Seizures in WHO grade 2 glioma: semiology and significance

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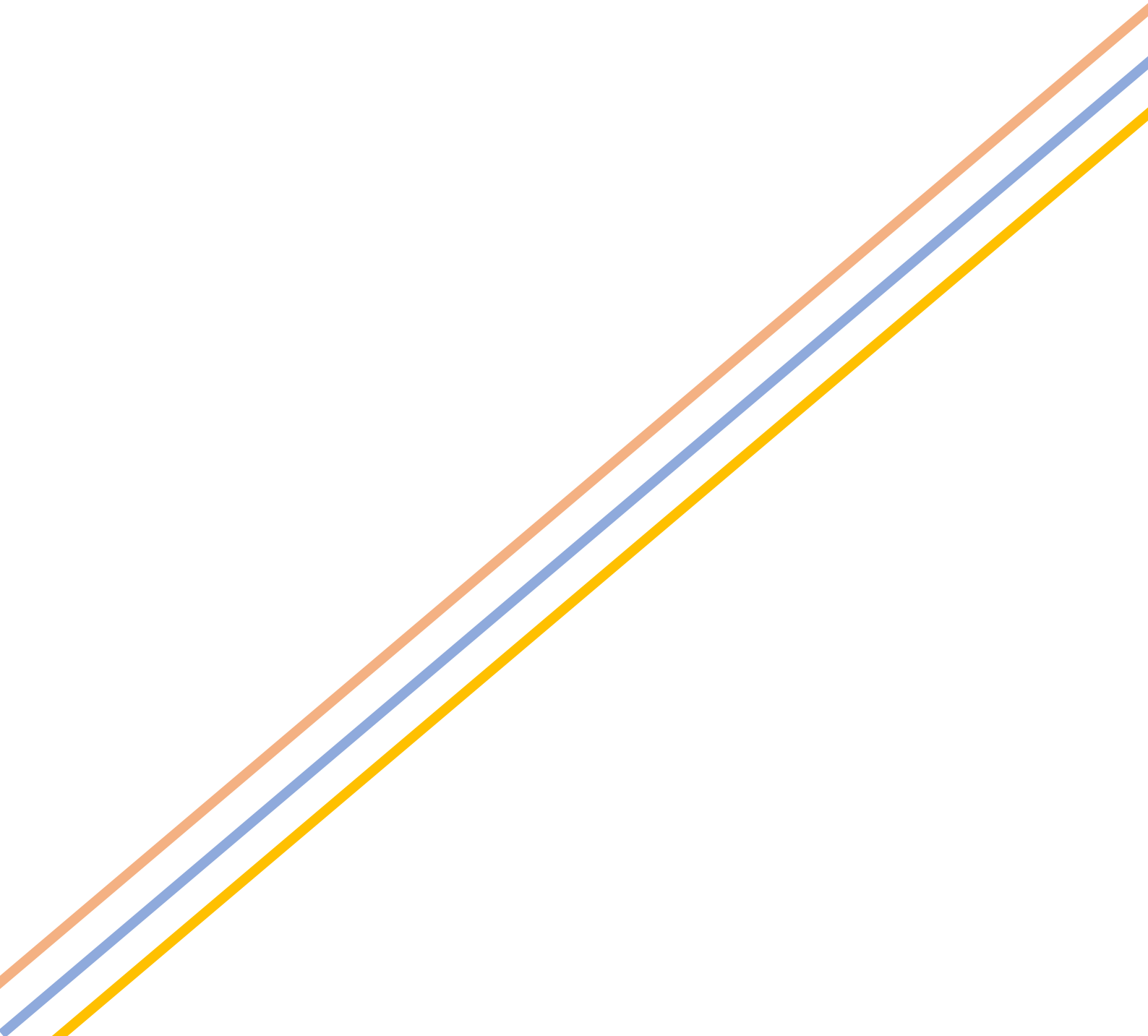

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Abstract

Background: WHO Grade 2 gliomas are a group of highly epileptogenic brain tumours. Seizures occur in the majority of patients and often at an early stage. How exactly these seizures are generated, their characteristics and their optimal management are not well understood. In addition, we also consider how experiencing seizures may inform tumour course and prognosis and how this may influence management. We review the existing literature before presenting a series of original research studies in an attempt to better understand this interplay and its clinical consequences.

Methods: Retrospective observational studies were performed on a patient cohort attending a low-grade glioma clinic at a large regional neurosciences hospital. Statistical methods employed predominantly included survival modelling with Kaplan-Meier plots and Cox-proportional hazard models as well as regression modelling.

Results: We demonstrate several novel relationships between gliomas and the seizures they generate in a clinical patient cohort. Experiencing predominantly motor seizures improved overall survival, despite controlling for known prognostic factors. Tumour laterality (left hemisphere) is also shown to significantly increase the risk of seizure generalisation, as is male sex. We show that levetiracetam is an effective first choice antiseizure medication in this patient population, though it does not appear to modulate MGMTp methylation, at least in grade 2 glioma.

Conclusions: We demonstrate that a complex relationship exists between grade 2 gliomas and the seizures they generate influencing their course and treatment. We outline further research directions via which an even greater understanding of these dynamics may be possible and allow advances in patient care.

Contents

List of abbreviations

2HG – 2-hydroxyglutarate

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ASM – Antiseizure medication

BBB – Blood-brain barrier

CA IX – Carbonic anhydrase IX

CI – Confidence interval

CT – Computerised tomography

DNET– Dysembryoplastic neuroepithelial tumours

EEG – Electroencephalogram

EPO – Erythropoetin

FwP – Focal without progression

FBT-C – Focal to bilateral tonic-clonic

HIF-1α – Hypoxia inducible factor-1 α

HR – Hazard ratio

IDH-1 – Isocitrate dehydrogenase 1

GABA – Gamma-aminobutyric acid

GTCS – Generalised tonic clonic seizure

ILAE – International League Against Epilepsy

KPS – Karnofsky Performance Status

LRT – Likelihood ratio test

MDT – Multi-disciplinary team

MGMT – O-6-Methylguanine-DNA methyltransferase

MR – Magnetic resonance

MRS – Magnetic resonance spectroscopy

NHS – National Health Service

PCV – Procarbazine, Lomustine and Vincristine

QALY – Quality adjusted life year

rCBV – Ratio of cerebral blood volume

RCT – Randomised controlled trial

SV2A – Synaptic vesicle protein 2A

TERT – Telomerase reverse transcriptase

TMS – Transcranial magnetic stimulation

VEGF – Vascular endothelial growth factor

WHO – World Health Organisation

Key findings

- Patients with predominantly focal to bilateral tonic clonic seizures are more likely to achieve seizure freedom at any point within 10 years of diagnosis compared to those with purely focal seizures (paper 1).
- Men with grade 2 glioma are more likely than women to be diagnosed with epilepsy and are more likely to experience at least one focal to bilateral tonic-clonic seizure (paper 2).
- Left hemisphere grade 2 gliomas were twice as likely to ever have resulted in a focal to bilateral tonic-clonic seizure compared with right hemisphere gliomas (paper 2).
- Experiencing predominantly motor seizures inferred an increased chance of survival at 10 years post diagnosis compared with patients with predominantly non-motor seizure semiology (paper 2).
- The use of levetiracetam as a first line antiseizure medication results in roughly one-third of patients reaching seizure freedom at 12 months, with over two-thirds having achieved seizure freedom at 24 months (paper 1).
- Levetiracetam was more effective at inducing seizure freedom at 2 years ($p=0.02$) compared with a cohort taking an alternative antiseizure medication (paper 1).
- Concurrent use of levetiracetam at the time of histological sampling is not associated with an increase in the mean methylation percentage of the MGMT promoter region (paper 3).

Introduction and background

Glioma epidemiology and grading

Gliomas are a heterogenous group of primary brain tumours arising from glial cell types. According to Cancer Research UK, there are over 12,000 people diagnosed with a primary brain tumour in the UK each year.[1] The World Health Organisation (WHO) categorise brain tumours by grade from 1 through to 4 using the 2021 classification. Grade 1 gliomas often exhibit benign behaviour, rarely transforming to a higher grade, with the most commonly occurring being neuroglial entities such as dysembryoplastic neuroepithelial tumours (DNET) and gangliogliomas. These tumours may be cured with surgery or, if a patients symptom burden is low or non-existent, managed with a watch and wait strategy. Although grade 2 gliomas are also often slow growing, frequently advancing over several years, they invariably infiltrate surrounding brain tissue meaning that curative resection is not thought possible. The most common grade 2 entities encountered in adults are diffuse astrocytoma and oligodendrogliomas. Tumours with a grading of either 1 or 2 are sometimes collectively referred to as ‘low-grade’ gliomas. Grade 2 gliomas eventually transform into ‘high-grade’ lesions be it either anaplastic astrocytomas/oligodendrogliomas (grade 3) or glioblastoma (grade 4). Sometimes the term ‘lower grade glioma’ is utilised to describe grade 2-3 gliomas.[2] The overall survival from diagnosis in grade 2 glioma is often several years.[3] This is in stark contrast to high grade tumours such as glioblastoma, where overall survival is almost universally very poor, averaging about 12-15 months despite treatment. Even within the same grade, survival can vary depending on the specific tumour subtype. In patients with an oligodendroglioma, 5-year survival may be around 80%, whereas in those with a diffuse astrocytoma survival at 5 years can dip as low as 47%.[4] Grade 2 gliomas are relatively

uncommon compared with high grade lesions, with three glioblastomas diagnosed per single astrocytoma or oligodendroglioma in most UK centres.[1,5]

The nature of grade 2 gliomas is often such that they are seemingly slow growing, but early on in their existence are able to subtly but widely invade beyond visible radiological boundaries, rendering surgery non-curable.[6] The growth rate of grade 2 gliomas (both before and after surgery) is usually slow and steady (radiologically measured) until malignant transformation to a higher grade takes place, at which point there is invariably a significant acceleration.[7]

Tumour genetics

Until the fourth edition of the WHO classification of gliomas in 2016 (with the updated fifth edition in 2021), gliomas were classified primarily according to their histological appearances.[8] However, the rapid rise of tumour genotyping has revolutionised tumour classification. Genetic mutations exhibited by the tumour (or lack thereof) are now the prime means by which tumours are classified making the classification an integrated molecular and histological diagnosis. Through the use of tumour molecular markers, some of the heterogeneity previously seen within the glioma population has been removed and overall survival is now better predicted by subtype. Previously, a significant portion of grade 2 gliomas were labelled as seemingly ambiguous subtypes such as ‘mixed oligoastrocytoma’ when the histology alone wasn’t clear. Using the 2016 and beyond WHO classifications this label is all but gone. Despite the official classification changes coming in 2016, many centres performed tumour genotyping as routine throughout the 2000’s and so gliomas can often be quite readily reclassified retrospectively. This ‘legacy grading’ is sometimes

not possible due to the lack of testing, as only certain tests may have been chosen based on histological appearances.

The isocitrate dehydrogenase 1 (IDH-1) mutation is one of the most important in glioma tumour genetics. Diffuse astrocytomas with the IDH-1 mutation show better overall survival than those returning as wild-type. They were previously considered grade 2 tumours and labelled as either IDH-1 mutated or wild-type. However, as of the 2021 update, IDH-1 wild-type diffuse astrocytomas are now denoted as high-grade (grade 4), such is the detrimental effect on overall survival and their differing biology. Codeletion of 1p/19q is a chromosomal alteration considered the hallmark of an oligodendroglioma and thus infers improved overall survival compared with an IDH-1 mutation alone.[9]

As well as informing prognostication, tumour genotyping plays a major role in determining best treatment, most notably in glioblastoma. The alkylating agent Temozolomide is the most commonly used chemotherapeutic agent in glioma. Alkylation at the *O6* position of guanine is a mutation that leads to cell death.[10] O-6-Methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that is able to reverse this alkylating process, thereby repairing the guanine nucleotide. Functioning MGMT protein may therefore temper the cytotoxic effects of temozolomide. . The MGMT promoter (MGMTp) region is the area to which transcription factors bind in order to initiate transcription. This region can become methylated, preventing transcription and silencing the gene. The degree of MGMT promoter methylation (MGMT methylation status) can be measured and expressed as a percentage. Glioblastomas displaying a ‘methylated’ MGMT status (commonly taken as a mean methylation >15%) have a favourable outcome compared to those that are unmethylated (<10% methylation).[11] Different assays can be used to

measure MGMTp methylation including pyrosequencing and methylation specific PCR amongst others.[12] These differing methods result in some uncertainty as to where the optimal cutoffs should be and variability between centres. In addition, a numerical methylation percentage is often not reported in some methods. The significance of MGMTp methylation status in grade 2 glioma is unclear as it is often associated with other positive genetic prognostic markers.[13] MGMT status is probably prognostic in wild-type astrocytoma, however, as discussed these are now to be considered more similar to glioblastoma in nature. Other genetic markers are often tested for in most routine glioma genetic panels, including mutations in telomerase reverse transcriptase (TERT), though these do not currently play a major role in classification and its relevance to prognosis is not well understood. P53 mutations are often found in glioblastoma.[14] They occur much less frequently in grade 2 gliomas and have previously been associated with astrocytic tumours.[15] Although the WHO 2021 classification lists a P53 mutation as a key diagnostic gene in diffuse IDH-1 mutated astrocytoma, in reality it is rarely of importance and is superseded by other genetic markers.

Glioma related seizures

Seizures may occur in all types of glioma, however, the proportion of patients with epilepsy varies by tumour grade. Between 30-60% of patients with high grade gliomas, such as glioblastoma, will experience seizures.[16] By comparison, 60-90% of patients with low grade gliomas will develop seizures.[17] Outside of glioma, seizures are experienced by about 20-30% of people with brain metastases whereas they are reported in about a third of those with pre-operative meningioma.[18] This pattern of

epilepsy incidence being higher in less aggressive gliomas continues within the low grade classification itself, with the most benign grade 1 entities, such as DNETs, reported as being associated with epilepsy in near 100% of patients (though it is debatable whether only the symptomatic DNETs are the ones picked up).[16] Many patients with low grade gliomas come to medical attention for the first time due to the occurrence of a seizure (unlike many other neurological diseases- whereby seizures are often a late presenting and poor prognostic sign). Seizures may be the presenting symptom in over 70% of patients with low grade gliomas.[19-21] Often, if patients do not have seizures at presentation they rarely go on to develop them later on in the disease course, again suggesting that if they are to occur they invariably occur early.[22] As would be expected (due to the lower seizure incidence) close to a quarter of patients with glioblastoma have seizures as the initial symptom.[23] Neuroimaging is almost always performed at some point during the diagnostic work up of new seizures and it is often via this imaging that a glioma is first diagnosed. All new seizures should be investigated with appropriate neuroimaging according to UK guidelines.[24]

A variety of seizure types may occur in glioma related epilepsy. The International League against Epilepsy (ILAE) classifies seizures based on their semiology.[25] Current practice is that they are first categorised by their onset (focal, generalised or unknown). Focal seizures can then be subdivided into whether awareness is retained (previously termed ‘simple partial seizures’) or lost (previously ‘complex partial seizures’) as well as whether the resulting seizure activity is motor or non-motor. Generalised seizures invariably involve loss of awareness but can also be split by activity into motor or non-motor (previously termed ‘absence seizures’). Due to the nature of glioma related epilepsy being ‘lesional’ it is likely that all glioma

related seizures are focal in onset. Some however progress so rapidly to generalised seizure activity they may appear generalised from the start to the observer due to imperceptible or unreported initial focal signs. Previous studies of glioma related epilepsy have used the term ‘generalised’ to describe the seizure onset of some patients. Throughout our work we have taken the view that these are ‘focal to bilateral onset seizures’ (previously termed ‘secondary generalised seizures’) and will use this term to describe them forthwith.

