapeutic regimen for GBM improves survival 2.5 months (Stupp *et al.*, 2005).