The impact of tumour location upon the incidence of seizures in grade 2 glioma is a difficult relationship to unpick. Although work has frequently been undertaken to evaluate whether tumours in a particular region are associated with a higher seizure incidence, the fact that gliomas are often large and spanning multiple lobes/structures makes this somewhat difficult. The majority of studies looking at the effect of tumour location on seizures report that low grade gliomas in practically all supratentorial cortical regions result in high rates of seizures.[26] Many of these studies simply denote tumour location by lobe, with tumours frequently being ‘involved’ in multiple lobes due to the often large and spreading nature of these lesions. More sophisticated approaches have been attempted, such as using voxel-based mapping to more accurately reflect tumour presence and volume in specific brain regions. This technique suggests, amongst other things, that tumours involving the left pre-motor area increase the risk of all seizure types, particularly seizures with focal to bilateral activity.[27]

A proportion of patients with glioma related epilepsy never achieve seizure freedom. Medically intractable epilepsy is usually defined as seizures that are not controlled by a minimum of two appropriately selected and well-tolerated antiseizure medications. Previous studies have identified temporal lobe lesions, a long period of time since

seizure onset and the presence of focal aware seizures as risk factors for uncontrolled epilepsy.[21] Tumours involving the insula may also be associated with seizures that are more likely to be refractory to treatment.[28] Ki-67 expression is commonly used as a marker of proliferation. The Ki-67 protein can be detected when a cell is in an active phase of cell division and is undetectable during the resting (G0) phase.[29] Cells are designated as either positive or negative (meaning they are either actively dividing or are not) and the result is usually given as a percentage range of the total cells in the sample. There may be a correlation between a higher Ki-67 percentage and poor post-operative seizure control in grade 2 glioma.[30]

Seizure generation in grade 2 glioma

The profound epileptogenic tendency seen in low grade compared with high grade glioma suggests a mechanism of seizure generation beyond that of local mass effect, hypoperfusion or deafferentation.[31] In terms of the tumour itself, it is broadly thought that the tumour margin is the critical region for seizure generation in low grade glioma.[32] These margins may include a mixture of extra-tumoural tissue as well as malignant cells. It is possible that this ‘integrative’ set-up is a contributing factor to the high epilepsy burden seen in grade 2 glioma (and its lower grade counterparts) compared with perhaps the more ‘destructive’ behaviour of tumours with a lower incidence of seizures. Patients are more likely to experience seizure freedom post-operatively the greater the extent of resection.[33] In some cases, where anatomy and function allow, the performed resection may be supra-marginal (i.e. beyond the radiologically defined boundary of the tumour).[34] Resection of as much of the tumour margin as possible may be critical in reducing post-operative seizure risk. Sub-marginal or partial resections may explain why some patients go on to continue

to experience recurrent seizures despite surgery. Post-operatively, 64-82% of patients with grade 2 gliomas become seizure-free, compared with 80% of grade 1 glioneuronal patients and 77% of high-grade patients.[35-37]

Various physiological mechanisms by which grade 2 gliomas may cause frequent seizures have been suggested. In the general epilepsy population, it was previously postulated that seizures may arise due to an imbalance between the excitatory action of neurotransmitters such as glutamate and inhibitory actions such as those mediated by Gamma-aminobutyric acid (GABA). The mainstream of epilepsy thinking now prefers a 'network model' of epilepsy, and has moved away from focal abnormalities resulting in seizures. However, it is still not well understood how this network arises or is hijacked to cause seizures and alterations in neurotransmitters could cause the emergence of a seizure prone network.[38] The IDH-1 mutation, now seen to be one of the genetic hallmarks of grade 2 glioma, could provide a physiological explanation for the high seizure burden. IDH-1 is an enzyme that normally catalyses the conversion of isocitrate to α-ketoglutarate. Mutant IDH-1 goes further than this, reducing α-ketoglutarate to 2-hydroxyglutarate (2HG). 2HG is structurally very similar to glutamate and so may be able to mimic its excitatory action in vivo resulting in seizures by agonism of glutamate receptors.[39,40]. The presence of 2HG in IDH-1 mutated tumours is in little doubt and levels of 2HG can be measured by magnetic resonance spectroscopy (MRS) in said lesions.[41] Whether this presence translates to function in vivo via receptor agonism is less clear. Preoperative seizures may be more common in IDH-1 mutated gliomas than those with wild type.[40,42] Going against this being a causal relationship is the finding that only in grade 2 gliomas does the IDH-1 mutation appear to convey an increased seizure risk, with IDH-1 mutated grade 3 and 4 tumours not displaying a significant

increase compared to their wild type counterparts.[43] Furthermore, the highly epileptogenic grade 1 gliomas do not display IDH-1 mutations.

Alterations in glutamate transporter expression resulting in an excess of extracellular glutamate have also been suggested as an epileptogenic mechanism in glioma. The system Xc-transporter imports cystine into cells in exchange for glutamate release. This transporter is over-expressed in many types of cancer. This overexpression and subsequent excess of glutamate may contribute to the high seizure burden by facilitating an excitatory environment around the tumour.[44] The Xc-transporter utilises cysteine, a non-essential amino acid used to produce the antioxidant glutathione, which may also aid tumour survival. This theory has led to interest in the possible repurposing of the disease-modifying anti-rheumatoid drug sulfasalazine as it is an inhibitor of the system Xc-transporter. To date it has not been shown to be effective in vivo, possibly due to issues with tolerability, bioavailability, potency and its ability to cross the blood-brain barrier (BBB).[45,46] A retrospective study of 229 gliomas of varying grades 2-4 showed that over-expression of Xc-transporter was associated with seizures at presentation in grade 4 gliomas but not in grade 2.[47]

Reduction in the expression of the potassium-chloride transporter KCC2 has been demonstrated in peritumoral neurons in mouse models of glioma (as also occurs in the developing foetal brain).[48] KCC2 pumps chloride out from cells. Elevations in intracellular chloride concentrations can cause GABA responses to become depolarising leading to a loss of inhibition and further imbalance towards a pro-excitatory environment. Expression of GABA may be lower in the tumour-affected hemisphere in glioma when compared with the opposite hemisphere.[49] None of the aforementioned physiological processes appear to explain fully the high seizure burden in grade 2 gliomas. It is therefore likely that, whilst they may be contributors

towards a seizure-favourable environment, many other drivers of seizure generation are yet to be discovered and that epilepsy in grade 2 glioma is very likely to be multifactorial.

Prognostic factors in grade 2 glioma

Several factors have been identified as prognostically significant in grade 2 glioma. Tumour histology has a significant influence on overall survival. As previously discussed, this is now primarily assessed using molecular genotyping. Since the change in the WHO classification, all grade 2 gliomas now possess an IDH-1 mutation. The presence or absence therefore of a 1p19q co-deletion separates oligodendrogliomas from diffuse astrocytomas respectively. Oligodendrogliomas appear to have a significant survival advantage over astrocytomas.[9,50] Reported mean overall survival in oligodendroglioma and diffuse astrocytoma tends to vary quite significantly in the literature. Some of this variation may come from the change in focus of the classification as many of the newer studies use primarily molecular genetics to categorise gliomas. Patients with a diffuse astrocytoma are likely to survive for an average of 5-7 years from diagnosis.[3,51] Oligodendroglioma overall survival is invariably longer, often up to double that of diffuse astrocytoma, with patients surviving anywhere between 7-17 years from diagnosis.[9,52-54] Evidence for MGMT promoter methylation and TERT mutations having a beneficial effect on overall survival and chemotherapy response mainly exists within the realm of glioblastoma.[13,55] Labs will often report grade 2 oligodendrogliomas with a TERT mutation as ‘triple positive’, though whether this represents a distinct prognostic group from ‘TERT negative’ oligodendrogliomas is controversial.[56] Now that IDH wild

type gliomas are no longer considered as grade 2 tumours this term may fall out of use.

Tumour volume at diagnosis appears to be an independent predicter of overall survival.[53,57-59] Most studies put down a relatively arbitrary line as to what denotes a large at presentation versus small at presentation tumour- usually above or below 5-6cm in diameter. It is readily assumed that larger at presentation tumours either are later in presenting or have a more rapid growth rate leading to poorer outcomes. It may also be that larger tumours may be harder to resect fully or supra-marginally due to involvement in a greater number of function critical structures.

Another factor commonly found to be linked with overall survival is the patients Karnofsky performance status (KPS).[52,59] Age too is often cited as a prognostic factor though is usually quite tightly correlated with the KPS. Increasing age has been reported as a worse prognosis in grade 2 glioma and overall survival in adults over 40 years old is lower than in those below 40.[4] This correlation between age and KPS is just one of the many correlations seen between all of these known and frequently reported prognostic factors. Many studies report a frontal tumour location as being prognostically beneficial. This is seemingly correlated with the fact that oligodendrogliomas most commonly occur in frontal regions.[60] A greater extent of resection is often cited as a positive prognostic factor.[53] Again, this is high correlated with the fact that frontal lesions are often more amenable to more aggressive surgical resection as well as the fact that tumour size may play a role in determining the feasibility of gross total resection.[61]

The association of seizures with altered glioma prognosis is a topic of much debate. Many studies have reported the presence of seizures at presentation as being a

positive prognostic factor, with patients who are seizure free pre-operatively having poorer overall survival.[62] This association doesn't appear to hold in the majority of glioblastoma populations.[63,64] The correlations seen with other well described prognostic factors also applies to seizures. Seizures appear to be more prevalent in patients diagnosed at a younger age.[26] If the hypothesis regarding the prognostically better off IDH-1 mutation conferring a seizure permissive environment is to be believed, then it is also logical that this would routinely mark out seizures as a positive prognostic sign. In examples such as this the seizures are simply the byproduct of another prognostic factor, rather than directly influencing proceedings themselves.

Rather than being a marker of tumour genetics or physiology, a way in which seizures could directly influence glioma prognosis would be if the electro-chemical physiology of a seizure were to impact upon tumour itself. Brain tumours do not exist in isolation from the rest of the brain. Changes to the neural microenvironment are likely to have an impact for better or for worse on the tumour as they would on non-infiltrated brain tissue. Understanding this interplay is critical to understanding the pathophysiology of grade 2 glioma.

The effect of seizures on glioma vascular supply and structure

Mechanisms by which seizures may directly impact tumour growth and progression are numerous. In a mouse model of a diffusely infiltrating glioma, glioma-infiltrated regions of the brain had significantly impaired neurovascular coupling compared with the healthy contralateral side.[32] This altered neurovascular coupling appeared to drive hypoperfusion of the tumour during seizures. In addition, glioma-induced inter-

ictal events were observed which also resulted in apparent induction of hypoxia within the tumour, despite the lack of a clinical seizure. Changes in blood flow to non-tumour-affected regions of the brain are also likely during and following a seizure. The short- and long-term consequences of this are not entirely clear and this topic would warrant a review of its own. It has been suggested that post-ictal phenomena in the general epilepsy population such as Todd's paresis, psychosis and amnesia could be a result of areas of the brain becoming hypo-perfused during or following a seizure.[65] The natural response to hypoxia in brain tissue is the upregulation of hypoxia-inducible factor 1-alpha (HIF-1α) by means of preventing it from being degraded.[66] This upregulation causes the transcription of multiple genes including vascular endothelial growth factor (VEGF) carbonic anhydrase 9 (CA IX) and erythropoietin (EPO). EPO upregulation may allow hypoxia resistance in glioma. In an animal model of glioma, EPO injection resulted in more rapid tumour spread and in human samples EPO receptor expression has been closely linked with expression of the proliferation marker Ki 67.[67] VEGF upregulation results in angiogenesis to increase blood flow to hypoxia-affected regions. This can promote tumour growth once its oxygen demand outstrips its original supply by facilitating new vessel formation. Vascular proliferation is a longstanding histopathological hallmark of a tumour which is beginning to progress and/or transform and of increasing WHO grade in glioma. VEGF as a target for therapy has been explored in recent years. The anti-VEGF drug Bevacizumab was trialled in glioblastoma patients to see whether restriction of its expression would result in reduced growth.[68] Unfortunately, no overall survival benefit was found, despite some possible improvements in progression free survival observed.

The ratio of cerebral blood volume (rCBV) is a value that can be assigned to a region of interest within or around a tumour using routine MR perfusion imaging. It is a ratio of the blood volume of a region of interest (the tumour core or margin for example) in comparison to the blood volume in a seemingly 'normal' area of white matter on the contralateral side. It is commonly used in clinical practice to differentiate between low and high-grade tumours with a sensitivity of 66-93% in the detection of low-grade glioma.[69] The widely accepted rCBV threshold for this distinction is <1.75 with a lower value more likely to indicate a low-grade glioma. How rCBV is affected by seizures is unclear. If seizures were to alter the vascular supply and/or structure in grade 2 glioma it might be expected that the rCBV would be altered. A literature search reveals very few results, with a single case report of transiently increased rCBV during focal status epilepticus in a patient with a stable anaplastic glioma.[70] A study of 18 suspected stroke patients who underwent computerised tomography (CT) perfusion scanning acutely (a modality which is also able to calculate rCBV), who were later given an alternative diagnosis of seizures, demonstrated that 14 out of 18 had unilaterally increased rCBV, predominantly in the temporal lobe.[71] Increasing rCBV is associated in glioma with transformation to a higher grade. This may reflect angiogenesis within and around the tumour. Theories already exist describing a cycle occurring within many types of tumours. Once they outstrip the pre-existing blood supply there is the promotion of angiogenesis to re-establish adequate blood flow.[72] If seizure-induced hypoxia over time causes a hastening in the upregulation of major angiogenic factors such as VEGF, then it is clear how they would influence how effectively a tumour can meet its metabolic needs. To our knowledge, no studies exist looking at the effect of seizures on measures such as the rCBV over time in glioma related epilepsy. Should seizures influence rCBV, it is

also possible that the ratio could be artificially inflated in patients with epilepsy versus those without. A pseudo-increase in rCBV could explain why some patients who have a rise in the ratio go on to show no discernible progression at biopsy.

Treatment of seizures in grade 2 glioma

The treatment of seizures in grade 2 glioma currently follows an approach similar to the general epilepsy population. Given the fact that novel mechanisms of seizure generation are likely to be at play in grade 2 glioma, whether or not specific antiseizure medications are effective in the glioma population needs to be further investigated. There is a lack of high-quality studies for even the most widely used antiseizure medications. The most recent Cochrane review on the subject 'Antiepileptic drugs for treating seizures in adults with brain tumours' identified only one small, randomised, unblinded trial in the glioma population.[73] It should be worth noting however, that the Cochrane review was performed in 2011 and so is now some years out of date. It is currently in the process of being updated by our group here in Leeds. The revision will permit a less strict inclusion requirement allowing data from well-designed prospective long-term observational studies to also be assessed alongside any newer randomised controlled trials (RCTs).

Studies that look to inform antiseizure medication choice are almost invariably retrospective observational studies and often include gliomas of different grades grouped together within treatment arms. There are fewer studies looking specifically at antiseizure treatment in grade 2 glioma alone. Although no widely accepted guidelines exist, various groups have sought to stratify antiseizure medication treatment options based on their specific suitability for use in patients with brain

tumours.[74] A prevailing theme of these suggested approaches is that antiseizure medications which are known to interact with other agents that are often administered to patients with brain tumours are best avoided. The primary examples of this are medications that interact with the cytochrome p450 system and thereby may disrupt metabolism of commonly used chemotherapeutic agents amongst other things. Notable examples of antiseizure medications with p450 activity include sodium valproate (p450 inhibitor), phenytoin (p450 inducer) and carbamazepine (p450 inducer). The alkylating agent temozolomide is only minimally affected by cytochrome p450 metabolism, however, the PCV (procarbazine, lomustine and vincristine) regimen which is also commonly used is heavily metabolised by the p450 system.[75] Commonly used antiseizure medications in the glioma population include levetiracetam, lamotrigine, topiramate, lacosamide, pregabalin, zonisamide, benzodiazepines, brivaracetam and perampanel. Pregnancy registers in the general epilepsy population suggest that only levetiracetam and lamotrigine (at conventional dosages) provide no additional risk of congenital malformations compared with the general population. All other antiseizure medications carry either known increased risk or unknown risks in pregnancy. In the grade 2 glioma cohort, the majority of who are middle aged, this may be an important additional consideration.

Levetiracetam is perhaps the most widely prescribed antiseizure medication in modern clinical practice (despite the results of the SANAD II trial discussed later p115). It has good efficacy in preventing both generalised and focal seizures and is generally well tolerated with a more acceptable side effect profile in comparison to older drugs.[76] In addition, both oral and intravenous forms are readily available and easily interchangeable in acute or emergency situations. It has also been adopted as a useful first-line antiseizure medication in status epilepticus when benzodiazepines

have failed. It does not induce or inhibit the p450 liver enzymes and has minimal pharmacokinetic interactions in general. A systematic review evaluating all studies reporting the efficacy of levetiracetam as monotherapy or as an add-on therapy in brain tumour related epilepsy concluded that levetiracetam was both safe and effective in reducing seizure frequency.[77] As with many assessments of antiseizure medications in brain tumours this review included gliomas of all grades. Relative effectiveness of levetiracetam was hard to decipher as different measures of seizure reduction were often used.

Both lacosamide and pregabalin have small studies supporting their use in brain tumour related epilepsy, as does topiramate.[78-80] In addition, serum Topiramate levels do not appear to be affected by temozolomide therapy.[81] Evidence for use of carbamazepine is limited and its use is likely to be infrequent due to drug-treatment interactions discussed.[82] Trials including oxcarbazepine are also limited. Perampanel has several trials suggesting it is an effective antiseizure medication in glioma related epilepsy and may have other, extra-epilepsy effects as discussed later on.[83,84] A PubMed search for the newest antiseizure medication, cenobamate, and “glioma” at the time of writing yields zero results, however, it may also soon see use in the glioma related epilepsy population. Phenobarbitone is no longer commonly used due to its side effect profile and only has a very narrow application refractory status epilepticus. For the same reason phenytoin is often avoided as a long-term option except in the most refractory of patients, though it still holds an important role in the treatment of status epilepticus.

The electroencephalogram in glioma

The role of electroencephalogram (EEG) in patients with gliomas is limited.[74] No consensus exists, for example, on how to approach a glioma patient without clinical seizures but with abnormal electrical activity detectable on EEG. For patients, the initiation of an antiseizure medication almost inevitably results in them receiving treatment for life. Much like in the general epilepsy population, there are few studies looking at whether withdrawal of antiseizure medication is reasonable given varying spells of seizure freedom. One small study was performed in patients with grade 1-3 glioma.[85] In the withdrawal group, 26% had further seizures (half of whom also experienced tumour progression) whereas 8% in the continuation group had breakthrough seizures. Decisions regarding withdrawal of antiseizure medication should be made by the treating clinician and the patient in partnership.

Prophylactic antiseizure medication

The practice of using prophylactic antiseizure medications in patients with all forms of glioma is often encountered around the world (though not so much in the UK). In such cases, antiseizure medication is prescribed regardless or not of whether patients have experienced a seizure attributable to their brain tumour. A Cochrane review on the topic in 2009 drew a neutral conclusion, neither for nor against prophylaxis.[86] At present, there is insufficient evidence to support the use of antiseizure medications in either newly diagnosed patients or as a means of reducing peri- or post-operative seizure risk, though large proportions of the evidence are of low quality.[87] The SPRING trial (Seizure prophylaxis in glioma) aimed to provide class I evidence in order to answer this question more

conclusively.[88] At the time of writing it is yet to report any results and may be being closed due to poor recruitment numbers.. Establishing a strong evidence base to answer this question is crucial as studies show wide variations in clinical practice with regards to prophylactic prescribing amongst clinicians in different centres.[89,90] Prophylactic antiseizure medication is not routinely prescribed in our centre or in many centres in the UK.

Antiseizure medications and progression- beyond seizure control

For several years, interest has persisted in whether many of the commonly prescribed antiseizure medications may have clinically significant effects on brain tumours beyond the control of seizures. The most widely studied has been the effect of sodium valproate on patients undergoing chemotherapy. Sodium valproate is thought to have action as a histone deacetylase inhibitor. This is of interest as altered expression of histone deacetylase may disrupt cell proliferation and differentiation.[91] It has been suggested that sodium valproate use may slow tumour growth and have a positive impact upon overall survival when given as an adjunct to chemotherapy or radiotherapy by inhibiting tumour metabolism.[92,93] This effect has only previously been suggested in glioblastoma and not grade 2 glioma. A large pooled analysis looking at survival in glioblastoma from commencement of chemotherapy with Temozolomide did not find that concurrent use of Sodium Valproate had any effect on overall survival.[94] Levetiracetam has also been touted as a potentially survival influencing agent in glioblastoma, again going beyond its ability to improve seizure control.[95] The mechanisms by which this may occur are not well documented. A potential theory is that levetiracetam increases the methylation percentage of the MGMT promoter region, thereby increasing the susceptibility of the

tumour to alkylating chemotherapy.[96] Experimental evidence in vitro suggests this may be the case though, barring a case series of 4 patients, in vivo data is lacking. The same pooled analysis that previously assessed whether Sodium Valproate may influence survival also reported no overall survival benefit to levetiracetam use. No trials exist to measure survival outcomes of these drugs in grade 2 glioma. Studies looking at whether various antiseizure medications (including both sodium valproate and levetiracetam) inhibit the growth of glioblastoma cell lines in vitro show none appear to do so at a dose that would be viable in human subjects.[97]

The novel mechanism of topiramate has also led to it being proposed as an agent with anti-tumour properties. Topiramate is a carbonic anhydrase inhibitor and is frequently used as an add-on treatment in many forms of epilepsy. Some tumours highly express carbonic anhydrase.[98] Non-invasive measurement of tumour acidosis is possible.[99,100] It has been demonstrated in animal models of glioblastoma that topiramate may potentially acidify the intracellular environment with a single dose.[101] This single study used implanted tumours in animals model, and not in vivo, human subjects. Whether topiramate causes tumour progression to slow in humans or not is unclear. Other drugs which may affect carbonic anhydrase include zonisamide, acetazolamide and possibly lacosamide.[102]

One of the newer antiseizure medications, perampanel, has a novel mechanism of action which may have wider effects on patients with brain tumours. Perampanel is an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptor antagonist.[102] Due to the traction of theories linking glutamate excess to seizure development in glioma, an agent with selective glutamate antagonism is an attractive treatment proposition. It has been explored as an add-on antiseizure medication in several studies and has improved seizure control in patients with

previously refractory epilepsy.[83,84,103] Furthermore, glutamate has been previously implicated in regulation of tumour cell growth and the high glutamate environment surrounding gliomas may facilitate this. Perampanel may reduce extracellular glutamate concentrations around tumour models in vitro, though some mouse models have failed to show an impact on tumour progression.[104,105] One study in humans with glioma reported a reduction in tumour volume at 6 months after treatment with perampanel, correlated with serum drug levels.[106] Caution should be taken however as the sample size of this study was 10 and it is yet to be repeated. Another study reported that, as well as improved seizure outcomes in patients with previously refractory epilepsy, cognitive performance improved.[107] This is possibly due to the treatment strategy being to discontinue another antiseizure medication at the time of perampanel initiation.

Seizures and their influence on anti-tumour treatments

Seizures may alter the efficacy of adjuvant treatments such as radio- and chemo-therapy. Radiotherapy requires the presence of oxygen to be effective and areas of hypoxia within tumours can lead to treatment resistance in many tumour types.[108] Treatment with radiotherapy is common practice in many forms of glioma. Due to the potentially hypoxic effect of seizures on the tumour, radiotherapy could be rendered less effective by ongoing seizures during a treatment cycle. Equally, it could be the case that previous episodes of repeated hypoxia have led to increased vascularisation within the tumour, resulting in improved oxygen delivery and blood flow, improving treatment efficacy.

Blood-brain barrier (BBB) permeability may also play a role in determining tumour growth. A novel study suggested the utilization of transcranial magnetic stimulation (TMS) to increase blood flow to the tumour during chemotherapy and thereby improve drug delivery.[109] They also demonstrated an animal model in which recurrent seizures resulted in increased glutamate release leading to increased BBB permeability, potentially marking out a way for this to occur naturally in vivo as a result of seizure activity. Whilst studies have previously indicated an overall reduction in seizure frequency after chemotherapy in a proportion of patients, there is little research into what effect seizures have on chemotherapy efficacy. Changes in vascular permeability around the tumour may allow improved chemotherapy delivery and seizure history and the current degree of seizure-control may be influential factors on chemotherapy success.

The interplay between seizures and the gliomas that generate them is poorly understood. We designed a project that culminated in a series of original research manuscripts aiming to shed further light on this unusual relationship and help to inform future directions of research.

Methods and Materials

Project design

An initial literature review was carried out. The search terms used in PubMed to acquire these studies were kept broad with a search of “epilepsy” and “glioma” yielding 2010 individual results. Only papers in English language were considered. In total 360 individual papers were identified as relevant and full copies of manuscripts

were retrieved and reviewed in full, informing all aspects of this work (preliminary reading, writing of the three manuscripts below and future research directions).

Following the review of the literature and preliminary reading, three key components were identified as key to better understanding the physiological and clinical relevance of seizures in glioma. These were 1) the glioma itself, 2) the seizures and 3) the treatments currently utilised to both manage seizures and prolong survival. It was decided that each paper should aim to better inform how one of these elements' influences another. This is represented in the below schematic (*figure 1A*).

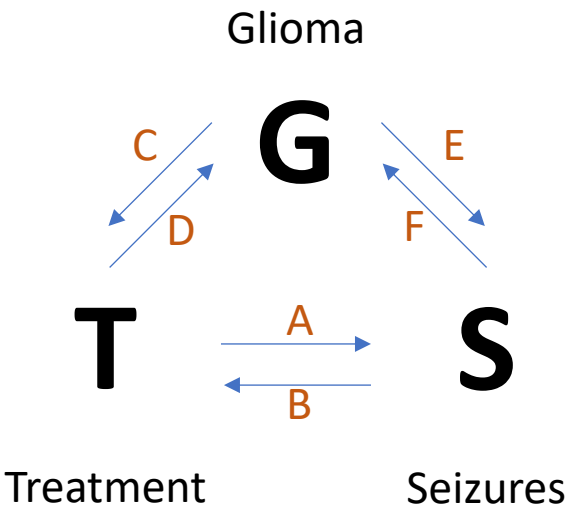

Figure 1A- Framework of factors in glioma related epilepsy

When summarising the existing literature, clear pre-existing research themes emerged. Arrow '*A*', the effect of seizure treatment on the seizures themselves, is regularly studied, despite the clear gaps in the range of antiseizure medications and predominantly retrospective designs as discussed above. This is most frequently studied by assessing changes in Engel classification (a widely used metric of seizure

frequency) following the introduction of various commonly used antiseizure medications, often as adjuncts. A smaller body of research also touches upon whether either chemotherapy or radiotherapy improves seizure control. A large body of literature exists looking at whether chemotherapy and radiotherapy are effective in grade 2 glioma. This is represented by arrow '*D*', the effect of treatment on gliomas, and is mostly outside of the scope of this work. Also included with arrow '*D*', and within the scope of this work, are papers looking at antiseizure medications, predominantly sodium valproate, and whether they may have a chemo-sensitising effect on gliomas, rending them more vulnerable to anti-tumour therapies. Finally, arrow '*E*' reflects a body of work, mostly theoretical, suggesting potential mechanisms of seizure generation in glioma as well as work on the potential influence of IDH mutations and tumour characteristics on seizure risk.

Each of the current manuscripts included later within this work, also inform one of these six relationships. Arrow '*A*' is added to significantly by Paper 1 and also the subject of the future work 'Study 4'. Arrow '*E*' is also frequently explored throughout all works, though predominantly in Paper 2, which also looks to inform arrow '*F*' (the effect of seizures on the tumour). In addition, less well understood relationships between ASMs and the tumour in arrow '*D*' (Paper 3) are explored, as well as ways in which arrow 'B' (Studies 5 & 6) may be looked at in our institution in the future, building on our work to date. Through this approach we aim to build on existing themes in the literature in novel ways, as well as pursue new directions of research.

Data acquisition and storage

All three studies were designed as retrospective observational studies. The setting of the studies was a large National Health Service (NHS) hospital trust in the United Kingdom with a catchment area for neurosurgical care comprising over 2 million patients. The identification of patients in the study was not performed by searching either clinic lists or for keywords in the medical records. Instead, a pre-existing database was used which had been collected for a historical, completed study and comprised of patients attending a low-grade glioma clinic over two decades. This database was used in some way for all three of the papers below. This database was originally ascertained by neurosurgeons for an observational study of all low grade gliomas at our institution and their surgical/histological characteristics. The earliest record used came from Jan 1997 with the latest from Dec 2021. In total there were 544 patients in the original database. Of these, 132 were removed due to either large amounts of missing data, duplication or an eventual diagnosis of something other than a glioma. All data within the study was collected retrospectively from the medical records. No new data was generated. All data in the study is routinely collected data pertaining to patients care. The study had no influence on patient treatment or level of follow up. Data was collected by one researcher manually looking through each of the patients records to add additional parameters not routinely collected in the original study. These additional parameters include all epilepsy/seizure data, medication history, chemotherapy and radiotherapy treatments, up to date survival status, last known follow up dates and cross checking of all tumour genetics in the original database as well as updates based on the most recent 2021 WHO classification. Collection of this data took one full time researcher 9 months to collect and organise. Each patient went from having 30 recorded parameters to almost 200. Data remained within the immediate patient care team

(neurosurgery and neurology departments). Data was kept securely at all times on secure trust devices and was not transferred to any external or portable drives. No paper copies of the data were made at any point. As part of improving transparency, post publishing the findings as manuscripts an anonymised version of the data with only the variables included in the statistical analyses of the papers would be available to the wider scientific community for scrutiny on a request only basis and at the discretion of the authors. Review of the study methods and ethics was performed by the local research and innovation department and a Caldicott letter of approval to confirm that the data was being used responsibly was issued (*Appendix 1*). Project data remains stored on secure NHS computers and is planned to be used for at least 2 further projects as outlined below in the 'Future work' section. Following completion of the intended projects any non-anonymised data on NHS servers will be deleted and only the minimal anonymised datasets will be kept for purposes of statistical scrutiny by peers as outlined above. It is likely these will be upload to an online repository though the suitability of this will be decided upon nearer the time of completion.

Statistical analysis

Statistical analysis for all studies was undertaken using the freely available open-source statistical software *R* (available to download from https://cran.r-project.org/, version 4.1.2). All analysis was undertaken and written in the native code of *R* (termed 'S syntax'). Anonymised data was imported from *Microsoft Excel* (Microsoft 365) using the '*readxl*' package. The unfiltered data for the projects below contained the observations of 412 individuals with up to 244 variables for each observation. Creation of a sub-database containing all observations using the '*data.frame'*

command reduced the number of variables due to duplication or irrelevance down to 219. As the original data set included a mixture of both grade 1 and grade 2 gliomas, as well as patients with a non-histological/molecular (i.e. purely radiological) diagnosis a further sub-database (referred to in code as *gr2*) was created again using the *'data.frame'* command to only include biopsy proven grade 2 gliomas. This resulted in a total of 228 observations across 219 variables. Whilst performing statistical analysis, data frames were left detached and referred to using the '*$*' symbol to ensure at all times that the correct data frame was being worked on and that this could easily be deduced by referring back the code. In addition, all *objects* in *R* were given unique names using only lower-case letters to avoid confusion as *R* is case-sensitive. The data frame *gr2* was used as the starting database for Paper 1 and Paper 2 as seen below. Paper 3 was coded separately as extra observations (anti-seizure medication at time of surgery and MGMT promoter methylation levels) were recorded in a separate *Excel* sheet and so a separate data frame (termed '*mgmt'*) was created via the same process as above.

Several statistical tests and models were used to analyse the data in the three papers. These are outlined below:

Cox proportional hazards model: The Cox proportional hazards model is a regression model primarily used to analyse survival times and their association with various predictor variables. In Paper 1, 'time to seizure freedom' was used as the survival time (t) with variables such as choice of antiseizure medication and seizure onset type as predictor variables. A sub analysis was performed with 'time to treatment failure' as *t* to see whether the choice of antiseizure medication or seizure type influence treatment failure. In Paper 2, a Cox proportional hazards model was similarly produced with 'overall survival' as *t* and predictor variables as 'tumour

histology', 'time to surgery', 'extent of resection' and 'presence of motor seizures'. For both papers it was vital that the assumptions made by a Cox proportional hazards model were met. Firstly, only variables that significantly improved the model's predictive power were included in the final model. In early analyses additional predictor variables were included, however, by use of the likelihood ratio test (uses ANOVA testing with a $p<0.05$), these were discarded due to insignificant effects. To allow the inclusion of two of the variables that improved the predictive power of the model in Paper 2, a stratification technique was used. Stratification allows for the inclusion of variables with non-proportional effects. The two variables stratified were the 'time the surgery' and 'extent of resection' variables. This was performed in *R* by prefixing variables with the '*strata*' command. An assumption of Cox proportional hazards is that the hazards remain constant over time. In order to check that this assumption is not being violated within a model, Schoenfeld residuals must be calculated and shown to be non-significant. This was done in *R* using the '*cox.zph*' command and all variables were appropriately non-significant for both papers.

Kaplan-Meier models: Initial Kaplan-Meier curves were drawn for each of the survival time measures and a predictor variable of interest. The prime reason for this was to identify any potential variables of interest to later include in the more powerful Cox proportional hazards models. The curves were drawn in *R* using the '*survfit*' function. For plots containing more than one curve (i.e., when a predictor variable is applied resulting in two or more groups rather than simply following the survival of one group) it is then possible to calculate a p value denoting significance between groups. This is performed using a chi squared test in *R* using the '*survdiff*' function. Whilst Kaplan-Meier with subsequent chi squared testing only allows the inclusion of

one variable, its advantage is that it provides easy to interpret visual representation of the data. For this reason, Kaplan-Meier curves were included in Paper 1, particularly in order to show the natural history of seizure freedom in this patient population over time.

Two sample t-test: In Paper 3, as there are essentially two groups of interest (those taking levetiracetam at the time of surgery and those not) for whom average MGMT promoter methylation is measured as a percentage. As this is assumed to be parametric data, a t-test was chosen to analyse for differences between the groups. This was performed in *R* using the '*t.test*' function. This average methylation data was also visually displayed in Paper 3 in a box plot using the '*boxplot*' function. A scatter plot was also produced using the '*ggplot*' package.

Fisher exact test: To allow the calculation of a p-value and odds ratio when comparing the risk of seizure generalisation in left versus right hemisphere tumours in Paper 2 a Fishers exact test was used. As this was analysis of a 2x2 table with no frequencies <5 either a Fishers test or chi squared test could have been used. In order to also calculate an odds ratio which can be a useful statistic when presenting data, a Fishers exact test was chosen.

SMOTE analysis: A SMOTE (synthetic minority oversampling technique) analysis was performed in Paper 2 following a suggestion from a reviewer. It allows oversampling of small cohorts. It was performed using the *ROSE* package in *R*.

Completed manuscripts and initial observational studies

Preliminary observational work (unpublished)

Once the dataset had been collected and formatted into a useable form, preliminary work was undertaken to better understand the basic epidemiological traits of the study population. This was done post the removal of all duplicate, missing and ineligible patients as described above (see *data acquisition*). Of an initial 414 patients with low grade glioma, 230 were diagnosed following surgical biopsy and analysis of tumour genetics with either a diffuse astrocytoma (n=132) or oligodendroglioma (n=98). The remaining patients (n=184) had either an alternative low-grade glioma on biopsy (a DNET for example) or lacked a conclusive histological diagnosis due to their management not including a biopsy or the results returning as ambiguous. Demographics of the preliminary cohort are listed below (*Table 1A*). Following the preliminary study, 2 patients were removed due to uncertainty regarding subsequent biopsies, hence the slightly smaller 'initial sample' populations seen in the published works.

	Total (n=230)	**Percentage (%)**
Gender		
- Male	146	67.5
- Female	84	36.5
Histology		
- Diffuse astrocytoma	132	57.4
- Oligodendroglioma	98	42.6
Tumour laterality		
- Left	99	43.0
- Right	125	54.3
- Midline/bilateral	6	2.6
Radiotherapy		
- Yes	111	48.3
- No	119	51.7

Chemotherapy		
- Yes	88	38.3
- No	142	61.7
Presenting complaint		
- Seizure	182	79.1
- Headache	22	9.6
- Trauma scan	4	1.7
- Focal neurology	6	2.6
- Confusion	2	0.9
- Dizziness	1	0.4
- Other	13	5.7
Epilepsy diagnosed		
- Yes	199	86.5
- No	31	13.5
If Epileptic (n=199)		
Seizure type*		
- Focal	105	52.8
- Focal to bilateral	84	42.2
- Unknown	10	5.0
Seizure awareness		
- Aware	58	29.1
- Unaware	135	67.8
- Unknown	6	3.0
Seizure activity		
- Motor	150	75.4
- Non-motor	37	18.6
- Unknown	12	6.0
Ever had a GTCS?		
- Yes	120	60.3
- No	67	33.7
- Unknown	12	6.0
Number of ASMs tried^		
- 0	4	2.0
- 1	110	55.3
- 2	50	25.1
- 3+	33	16.6
- Unknown	2	1.0

*Table 1A- Patient demographics, tumour histology and seizure history of grade 2 glioma patient cohort. GTCS = generalised tonic clonic seizure, ASM = antiseizure medication. *Patients were categorised as either 'focal' or 'focal to bilateral', not both. ^At last known follow up*

Roughly two thirds of the patients in the cohort were male. The median age at diagnosis was 38.0 years (range 17.7-73.8). Diffuse astrocytoma accounted for 57.4% of histological diagnoses. The vast majority of patients (86.5%) received a diagnosis of epilepsy at any stage during follow up. Seizures appeared to occur early in the majority of patients as 79.1% had them at presentation. Half of those patients with epilepsy had predominantly focal type seizures. Approximately two thirds of patients experienced seizure semiology mainly involving loss of awareness (67.8%) and three quarters had seizures with motor activity (75.4%). Those with epilepsy were more likely than not to have ever experienced a generalised tonic clonic seizure (GTCS, 60.3%). The breakdown of ASMs tried by each patient roughly reflects that of the general epilepsy population, with over half presumably having total or sufficient control with a single ASM, a further 25% gaining benefit from the second ASM and a fraction of patients likely displaying refractory seizures despite treatment.

Paper 1: Levetiracetam as a first line antiseizure medication in WHO grade 2 glioma: time to seizure freedom and rates of treatment failure

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Levetiracetam as a first line antiseizure medication in WHO grade 2 glioma: time to seizure freedom and rates of treatment failure

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Conflict of Interest:

SF has delivered a paid lecture of his own work for UCB Pharma. All other authors have no declarations to make in relation to this work.

Authorship:

The study was designed by SF, RM and MM. SF, JG, PC and RM collected the data. Data analysis was performed by SF and interpreted by SF, RM and MM. All authors were involved in drafting the manuscript and have read and approved the final version.

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Short title: Levetiracetam first line in WHO grade 2 glioma

Abstract

Objective: The high seizure burden seen in WHO grade 2 gliomas is well documented. This study aims to identify factors which influence the probability of seizure freedom (12 months of seizure remission) and treatment failure (ASM cessation or introduction of an alternative) in patients with WHO grade 2 glioma.

Methods: This is a retrospective observational analysis of patients from a regional UK neurosurgical centre with histologically proven (n=146) WHO grade 2 glioma and brain tumour related epilepsy. Statistical analyses using both Kaplan-Meier and Cox-proportional hazards models were undertaken, with a particular focus on treatment outcomes when the commonly prescribed ASM levetiracetam (n=101) is used first line.

Results: Treatment with levetiracetam as a first line ASM resulted in a significant increase in the probability of seizure freedom ($p<0.05$) at 2 years compared with treatment with an alternative ASM. Individuals presenting with focal seizures without bilateral tonic-clonic progression were between 39-42% significantly less likely to reach seizure freedom within 10 years ($p<0.05$) and 132% more likely to fail treatment by 5 years ($p<0.01$) when compared to those that had seizures with progression to bilateral tonic-clonic activity. ASM choice did not significantly affect treatment failure rates.

Significance: Over two-thirds of WHO grade 2 glioma patients treated with levetiracetam first line achieve seizure freedom within 2 years and it is a reasonable first choice agent. Experiencing mainly focal seizures without progression infers a significant long-term reduction in the chance of seizure freedom. Further studies are needed to inform ASM selection.

Keywords: Glioma, epilepsy, levetiracetam, seizures, neuro-oncology

Key points

What is already known on this topic-

• There currently exists little consensus with regards to the optimal first line antiseizure medication in epilepsy secondary to WHO grade 2 glioma.

What this study adds-

• The use of levetiracetam first line results in roughly one-third of patients reaching seizure freedom at 12 months, with over two-thirds having achieved seizure freedom at 24 months.

• Patients with focal to bilateral tonic-clonic seizures have a significantly increased chance of reaching seizure freedom in the long term.

How this study might affect research, practice or policy-

• Levetiracetam is an appropriate first-line agent in this specific patient population.

• Seizure type should be considered as a strong indicator of the probability of future seizure freedom.

Introduction

Gliomas are a heterogenous group of primary brain tumours arising from glial cell types. According to Cancer Research UK, over 12,000 people are diagnosed with a primary brain tumour each year in the UK.[1] Primary brain tumours are further defined by the World Health Organisation (WHO) Glioma Classification, with grades 1 and 2 classed as 'low-grade' and grades 3-4 as 'high-grade'.[2] WHO grade 2 gliomas are considered 'low-grade' in nature but are thought to inevitably progress to high-grade with time. Until the updated WHO classification of 2016, tumours were primarily identified by histological appearances. More recently, the rise of tumour genotyping has become the dominant factor in determining how tumours are classified. Two major histological subtypes of WHO grade 2 tumours are diffuse astrocytoma and oligodendroglioma.

Epilepsy is a common manifestation in WHO grade 2 glioma. Despite their usually slower trajectory of growth there is a strikingly high incidence of tumour associated seizures in this patient population. Estimations of epilepsy prevalence in WHO grade 2 glioma vary, though in the range of 60-90% of patients are thought to suffer with tumour related seizures.[3 4] In contrast, only 30-60% of patients with high-grade tumours such as glioblastoma multiforme have a diagnosis of epilepsy.[5] This stark contrast in rates of seizures suggests that either different or additional mechanisms of seizure generation exist in low-grade tumours compared with their high-grade counterparts. Tumour invasion and destruction of cortical networks via deafferentation or compromise of vascular supply alone are unlikely to be to sole processes by which seizures are generated or provoked.[6] Glioma specific mechanisms of epileptogenesis have been proposed involving the excitatory transmitter glutamate. These range from impairment in transport and clearance of

glutamate to molecular mimicry of its action following mutations in the isocitrate-dehydrogenase 1 gene (IDH1) leading to production of aberrant compounds such as 2-hydroxyglutarate (2-HG).[7 8]

As this patient group experiences a high seizure burden and considering the high likelihood of novel and tumour specific pathophysiology driving this, attention has turned to how best to treat symptomatic seizures in low-grade tumour related epilepsy. At present, there is no universally accepted protocol in terms of which antiseizure medication (ASMs) may be most appropriate in terms of efficacy and tolerability in seizures of this nature. Levetiracetam, lamotrigine and topiramate have all been suggested as potentially appropriate first line ASMs in tumour-related epilepsy.[9-11] Due to the high frequency of concurrent prescribing of both chemotherapeutics and other drugs such as corticosteroids certain ASMs are less utilised due to drug-drug interactions, though they are still be prescribed in tumour-related epilepsy in select patients. ASMs such as carbamazepine and phenytoin may be effective but are likely to interfere with other treatments, predominantly due to their interactions with hepatic enzymes. Sodium valproate has fewer drug interactions and has been used effectively in tumour-related epilepsy though it may increase the risk of chemotherapy induced thrombocytopaenia, leukopaenia and neutropaenia when taken in combination with temozolomide chemotherapy.[12] Other, less commonly used ASMs, have been trialled in small retrospective and prospective studies including lacosamide, pregabalin and perampanel.[13-15]

In most trials of the above ASMs, patient selection with regards to brain tumours is relatively broad. Many group together both low- and high-grade gliomas as ‘brain tumour related epilepsy’ within the treatment arms. Given that high-grade glioma is more common than grade 2 glioma, plus the probable differences in tumour

physiology described previously, there is a relative lack of information on ASM efficacy, specifically in patients with WHO grade 2 glioma.

Levetiracetam is one of the most commonly prescribed ASMs. It is thought to exert its action by targeting the synaptic vesicle protein 2A (SV2A), though the precise function of this protein and how levetiracetam is able to utilise it remains unclear.[16] Due to its efficacy against a range of seizure types it is a first line agent in generalised epilepsy syndromes in the broader epilepsy population. It is generally well tolerated in terms of drug induced side effects and is relatively simple to commence and titrate. It is also included in many protocols for status epilepticus. In focal epilepsy, levetiracetam has also long been considered effective, though, a recent randomised controlled trial (SANAD II study) comparing levetiracetam, lamotrigine and zonisamide in newly diagnosed focal epilepsy, ultimately found lamotrigine to be the most optimal ASM.[17] In addition, the other arm of the SANAD II study compared levetiracetam with sodium valproate in generalised or unclassified newly diagnosed epilepsy.[18] It found that levetiracetam did not meet the criteria for non-inferiority compared with sodium valproate.

In patients who experience seizures secondary to a low-grade glioma, focal seizures without progression to bilateral tonic-clonic activity (FwP seizures) are most common.[19] it is unclear as to whether FwP seizures secondary to a tumour respond in a similar way to ASM treatment as other focal epilepsy syndromes. The term ‘generalised onset’ implies a seizure which according to the ILAE classification ‘rapidly engages bilaterally distributed networks’.[20] Seizures may also be classified as ‘focal to bilateral tonic-clonic’ (FBT-C seizure), previously termed seizure with secondary generalisation, where the initial manifestation of the seizure is focal (for example déjà vu or unilateral motor activity) with subsequent progression to

generalised tonic-clonic activity. For our study, we have distinguished between patients who have FwP seizures and those who progress to have a FBT-C seizure with the assumption that all seizures have a focal onset at least initially.

We aimed to examine how levetiracetam performed with regards to treatment outcomes (probability of inducing seizure freedom, the time to seizure freedom and rates of treatment failure) in a population with histologically proven WHO grade 2 glioma. Moreover, we examined the relationship between the seizure semiology and treatment outcomes as well as histological, surgical and patient demographic factors.

Materials and methods

Patient selection: We undertook a retrospective analysis of patients presenting to a low-grade glioma clinic at an NHS regional neurosurgical centre. Inclusion criteria for the study were as follows: 1) Age ≥16 at the time of diagnosis, 2) histologically proven either diffuse astrocytoma or oligodendroglioma, 3) a diagnosis of epilepsy presumed to be related to the glioma and 4) use of an ASM with the intention to treat brain tumour related seizures. Of 412 patients recorded as attending the low-grade clinic, 146 fit the study criteria. A flow diagram of the patient selection process can be seen in *Figure 1*.

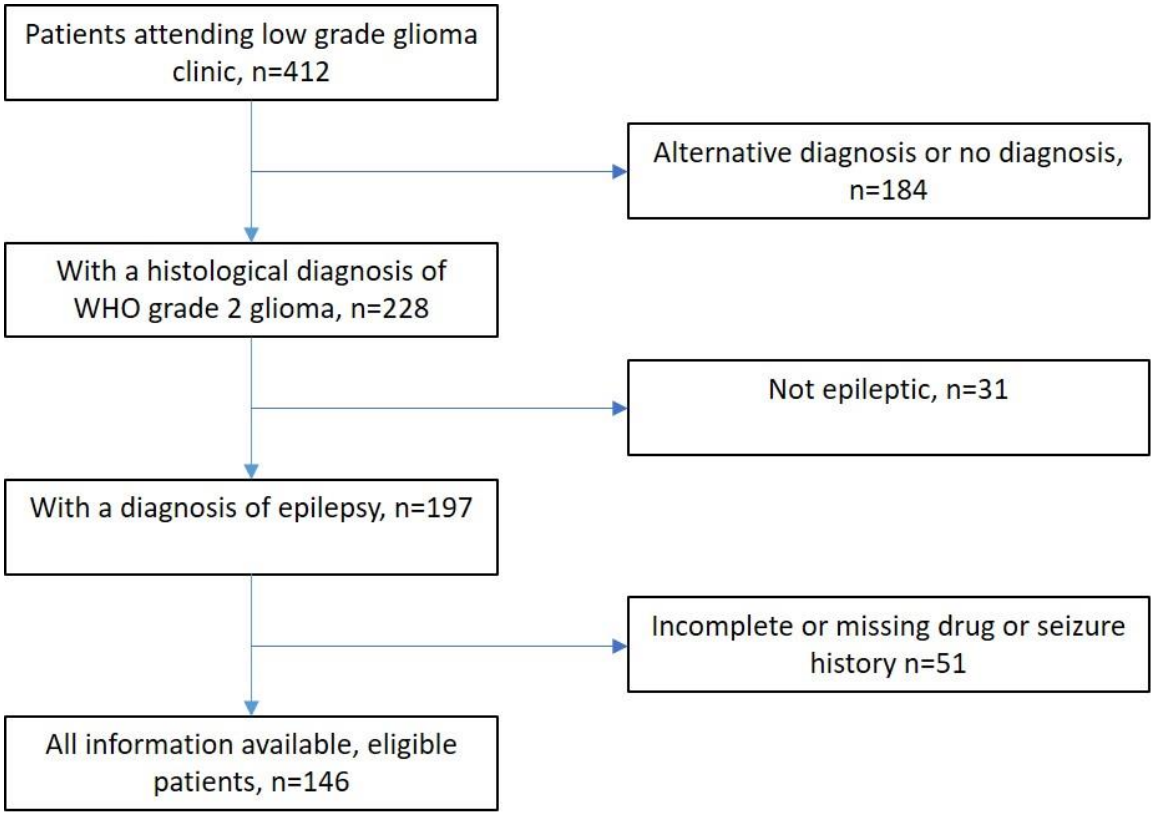

Figure 1- Flow chart demonstrating the patient selection process

Data collection and study design: This is a retrospective observational study. The electronic medical records of each patient were searched in order to establish basic patient demographics, a timeline of the disease course (age at diagnosis, survival time, time of surgery and follow up period), histology/genetic results (tissue/genetic diagnosis), epilepsy information (date of epilepsy diagnosis and predominant seizure type) and ASM use (first ASM prescribed, treatment failure time, reason for treatment failure and number of ASMs tried in total). The date distribution of records searched was Jan 1997 to Dec 2021. Preliminary data included coarse lobe-based location tags for each tumour, however, early analysis showed that all regions except brainstem or cerebellar tumours were more likely to be associated with seizures. In

addition, the size of these tumours often caused several locations to be involved making categorisation difficult. Tumour volume was not assessed.

Patient age at diagnosis ranged from 18yrs to 71yrs. Seizure type was coded as either ’FwP’, ’FBT-C’ or ‘unknown’ based on descriptions from clinic letters, ambulance records and in hospital notes, with the most predominant type (not the first) experienced by the patient being used.

Statistical analysis software: The study data was anonymised at collection. It was then coded to allow analysis in the statistical software package ‘R’ (available via https://www.r-project.org/, version 4.1.2).

Model and variable selection: Kaplan-Meier analysis was performed in R to provide a visual depiction of rates of a) seizure freedom and b) treatment failure between levetiracetam and alternative ASM groups. Seizure freedom was defined as 12 months without seizures. Patients were censored if lost to follow up. An intention to treat approach was taken meaning patients remained in their original cohort regardless of treatment failure. Treatment failure of a first ASM was defined as either cessation of the ASM for any reason or addition of another ASM for reasons of seizure control (polytherapy). A Kaplan-Meier model with censoring at treatment failure was considered, however, in order for Kaplan-Meier analysis to be valid, an assumption is made that censoring is non-informative – that it occurs not because of any factors that could influence the outcome. Seeing as treatment failure due to uncontrolled seizures does have an obvious impact on a patient’s risk of seizure freedom this method would likely be invalid.

Further analysis was undertaken using a Cox-proportional Hazards (CPH) model to explore variables which may influence a) seizure freedom and b) treatment failure.

Several variables were chosen to include in an initial CPH model for both outcomes to assess for differences between the levetiracetam and alternative ASM cohorts. The additional variables included were: seizure type (FwP vs FBT-C), sex, age at diagnosis, histology (diffuse astrocytoma vs oligodendroglioma), type of surgery performed (debulking vs biopsy) and time to first surgery from diagnosis. After generation of initial models, and in order to ensure appropriate variable selection, ANOVA testing was carried out using the 'likelihood ratio test' (LRT) to see whether inclusion of specific variables led to any improvement in the model. Using this method, it was found that the type of surgery performed, sex, histology and age at diagnosis variables all failed to make any significant contribution to either model and so these were excluded from further modelling. That surgery type did not influence outcome likely reflects the fact a vast majority of patients had debulking surgery. The time to first surgery variable made no significant contribution to the treatment failure model and was also excluded from this section.

An assumption of the CPH model is that the hazards remain constant over time. We checked this in our models by calculating Schoenfeld residuals in R. The time to first surgery variable appeared to violate the presumptions of proportional hazards but improved the model estimates when included in the time to seizure freedom model. In order to overcome this the time to first surgery variable was stratified so that it could be included in the final model. All remaining variables in the models produced Schoenfeld residuals with non-significant p-values, allowing the use of a proportional hazards approach.

Ethical considerations: No new data was being generated by this study and this was a retrospective review of medical case notes. The study methods were reviewed by the local Information Governance and Caldicott teams with a Caldicott letter of

approval being issued sanctioning the study. All patient sensitive data was stored password protected via secure NHS servers and according to local policy.

Results

Patient demographics and seizure type

The average age at diagnosis at our centre was 39.9 years. The majority of patients were male (102 male, 44 female). Average follow-up time was 6.29 years with the median year of diagnosis being 2015 (only 9 patients were diagnosed pre-2005). The median time from first seizure to ASM initiation was 38 days and from radiological diagnosis to ASM initiation 11 days, likely reflecting the average time to neuroimaging. Predominantly FwP seizures were experienced by 79 patients (54.1%) whereas 67 had mostly FBT-C (45.9%). Histologically, 71 patients (48.6%) had an oligodendroglioma and 75 (51.4%) diffuse astrocytoma. Chemotherapy was received by a similar number of levetiracetam group patients (35/101, 34.7%) and alternative ASM group patients (17/45, 37.8%) at some point during follow-up. carbamazepine or phenytoin was given to 5 patients who also received chemotherapy. All 5 had either reached seizure freedom or failed treatment by the time of chemotherapy, negating this potential interaction. The median time from ASM initiation to administration of chemotherapy in those that received it was 1037 days.

Kaplan-Meier analysis of 2-, 3- and 5-year seizure freedom

Of 146 patients with confirmed diffuse astrocytoma or oligodendroglioma, 101 were prescribed levetiracetam first line with the remaining 45 patients prescribed an alternative ASM. The ‘alternative ASM’ group were prescribed various commonly

available ASMs (carbamazepine n=16, lamotrigine n=15, sodium valproate n=9, topiramate n=3, phenytoin n=2).

Kaplan-Meier analysis was performed to compare seizure freedom times between the levetiracetam as a first ASM group and the alternative first ASM group (*Figure 2*).

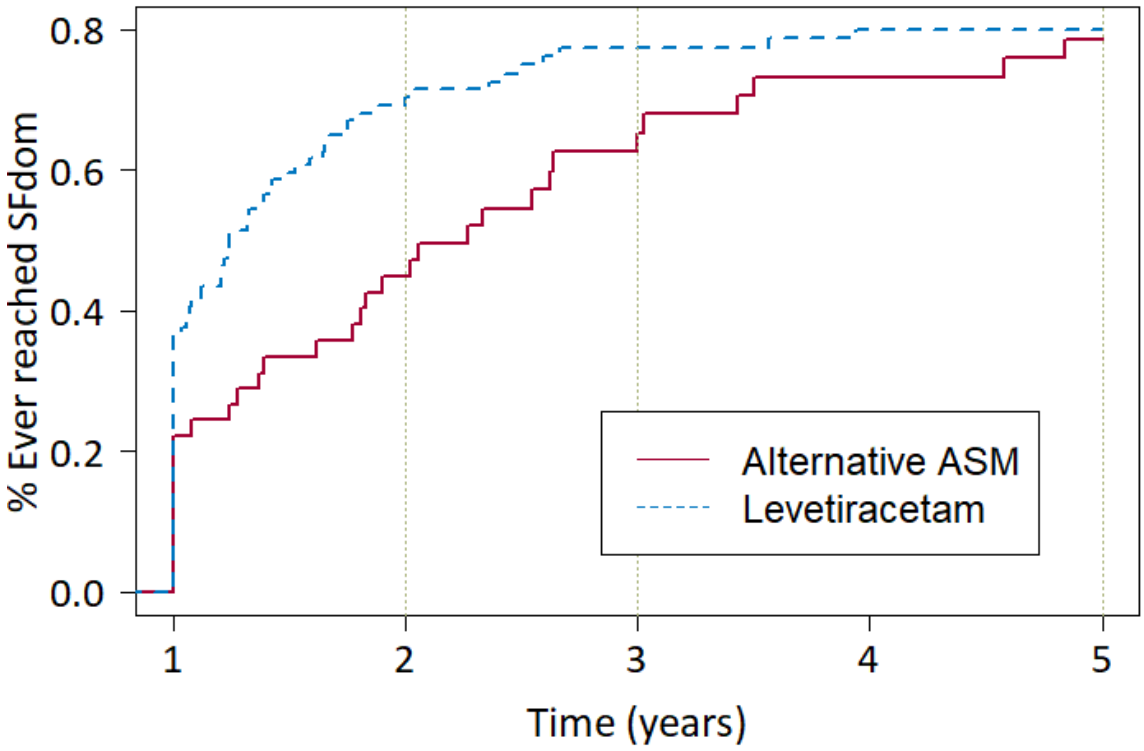

Figure 2- Kaplan-Meier analysis of seizure freedom when given levetiracetam vs an alternative first-line ASM in grade 2 glioma. Dotted lines (green) at 2-, 3-, and 5-year intervals from first ASM administration. ASM, antiseizure medication; SFdom, seizure freedom

Chi-square testing was performed to assess for significance between the two groups at 2-, 3- and 5-year intervals. A significant difference was detected at 2 years (p=0.005, 1df) and 3 years (p=0.01, 1df) with levetiracetam increasing the probability of seizure freedom. No significant difference was seen at 5 years (p=0.08, 1df).

Kaplan-Meier analysis of treatment failure

In total 33 of the 101 levetiracetam patients failed treatment within 5 years of ASM commencement (with treatment failure defined as cessation of the first ASM or addition of a second agent). Of those that failed treatment, 7 (21.2%) had side effects or an adverse event listed as the cause with 26 (78.8%) failing due to poor seizure control. In the alternative ASM group 19 participants failed treatment within 5 years. Of these, 3 (15.8%) were due to side effects or adverse events and 16 (84.2%) due to poor seizure control. In both groups, roughly 4 in 5 patients that failed treatment did so for reasons of seizure control (figure 3).

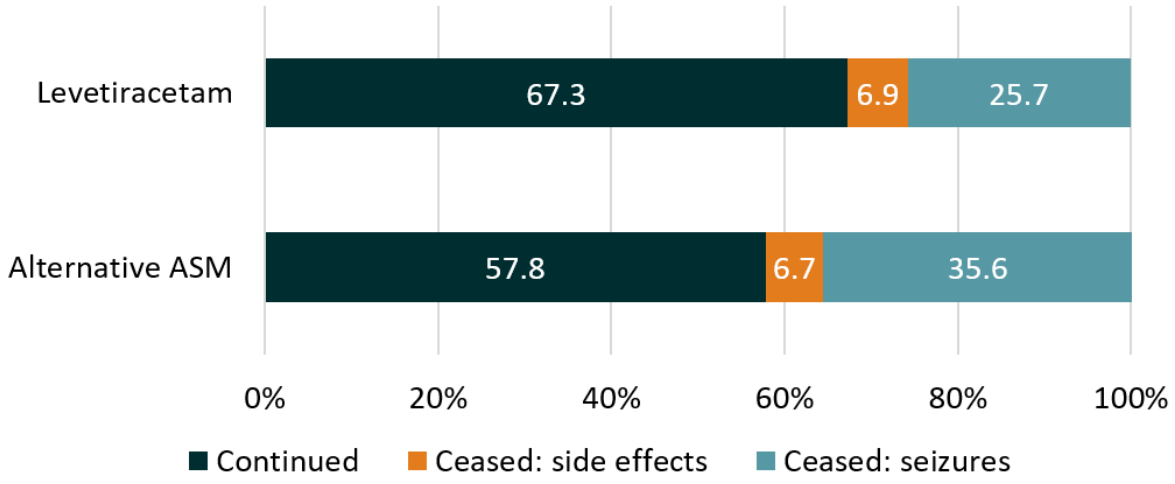

Figure 3- Reasons for treatment failure over 5 years in levetiracetam groups vs alternative ASM group. ASM, antiseizure medication

Treatment failure rates between the two groups were plotted in a Kaplan-Meier analysis (figure 4).

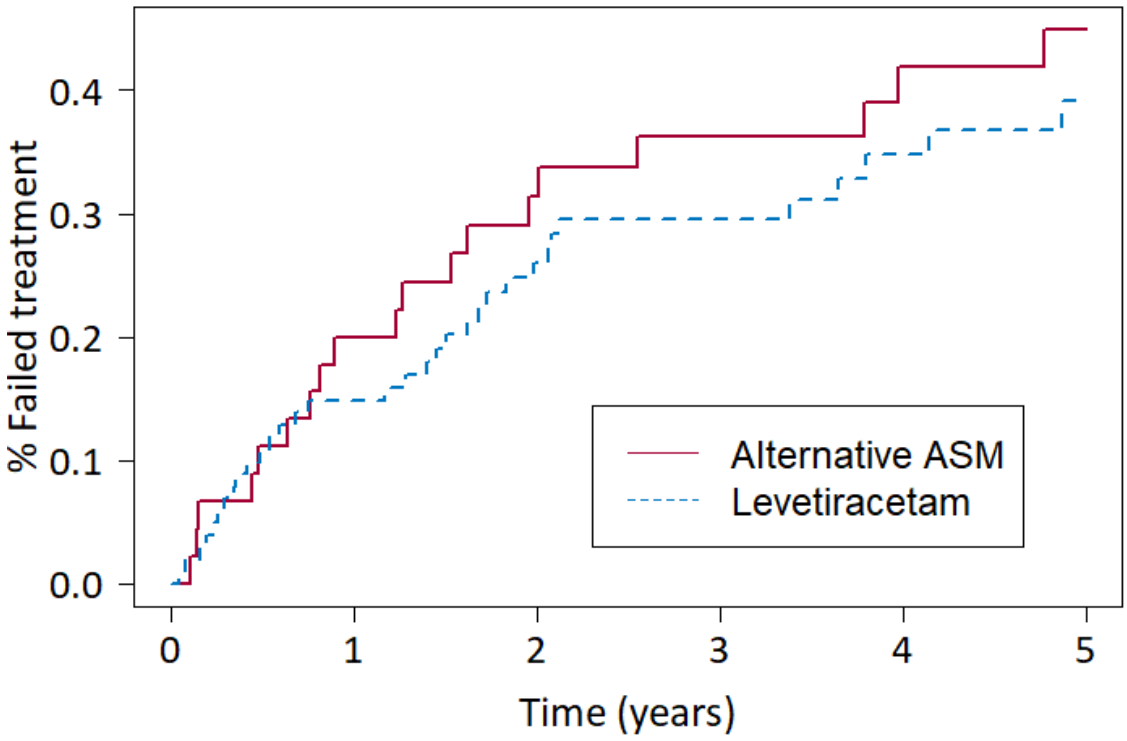

Figure 4- Kaplan-Meier analysis of ASM treatment failure in grade 2 glioma. ASM, antiseizure medication

Figure 4 shows that at all time points over 5 years a similar proportion of patients in each group failed treatment (roughly 15% at 1 year and 25% at 2 years for example). Chi-square testing here gives p=0.5 (1df), suggesting that no significant difference in the rate of treatment failure exists between the two groups.

The similar treatment failure rates (and similar reasons for failure), coupled with the lack of a significant statistically significant difference between the groups, strengthens the notion that the difference demonstrated in figure 2 is not being distorted by excessive rates of treatment failure or ASM intolerance in one treatment group more than the other.

Cox-proportional Hazards model of seizure freedom

Using a Cox-proportional hazards model, the influence of included variables on the time taken to reach seizure freedom over a 2-, 3-, 5- and 10-year period was assessed. These results are outlined below in *Table 1*.

	p-value	Hazard ratio and 95% CI
Levetiracetam as 1st line ASM		
SFdom 2yrs post ASM start	0.02*	1.76 [1.06 – 2.94]
SFdom 3yrs post ASM	0.06	1.57 [0.99 – 2.50]
SFdom 5yrs post ASM	0.21	1.32 [0.86 – 2.03]
SFdom 10yrs post ASM	0.16	1.36 [0.88 – 2.09]
FwP seizure type		
SFdom 2yrs post ASM	0.02*	0.61 [0.40 – 0.94]
SFdom 3yrs post ASM	0.009**	0.59 [0.39 – 0.88]
SFdom 5yrs post ASM	0.006*	0.58 [0.39 – 0.86]
SFdom 10yrs post ASM	0.006*	0.58 [0.39 – 0.85]

Table 1- Influence of levetiracetam as a first line ASM and seizure type on the probability of seizure freedom at 2, 3, 5 and 10 years from commencement of ASM. ASM = antiseizure medication, CI = confidence interval, FwP = focal seizures without progression to bilateral tonic-clonic activity, SFdom = seizure freedom

At the 2-year time interval, levetiracetam as a first ASM was associated with a significant increase in the probability of seizure freedom (p=0.02) with 69 of 96

patients followed up (5 censored) reaching seizure freedom. This compares with 20 of 44 patients (1 censored) becoming seizure free in the alternative ASM group. Being given levetiracetam meant patients were 76% more likely to be seizure free at 2 years than with an alternative ASM. This effect was not significant at the 3-, 5- and 10-year intervals. Predominant seizure type was highly significant when predicting seizure freedom at all time intervals. At 2 years following first ASM administration, those with predominantly FwP seizures were 39% less likely than those with FBT-C seizures to have reached seizure freedom. The improved seizure prognosis seen with FBT-C seizures appeared to be fairly constant across all 10 years of follow up.

Cox-proportional hazards model of treatment failure

A CPH model comparing the risk of treatment failure over a 5-year period was produced. Seizure type was shown to have a significant effect on the likelihood of treatment failure, with patients experiencing predominantly FwP seizures much more likely to fail treatment than those reporting FBT-C seizures ($p=0.006$, $HR=2.32$, CI [1.27 – 4.26]). This translates to patients with FwP seizures being 132% more likely to fail treatment within 5 years. Use of levetiracetam as a first line ASM did not demonstrate a significant change to the risk of treatment failure when compared to alternative ASMs ($p=0.82$, $HR=0.94$, CI [0.53 – 1.66]).

Number of ASMs prescribed

For each patient the total number of different ASMs prescribed was recorded. However, these values needed to be adjusted as they are amassed over a total follow up period which for some patients can be 10 years plus (unlike the previous analyses calculated over the first 5 years for both groups). When an ASMs/year of follow up average was calculated, the levetiracetam group was prescribed roughly

0.31 ASMs/year of follow up with 0.24 ASMs/year for the alternative ASM group. This could suggest that polytherapy may eventually be more common in the levetiracetam group than the alternative ASM group.

Discussion

Levetiracetam is the most widely prescribed first ASM in patients with grade 2 glioma at our institution and this is likely mirrored elsewhere.[21] There is a need for further evidence as to which of the commonly prescribed ASMs is most effective in this patient group and whether applying similar treatment strategies informing ASM choice in other forms of epilepsy is reasonable.

We found a significant treatment effect for levetiracetam at 2- and 3-year intervals in the Kaplan-Meier analysis and at only 2 years with the CPH approach. In the Kaplan-Meier analysis of seizure freedom rates between the two groups we have opted to use an intention to treat approach, meaning that patients stay in their original cohort despite treatment failure at any stage. If patients are not seizure free after a few years on any ASM it is likely an additional or alternative agent will have been prescribed. This makes it likely that the majority of the patients with ongoing seizures beyond 2-3 years in figure 2 will have been switched to an alternative or additional ASM. This may explain the narrowing seen in the difference between the two cohorts, starting at roughly 2.5 years, as patients with ongoing seizures in the 'alternative ASM' group are likely to have been switched to a different first line ASM (which may be levetiracetam). It may be that the alternative ASM group contains one or more ASMs that are much less effective in this patient group. For this reason, the better performance of levetiracetam overall should not be taken as showing its

superiority over all ASMs in the alternative group. Instead, this study adds important information with regards to the likely efficacy of levetiracetam on seizure outcomes at various time points when given first line to this specific patient population. Our study suggests that roughly two-thirds of patients treated with levetiracetam first line could expect to be seizure free at 2 years. It is also worth considering that despite meeting the criteria for 'treatment failure' in our study, an ASM may be continued alongside a new introduction and still contribute in part to reduction of overall seizure risk. For this reason, there is a possibility that polytherapy may play a part in the results seen in *Figure 2*. Despite treatment failure rates being similar between the two groups, analysis of the average 'ASMs/year of follow up' data suggests that levetiracetam may be more likely to be continued alongside a newly introduced agent than one of the alternative ASMs which could be more likely to be simply swapped out. This may be a reflection of local practices at our institution.

The CPH model of seizure freedom also demonstrated a higher rate of seizure freedom in the levetiracetam group at the 2-year mark. When creating the models, we found that time to seizure freedom was influenced by how soon after diagnosis the patient had surgery. This makes sense given the well documented antiseizure effect of surgery in grade 2 glioma which appears to increase with extent of resection.[22] The Kaplan-Meier model does not control for any additional variables unlike the CPH method. For this reason, the CPH model is likely more reflective of reality as it was able to take into account both the time to surgery and seizure type.

Focal seizures can often be more difficult to treat when compared with generalised seizures in many forms of epilepsy. It is therefore interesting to see that our study demonstrates via the CPH model that seizure type appears to give a fixed effect over time influencing the likelihood of both seizure freedom and treatment failure. At each

time interval a patient was between 39-42% less likely to attain seizure freedom if they had predominantly focal seizures without bilateral tonic-clonic spread. It is worth noting that when collecting the data, we found that most patients in this population had very stereotyped seizure semiology, with very few exhibiting a mixed picture that proved difficult to categorise. The fact that this difference between seizure types persists for up to a decade suggests that not only is ASM therapy much less effective against FwP seizures but that debulking surgery may also have more antiseizure effect against seizures that generalise to involve bilateral tonic-clonic activity vs those that do not. As a debulking procedure is likely to cause widespread disruption to cortical networks in the vicinity of the tumour it is possible to see how bilateral tonic-clonic seizures may be more readily averted via a post-operative reduction in connectivity- even though not all tumour tissue can be resected due to their infiltrating nature. Whether patients convert from seizures with bilateral tonic-clonic activity to FwP seizures post-surgery is not deducible from the data collected here.

Levetiracetam is widely seen as a generally well tolerated ASM. In our analysis we found that roughly 1 in 5 patients were unable to tolerate it due to either side effects or an adverse event. There was no difference ($p=0.63$) between the two groups in terms of reasons for failing treatment and therefore levetiracetam didn't stand out as an ASM with superior tolerability as might commonly be assumed. We did not collect dose information for the various ASMs. There will certainly be a range of doses on which a variety of patients (weights, renal function, hepatic function, competing drugs) will be maintained. We have no reason to suspect our institution has any atypical policies regarding ASM dosing. Furthermore, this patient cohort is, in our experience, often proactively managed by epilepsy specialist nurses and so instances of underdosing are likely to be uncommon.

Preliminary data included location tags for each tumour. Early analysis suggested that all glioma locations, except for those in the brainstem or cerebellum, were likely to be associated with the development of seizures (including occipital lobe tumours). Tumour location was therefore not included as a variable in our final analysis.

Conclusion

Our study suggests that levetiracetam is a suitable first line ASM in the treatment of seizures in grade 2 glioma. Over 60% of patients prescribed levetiracetam achieved seizure freedom within 2 years. Levetiracetam was more efficacious at 2 years when compared with a group of alternative first line ASMs, though further comparative prospective studies are needed in order to ascertain which drug is the most efficacious initial treatment. A history of predominantly FBT-C seizures in patients with grade 2 glioma is a significant predictor of seizure freedom which seemingly persists for up to a decade and despite surgery.

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Paper 2: Motor seizures confer overall survival benefit in grade 2 glioma

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Motor seizures confer overall survival benefit in grade 2 glioma

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Conflict of Interest:
All authors have no declarations to make in relation to this work.

Authorship:
The study was designed by SF, RM and MM. SF, JG, PC and RM collected the data. Data analysis was performed by SF and interpreted by SF, RM and MM. All authors were involved in drafting the manuscript and have read and approved the final version.

Short title: Motor seizures confer survival benefit in grade 2 glioma
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Abstract

Objective: The prevalence of epilepsy in WHO grade 2 glioma is high, with seizures being the presenting symptom in 60-90%. We explore the epidemiology of seizures in this patient population in a regional neurosurgical centre.

Methods: Electronic health records of patients with histologically-proven WHO grade 2 glioma (n=228) were reviewed between 1997-2021 with data collected including patient demographics, epilepsy prevalence and seizure semiology. The influence of seizure type on overall survival was calculated using a Cox proportional hazards model.

Results: Overall, 197/228 (86.4%) patients were diagnosed with epilepsy - either at presentation or during the course of their disease. Males were more likely than females to be diagnosed with epilepsy (91.1% vs 77.1%, p=0.003) and, in those with epilepsy, more likely to experience at least one focal to bilateral tonic-clonic seizure (69.4% vs 54.1%, p=0.05). Patients with left sided tumours were twice as likely to have experienced a focal to bilateral tonic-clonic seizure (p=0.02, OR=0.47). Predominantly experiencing seizures with motor activity appeared to confer better overall survival with a 65% decrease in the risk of death 10 years post diagnosis (HR=0.35, p=0.02). This is despite accounting for previously described prognostic markers including tumour histology/genetics, time from diagnosis to surgery and the extent of tumour resection.

Significance: Motor seizure activity is a frequent feature in WHO Grade 2 glioma and appears to confer a survival benefit regardless of histology or surgical factors. Seizures due to dominant hemisphere tumours may be more likely to propagate and cause bilateral tonic-clonic activity.

Key words:

Glioma, Epilepsy, Motor, Seizures, Survival

Key points:

- Experiencing predominantly motor seizure activity appears to confer an overall survival benefit accounting for histology and surgical factors.

- Left hemisphere gliomas are twice as likely to ever cause a focal to bilateral tonic clonic seizure than right hemisphere gliomas.

- Male sex is an independent risk factor for the development of epilepsy in grade 2 glioma.

Data availability:

Anonymised Data will be made available by the authors at reasonable request.

Introduction

Seizures are a common and often early manifestation in patients with a brain tumour. Gliomas are a group of tumours arising from the glial cell type. Glioma grading follows the World Health Organisation (WHO) classification for brain tumours and runs from grades 1 to 4.[1] In particular, lower grades of glioma (grades 1 and 2) are associated with a very high incidence of seizures in comparison with those of a high grade.[2] The incidence of tumour-related epilepsy in WHO grade 2 glioma is reported to be between 60-90%.[3,4]

Grade 2 gliomas are usually slow growing tumours and, despite most eventually progressing to a higher grade, overall survival from diagnosis is often several years.[5] This is in stark contrast to high grade tumours such as glioblastoma (WHO grade 4) where overall survival is almost universally very poor, averaging about 12-15 months despite treatment. The two most commonly encountered grade 2 gliomas in adults are diffuse astrocytoma and oligodendroglioma. Even within the same grade, survival can vary dependant on the specific tumour subtype. One large epidemiological study shows that in patients with an oligodendroglioma, 5-year survival was 80%, whereas in those with a diffuse astrocytoma survival at 5 years was 47%.[6]

The recent rise in tumour molecular subtyping now complements histological appearances. Accordingly, tumour classification is now an integrated molecular and histological diagnosis.[1] The use of tumour molecular markers to better classify grade 2 gliomas has helped to improve our understanding of the heterogenous outcomes previously seen in this patient population and critically helps better predict overall survival. Diffuse astrocytomas with the isocitrate dehydrogenase-1 (IDH1) mutation

show better overall survival than those returning as wild-type (and in fact, as of the 2021 WHO classification, wild-type astrocytomas are now classified as glioblastoma).[7] In addition, the 1p19q codeletion is considered the genetic hallmark of an oligodendroglioma, thereby also inferring a better prognosis.[8] Molecular markers may also predict better overall survival by way of response to treatment. Methylation of the promoter region of O-6-Methylguanine-DNA Methyltransferase (MGMT) is associated with a favourable response to the chemotherapy agent temozolomide, thought to be due to methylation hampering tumour self-repair mechanisms.[9] This association is stronger in glioblastoma, and less clear in grade 2 glioma.[10]

Other prognostic factors in grade 2 glioma include age, performance status and the extent of surgical resection. Increasing age infers a worse prognosis in grade 2 glioma and overall survival in adults over 40 years old is lower than in those below 40.[6] A higher Karnofsky performance status at diagnosis has also been correlated with better outcomes.[11] Surgical resection is now undertaken in the majority of patients with grade 2 glioma, although a watch-and-wait policy was often used in the past. Despite resection not being a curative treatment, it has been shown that earlier resection may delay malignant transformation and improve survival outcomes.[12] The extent of resection is important, with gross total resection more likely to result in longer progression free survival compared with sub-total resection or less.[13] A feature of all gliomas is that their spread within the brain often extends beyond the apparent tumour margins delineated on conventional neuroimaging. For this reason, supra-total surgical resection (resection beyond the perceived radiological margins) is often advocated and may lead to even better outcomes.[14] As the peri-tumoural margin is often thought to be the instigator of seizures in glioma-related epilepsy, it is

perhaps not surprising that a greater extent of resection is also associated with better seizure outcomes post-operatively.[15,16]

Grade 2 gliomas can occur in most parts of the brain. They are most frequent in the frontal lobes, followed by parietal then temporal lobes. Tumour size and location can inform prognosis. Patients with a glioma in a frontal cortical region have long been thought to display an improved overall survival and this may be in part to the higher likelihood of tumours in these areas being an oligodendroglioma, and more amenable to greater extents of resection.[17] A smaller tumour volume at presentation is also associated with better overall survival. The development of tumour related epilepsy appears more likely with frontal location and oligodendroglioma histology, with the exception of tumours situated in the midline.[4,18]

The presence of epilepsy at diagnosis may influence survival. Several studies report improved outcomes in patients who present with epilepsy versus those who do not.[19] The exact mechanisms underpinning this correlation are not clear. It may be that seizures lead to an earlier presentation and therefore earlier intervention. Others have looked to tumour physiology for an explanation. As previously noted, the presence of an IDH1 mutation is associated with an improved prognosis. IDH1 is an enzyme which normally catalyses the conversion of isocitrate to α-ketoglutarate. Mutant IDH1 has altered enzymatic activity, reducing α-ketoglutarate to 2-hydroxyglutarate (2HG). 2HG is structurally similar to glutamate, the primary excitatory neurotransmitter in the CNS, and it is hypothesised that it may mimic its excitatory action in vivo or alternatively cause local disruption via mTOR hyperactivation, resulting in seizures.[20] Patients of differing glioma grades have been shown to be more likely to experience seizures if they possess the IDH1 mutation compared to wild-type.[21] As the majority of patients with grade 2 glioma possess

both the IDH-1 mutation and usually develop seizures, this probable relationship does little to help prognosticate within the grade.

The International League Against Epilepsy (ILAE) provides a framework in order to classify seizure type in all forms of epilepsy.[22] This framework organises seizures by their onset (generalised or focal), level of awareness (aware or unaware) and additional descriptors such as the presence of motor activity (motor or non-motor). Seizures which are focal in onset but progress to a generalised tonic-clonic seizure (previously termed secondary generalised) are now referred to as 'focal to bilateral tonic-clonic'. Previous studies of low-grade gliomas have often reported the seizure onsets of patients using older seizure terminology.[18,23] Focal seizures appear to dominate and some studies do not use the notion of generalised onset at all in this patient group, terming all apparently generalised seizures as focal to bilateral tonic-clonic.[23] This approach seems reasonable given that these patients have a focal epileptogenic lesion, focal seizures appear to be the norm and seizures reported as generalised onset may simply have an unwitnessed or subtle focal onset. Seizure characteristics have been used to infer the likelihood of seizure freedom, with Chang et al, 2008 demonstrating in a cohort of 332 patients with low-grade glioma that both focal aware seizures preoperatively and a temporal lobe location increased the likelihood that seizures would be pharmaco-resistant.[18] Focal aware seizures also predicted poorer seizure outcomes post-operatively.

Little is known about whether these seizure types reflect something more fundamental regarding the tumour and its likely course. We sought to explore in more detail whether bilateral generalised tonic-clonic seizure spread, seizure awareness or motor activity was associated with improved survival outcomes in a population of patients who had undergone surgery for diagnostically proven grade 2

glioma. We also assess how patient demographics and tumour laterality may influence the seizure type experienced.

Materials and methods

Patient selection: We undertook a retrospective observational study of patients presenting to a multi-disciplinary low-grade glioma clinic at a UK National Health Service (NHS) regional neurosurgical centre. Patients included in the study were required to have diagnostically proven WHO Grade 2 diffuse astrocytoma or oligodendroglioma. Of an initial 412 patients attending a low-grade clinic, 228 were ultimately included. The effect of histology, presenting complaint, tumour genetics, sex and tumour laterality on survival (primary analysis) was analysed. A subgroup was then created which only included those patients with a diagnosis of epilepsy and in whom seizure semiology was evident. The effect of seizure semiology on survival was assessed in this smaller group of 185 (secondary analysis). Patient selection is depicted in *Figure 1*.

Data collection and study design: This was a retrospective observational study that examined the electronic medical records of patients between January 1997 and December 2021. Data collected included: patient demographics, tumour laterality, extent of surgical resection, disease course information (age at diagnosis, survival time, dates of surgery, follow up period), an integrated tissue/molecular diagnosis (according to WHO 2021 Tumours of the CNS criteria) and epilepsy descriptors (whether present at glioma diagnosis and seizure semiology including whether bilateral generalised tonic-clonic spread, awareness and activity). Extent of resection was categorised on post operative MRI (within 72 hours) as either gross total

(apparent complete resection), near-total (<3mm rim residual only), sub-total (nodular residual) or biopsy/gross residual. This was done by reviewing the neuroradiologist's report and, where there was uncertainty, the view of the multidisciplinary team with the hindsight of the next follow-up MRI. Whether a resection was supra total (beyond visible pre-op radiological margin) was not reported and so this wasn't considered. Seizure semiology information was extracted from clinic letters, pre-hospital/ambulance sheets, in-hospital notes and electroencephalography/video telemetry recordings. For each patient, the predominant (most typical/frequent) seizure type which they experienced was recorded. Records were searched between January 1997 and December 2021.

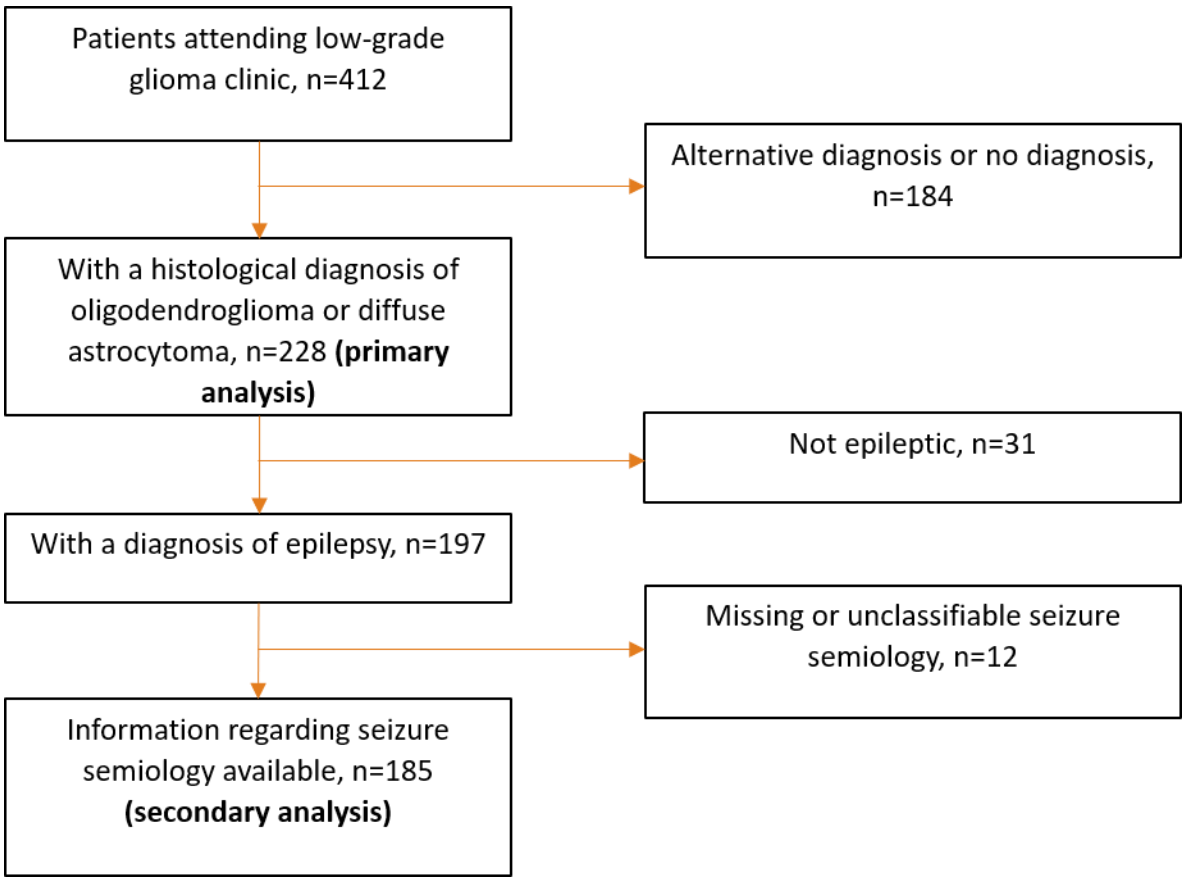

Figure 1- Flow chart demonstrating the patient selection process for both the primary and secondary analyses.

Statistical analysis software: Study data was anonymised at collection. It was then coded to allow analysis using the statistical software 'R' (available via https://www.r-project.org/, version 4.1.2). Most analysis was performed using base 'R' and the 'survival' package. The synthetic minority oversampling technique (SMOTE) sub-analysis was performed using the 'ROSE' package, also available in 'R'.

Model and variable selection: For the primary analysis, Kaplan-Meier analysis was conducted in R to provide a visual depiction and p-values via chi-square testing. This was done for several variables including tumour diagnosis (astrocytoma vs oligodendroglioma) and presenting complaint. Patients were censored if lost to follow up. Fisher's Exact Test ($p<0.05$) was used to assess the influence of tumour laterality on the risk of a focal to bilateral seizure. In the secondary analysis, a Cox-proportional hazards (CPH) model was applied to the data allowing the calculation of hazard ratios and p-values to assess the influence of each variable on 10-year overall survival. Variable selection was honed by performing ANOVA testing ($p<0.05$) using the likelihood ratio test (LRT) to see whether inclusion of specific variables led to an overall improvement in the model's predictive power. The likelihood ratio tests indicated that the CPH model needed to include the histology result, the time from diagnosis to surgery (to the nearest year) and the extent of tumour resection (coded as biopsy, subtotal, near total and gross total) as these all had a significant influence on 10-year overall survival. Both the time from diagnosis to surgery variable and the extent of resection variable were stratified in order to allow their inclusion. There was no significant difference in the extent of resection variable between the motor seizure vs non-motor seizure groups ($p=0.85$) with chi-square testing ('biopsy' 15.4% vs 16.7%, 'subtotal' 47.7% vs 52.8%, 'near-total' 23.5% vs 16.7% and 'gross total' 13.4% vs 13.9% respectively). Other variables (sex, awake or asleep resection,

TERT mutation status, patient age at diagnosis and tumour laterality) were discarded at this stage due to them not significantly improving the model when included. The MGMT methylation status variable contained too much missing data to be included. Patient age at diagnosis was omitted from the model having returned a p-value of 0.29 when included and not statistically improved the model (LRT p=0.29). Even when included, no change in the significance values returned for both tumour histology or seizure type on survival was observed. All patients had IDH-1 mutation positivity at some point although occasionally, after subsequent operations, their IDH-1 status changed. This was felt to be most likely due to surgical factors (normal brain tissue being sent for analysis) or laboratory factors such as tests/reagents failing. Patients with changing IDH-1 status were rare in our group. A sub-analysis, in which a variable stating whether or not IDH-1 mutation status changed, was temporarily added to the Cox model and this again did not change the significance of other variables or significantly contribute to the predicting power of the model ($p>0.05$). Schoenfeld residuals were calculated to ensure that the CPH model assumptions were met and these were appropriately non-significant.

Results

Primary analysis group (n=228)

Presenting complaint and tumour diagnosis on survival: A Kaplan-Meier analysis comparing the 10-year overall survival of patients presenting with seizures (n=180) versus those with other presenting complaints (n=48) did not demonstrate a benefit to seizures at presentation on chi-square testing (p=0.6, 1df). This is shown in *Figure 2*.

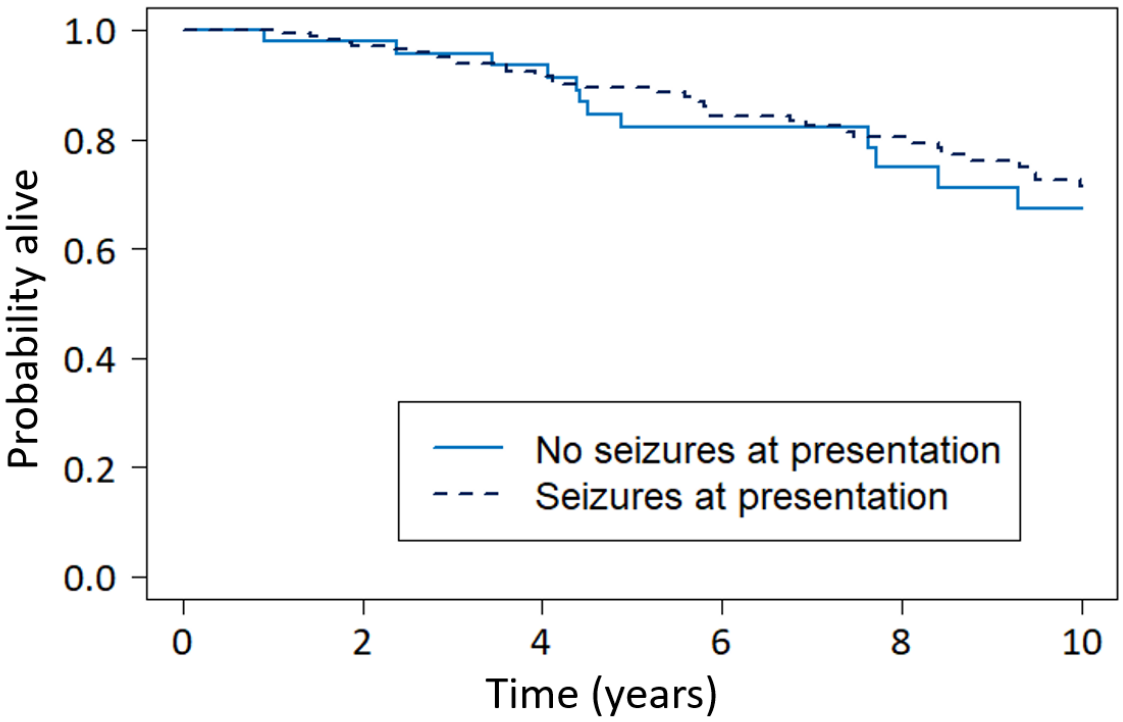

Figure 2- Kaplan-Meier analysis of overall survival from diagnosis depending on presenting complaint.

Although our patient cohort of 228 is a reasonable size, the high incidence of seizures in grade 2 glioma leads to the 'no seizures at presentation' group (21.2% of patients) still being relatively small (n=48). It may be that a much larger, national patient cohort is required to properly asses this relationship. In total 197/228 (86.4%) patients received a diagnosis of epilepsy, either at presentation or during follow-up. Ever having a diagnosis of epilepsy, either before or after glioma diagnosis, also failed to show any significant overall survival benefit (p=0.5, 1df). We performed a sub-analysis in which this 'never epileptic' population was oversampled using SMOTE. In this 'SMOTE'd' dataset, a Cox-proportional hazards analysis controlling for histology still demonstrated no significant survival benefit for the epileptic group

(p=0.87). Patients with a confirmed oligodendroglioma were significantly more likely to be alive at 10 years than those with an astrocytoma (p<0.001, 1df). TERT status did not significantly influence 10-year survival (p=0.2, 1df). Where tested, methylation of the MGMT promoter region (>15% methylation) was associated with increased overall survival compared with patients with an unmethylated (<10%) or equivocal (10-15%) result (p=0.03, 2df). The findings correlate with previously published literature and validate our data as representative of WHO Grade 2 glioma.

Risk of experiencing focal to bilateral seizures by sex and tumour laterality: Whether a patient had ever experienced a focal to bilateral seizure was coded as yes or no. Using Fishers Exact Test, it was found that males (133/146, 91.1%) were more likely to be diagnosed with epilepsy than females (64/83, 77.1%, p=0.003). In those with epilepsy and an auditable medical record (n=185, 12 patients had incomplete seizure data), males were more likely to have ever experienced a focal to bilateral seizure (p=0.05, 69.4% vs 54.1%). There was no difference in overall survival between the sexes.

Each tumour was assigned a laterality (left or right) with 18 patients omitted due to having either a midline or diffusely bilateral lesion (leaving n=210; 94 left-sided, 116 right-sided). Tumour laterality did not influence 10-year overall survival (Kaplan Meier Chi square, p=0.4) or the risk of developing epilepsy (p=0.84). The left/right distribution of tumours was similar in males and females (p=0.33). Epileptic patients with left-sided tumours were twice as likely to have ever experienced a focal to bilateral seizure than those with right-sided tumours (Fisher’s Exact Test, p=0.02, OR=0.47, CI [0.24-0.91]). The proportion of patients ever experiencing a focal to bilateral seizure by tumour laterality is displayed in *Figure 3*.

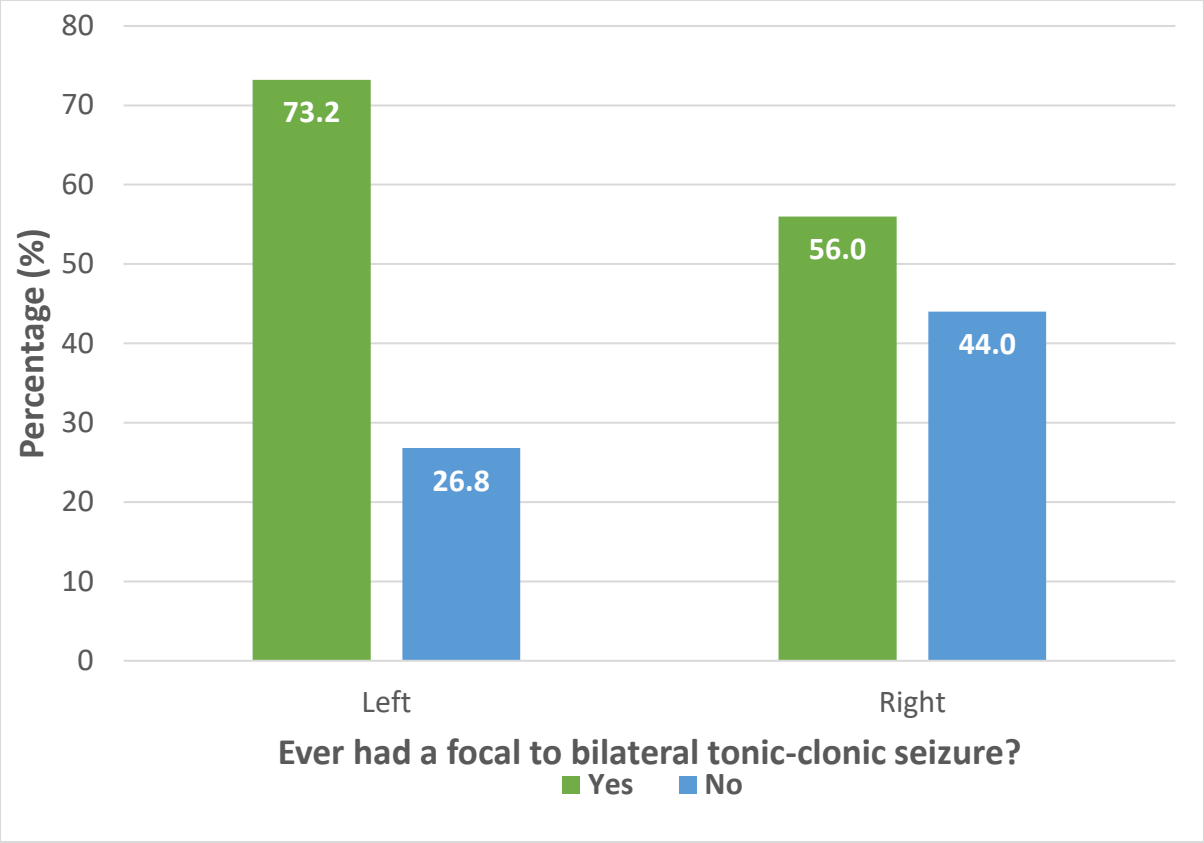

Figure 3- Bar chart comparing left and right sided tumours and the percentage of patients with each to have ever experienced a focal to bilateral tonic-clonic seizure.

Tumour histology did not influence the risk of focal to bilateral seizures (p=0.21).

Secondary analysis group (n=185)

Seizure semiology and 10-year overall survival: A sub-analysis was undertaken to assess whether certain features of seizure activity were associated with a change in 10-year overall survival. Initial Kaplan-Meier models with Chi-square testing did not show a significant difference in survival based on seizure type (focal vs focal to bilateral, p=0.3) or seizure awareness (aware vs. unaware, p=0.5). Seizure activity (motor vs non motor) did show a significant difference, with motor seizures

associated with better overall survival (p=0.05). Oligodendrogliomas were not more likely to cause motor seizures than astrocytomas (chi-square, p=0.99). Ever having a focal to bilateral seizure did not influence survival (p=0.7).

In order to investigate this relationship further, with a wider selection of variables thought to influence overall survival, a CPH model was produced using only variables which contributed to the model's predictive power as per a likelihood ratio test. This included the tumour histology as per genetic testing and the seizure activity type. In addition, both the time from diagnosis to surgery and the extent of tumour resection were accounted for using appropriate stratification methods (see 'model and variable selection' above). A summary of the CPH model results is displayed below in *Table 1*.

Confirmed astrocytoma resulted in a fourfold increase in the risk of death in 10 years (p=0.002) compared with oligodendroglioma at first biopsy. Having predominantly motor seizure activity showed a survival advantage with a 65% decrease in the risk of death at 10 years.

	p-value	Hazard ratio	95% Confidence interval
Oligodendroglioma on biopsy	0.002	0.21	0.07 – 0.56
Motor seizure activity	0.02	0.35	0.15 – 0.83

Table 1- The influence of histology and seizure activity type on overall 10-year survival as calculated by a Cox-Proportional Hazards model.

Discussion

To our knowledge, a correlation between the predominant seizure activity type which a patient experiences and overall survival has not previously been demonstrated or looked for. That patients with motor seizures are twice as likely to survive within a 10-year timeframe, controlling for histology, tumour diagnosis, extent of resection and time to surgery, raises questions as to whether this effect is due to the seizure activity type itself or related to another attribute of this patient group.

One possible explanation could come from tumour location. As discussed previously, the location of grade 2 gliomas may sometimes be difficult to categorise, especially when they span multiple lobes. Using simple methods to categorise these tumours (deciding in a binary fashion whether or not a tumour is involved in a particular region or lobe) proves challenging in such circumstances. It also doesn't reflect what we believe to be happening in these tumours with regards to seizure generation, namely that the tumour margin is the site of epileptogenesis rather than the core. It may be that future studies could better describe tumour location using volumetric measurements or by estimating tumour surface area within each region of interest. This may also however be a flawed methodology as proximity in the brain does not necessarily equate to connectivity. All of our patient cohort underwent a surgical procedure, with the vast majority undergoing maximal safe tumour debulking rather than biopsy alone. It may be the case that patients with motor seizures have a tumour in a more frontal location which in turn allows for a more aggressive surgical resection than a tumour closer to more eloquent areas. Our model was set up to account for the extent of resection (albeit with only 4 categories) and yet seizure activity still appeared to be a significant factor in predicting survival.

An alternative hypothesis may come from the differing timeframes with which patients present with motor vs non-motor seizures. Some of the non-motor seizure patients in our cohort manifested as intermittent sensory symptoms or emotional/behavioural phenomena. Both of these presentations could easily be labelled as alternative diagnoses in this relatively young patient cohort such as migrainous aura or psychiatric complaints. Frank motor seizures on the other hand are likely to be a greater cause for concern of neurological disease amongst both patients and the primary care clinicians they initially present to, likely resulting in earlier neuroimaging and a comparatively earlier diagnosis. Whilst we know that seizures are a very frequent occurrence, and often the first symptom to manifest clinically in grade 2 glioma, it is not clear how early they occur in tumourigenesis. It may be that whilst seizures are the first clinical manifestation, their appearance is still several years after the onset of tumourigenesis, negating the impact of slightly earlier discovery due to overt seizures. It doesn't appear from our modelling that experiencing a focal to bilateral seizure either regularly or at least once influences survival.

We found that patients with left sided tumours were more likely to have experienced at least one focal to bilateral seizure. In our study we were not able to record whether patients were right- or left-handed due to a lack of clinical information. We did not infer from functional imaging their dominant hemisphere. However, one would assume that the vast majority of patients within our cohort would be left hemisphere dominant, much like the general population. Our results suggest that dominant hemisphere tumours are more likely to result in seizure generalisation than those in the non-dominant hemisphere. The higher likelihood of generalisation suggests there may be faster propagation of seizures from dominant to non-dominant hemispheres

or a greater ability of the dominant hemisphere to resist involvement when there is uncontrolled activity in the non-dominant side. A recent study suggested that left hemisphere focal onset seizures may have a longer duration than right and this increased seizure duration may proportionately increase the risk of generalisation.[24] This may also be the case in tumour related epilepsy and many other lesional epilepsies. However, seizure length is not something we were able to measure within the scope of this study.

Improved survival outcomes in patients with oligodendroglioma have been previously reported. We re-demonstrate these again in our grade 2 glioma cohort. The significance of seizures at presentation on overall survival has remained controversial and, at least in this analysis, no significant link was demonstrated. This may simply be due to this being a relatively small effect and requiring a larger sample size than the one we present here. Patient sex appears to influence both the likelihood of epilepsy in grade 2 glioma as well and the risk of seizure generalisation with males more likely to have epilepsy and less likely to have only focal seizures. It is possible that seizures are underdiagnosed in females with glioma if they are more likely to have purely focal seizures, that are perhaps less apparent to the clinician. Neither of these findings appear to impact upon overall survival however, perhaps implying more fundamental differences in seizure generation and propagation in tumour affected brain between sexes.

Conclusions

The presence of motor as opposed to non-motor seizures appears to infer an overall survival advantage in grade 2 glioma; independent of diagnosis, time to surgery from

diagnosis and extent of resection. This correlation may be explained by tumour location within the electrical geography of the brain, motor seizures alerting a clinician earlier to their cause, or something more fundamental about tumour biology and neurophysiology. Left (and likely dominant) hemisphere seizures may increase the risk of seizure generalisation, either reflecting greater connectivity between the peri-tumoural margin and the rest of the brain or a tendency to provoke focal seizures of a greater duration. Further work is needed to better understand the complex biology and neurophysiology of tumour epileptogenesis and how this translates to the clinical course.

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Paper 3: Levetiracetam does not increase MGMT promoter methylation in WHO grade 2 glioma

Completed pending submission

Levetiracetam does not increase MGMT promoter methylation in WHO grade 2 glioma

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All authors have no declarations to make in relation to this work.

Authorship: The study was designed by SF, RM and MM. SF, JG, PC and RM collected the data. Data analysis was performed by SF and interpreted by SF, RM and MM. All authors were involved in drafting the manuscript and have read and approved the final version.

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Abstract

Background: Many patients with grade 2 glioma related epilepsy are prescribed the antiseizure medication levetiracetam. Some in vitro studies have suggested a beneficial effect of levetiracetam in glioma, beyond its role in the treatment of seizures. It is hypothesised that it induces the methylation of the promoter region of the DNA repair protein MGMT via the tumour suppressor protein p53. We investigated whether, at the time of biopsy, patients with proven grade 2 glioma taking levetiracetam had higher MGMT promoter methylation percentages than those who were not.

Method: We performed a retrospective observational study of 80 patients with grade 2 glioma (On levetiracetam=51, Not on levetiracetam=29) and recorded mean MGMT methylation percentages across CpG sites 72-79, antiseizure medication use at the time of biopsy and tumour histology/genetics at diagnosis. Analysis was undertaken using multiple linear and logistic regression.

Results: No difference in the mean MGMT methylation percentage was observed in patients on levetiracetam (p=0.72). Oligodendroglioma histology predicted an increase in methylation of 8.2% compared with diffuse astrocytoma (p=0.02). Increasing patient age at diagnosis was associated with increased methylation percentages (p=0.02).

Conclusions: Levetiracetam does not significantly alter the mean MGMT promoter methylation percentages in grade 2 glioma, based on this retrospective small cohort study. Further study of other antiseizure medications may be warranted given their frequent use in this patient group.

Introduction

Levetiracetam is one of the most commonly prescribed antiseizure medications (ASMs) in many forms of epilepsy. It is considered a first line ASM in idiopathic generalised or unclassifiable epilepsy alongside Sodium Valproate, in part due to its suitability of use in women of childbearing age.[1] It may also be considered in monotherapy or combination in focal epilepsy syndromes as well as acutely in the treatment of status epilepticus. Levetiracetam is distinct from many traditional ASMs in that its mechanism isn't related to voltage-gated Na+ channels or T-type voltage-gated Ca2+ channels. Its exact mode of anti-seizure action is as yet unclear. Synaptic vesicle protein 2A (SV2A) has been identified as a binding site of levetiracetam (and also its sister ASM Brivaracetam).[2] It does appear that levetiracetam exerts its antiepileptic action via these means, as in knockout mice heterozygous for SV2A (meaning they have 50% fewer binding sites available) levetiracetam is significantly less effective.[3] Mice that are homozygous for SV2A knockout have lethal seizures.[4] Levetiracetam may also have an effect on N-type Ca2+ channels and has been shown in vitro to modulate AMPA receptors.[5] As with many other ASMs, levetiracetam likely has multimodal action. Although no consensus exists as to the optimal ASM for treatment of seizures in people with brain tumours, levetiracetam is widely used for this purpose and appears to be broadly effective and well tolerated.[6,7]

World Health Organisation (WHO) grade 2 gliomas are typically slow growing tumours and overall survival from first diagnosis can be several years.[8] Despite their initially benign course, epilepsy is strikingly common with these tumours and an acute presentation to hospital with a seizure is often the sentinel symptom. The incidence of epilepsy in grade 2 glioma is reported to be between 60-90%.[9,10]

Molecular genotyping has revolutionised the WHO classification of gliomas.[11] The two most commonly encountered grade 2 gliomas are diffuse astrocytoma and oligodendroglioma. Diffuse astrocytomas are defined by their isocitrate dehydrogenase (IDH) 1 or 2 mutation status; tumours without IDH-1 type mutations are now classified as WHO grade 4.[12] Co-deletion of 1p/19q is a chromosomal alteration now considered the hallmark of an oligodendroglioma and infers an even longer survival time.[13]

Alkylation at the O6 position of guanine is an early mutation leading to cancer and cell death.[14] O-6-Methylguanine-DNA Methyltransferase (MGMT) is a DNA repair protein which is able to remove alkylation from this position. The MGMT promoter region (MGMTp) may become methylated which in turn reduces MGMT expression. Approximately half of grade 4 gliomas are found to have MGMTp methylation.[15] Methylation of the MGMTp has been shown to be a positive prognostic indicator in high-grade glioma.[16] It is hypothesised that MGMTp methylation lessens a gliomas ability to self-repair and may lead to malignant cell death, thereby hampering tumour growth. The alkylating agent Temozolomide is the most commonly used adjuvant chemotherapeutic agent in glioma, following trials showing improved survival in glioblastoma patients.[17] Silencing of the MGMTp by methylation may be a means of reducing chemoresistance to alkylating agents.[18] The prognostic significance of MGMTp methylation in low-grade glioma however, specifically grade 2 glioma, remains uncertain. There may be some interdependency between IDH status and/or the presence of a 1p/19q co-deletion and MGMTp methylation, causing difficulty in untangling the true impact of MGMTp status.[19] Large trials have previously attempted to retrospectively analyse this relationship but have given inconclusive results.[20,21] This may be partly due to the way in which MGMTp methylation is

reported, often with 3 categories termed methylated (>15% methylation), unmethylated (<10% methylation) and indeterminate (10-15% methylation). This is a fairly simplified method of categorisation as each result is an average of methylation percentages seen across several CpG sites. A CpG site refers to a section of the DNA sequence in which a cytosine nucleotide is followed by a guanine nucleotide. A CpG island consists of many CpG sites and a total of 98 CpG sites exist within the MGMT CpG island.[22] The island appears to become methylated in distinct blocks, with CpG sites 73-90 tending to show similar levels of methylation.[23] Different labs and groups test different sites though the majority of these lie within the CpG sites numbered 73-90. This region has previously been shown to be important in terms of transcription and gene expression. Methylation above 15% is thought to infer an improved prognosis in high-grade glioma.[16] The significance of 'weak' methylation seen in the indeterminate group (10-15%) is unknown though there may be a mild positive prognostic effect, at least in the glioblastoma population.[24]

There has long existed an interest in the use of ASMs in glioma patients beyond that of seizure control. Perhaps the most extensively researched of these is whether Sodium Valproate use improves survival in glioblastoma. It is suggested that as a histone deacetylase inhibitor Sodium Valproate is able to sensitise glioma cells to chemotherapy and/or radiation.[25] Although some cohort studies suggest a prognostic benefit to Sodium Valproate administration, larger epidemiological studies appear not to suggest any improvement in overall survival.[26-28] Levetiracetam has also been postulated to have effects on gliomas beyond seizure control though this is less well studied. A recent meta-analysis of over 5000 patients with glioblastoma suggested no overall survival benefit to levetiracetam administration.[29] A single study looking at potential tumour altering properties of levetiracetam comprising of both an in vitro

model as well as a case series of 4 glioma patients has been reported.[30] In vitro, levetiracetam was found to decrease MGMT protein and mRNA expression levels in glioblastoma cell lines. Furthermore, in 4 patients who underwent two separate biopsies 7-14 days apart (and were administered levetiracetam prior to the second biopsy but not the first), MGMT expression was universally reduced. It was therefore hypothesised that levetiracetam exerts an effect on MGMT expression. This effect was postulated to rely on the presence of the tumour suppressor gene p53, with in vitro studies suggesting that intact expression of p53 was a necessity for levetiracetam to reduce MGMT expression in this way. A trend for lower MGMT activity in p53 positive patients with glioma (i.e. those without a p53 mutation) has been observed previously.[31] Mutations of p53 are extremely common, occurring about 50% of the time in cancers of many forms.[32] Degrading the role of p53 as a tumour suppressor gene is likely advantageous to tumours of all types. As well as losing its ability as a tumour suppressor, mutations may sometimes cause 'gain-of-function' effects. When exactly p53 mutation occurs in glioma is not clear and it may be that for a period of time in the early life of the tumour p53 expression remains intact. As p53 mutations are so common, and as they do not currently inform clinical practice with regards to treatment or prognosis, they are often not tested for in routine glioma histology work-up.

We sought to assess factors that could influence MGMTp methylation in a population of patients with histologically proven WHO grade 2 glioma (2021 classification). Specifically, we looked at whether taking levetiracetam at the time of biopsy was likely to infer a greater or lesser methylation percentage compared with similar patients taking an alternative antiseizure medication or no antiseizure medication.

Materials and methods

Patient selection: We undertook a retrospective observational study of patients presenting to a multi-disciplinary low-grade glioma clinic at a UK National Health Service (NHS) regional neurosurgical centre. Patients included in the study were required to have diagnostically proven WHO Grade 2 diffuse astrocytoma or oligodendroglioma. Of an initial 412 patients attending the clinic, 80 were ultimately included in the analysis. Reasons for patients being excluded from the analysis are listed below in *Figure 1*.

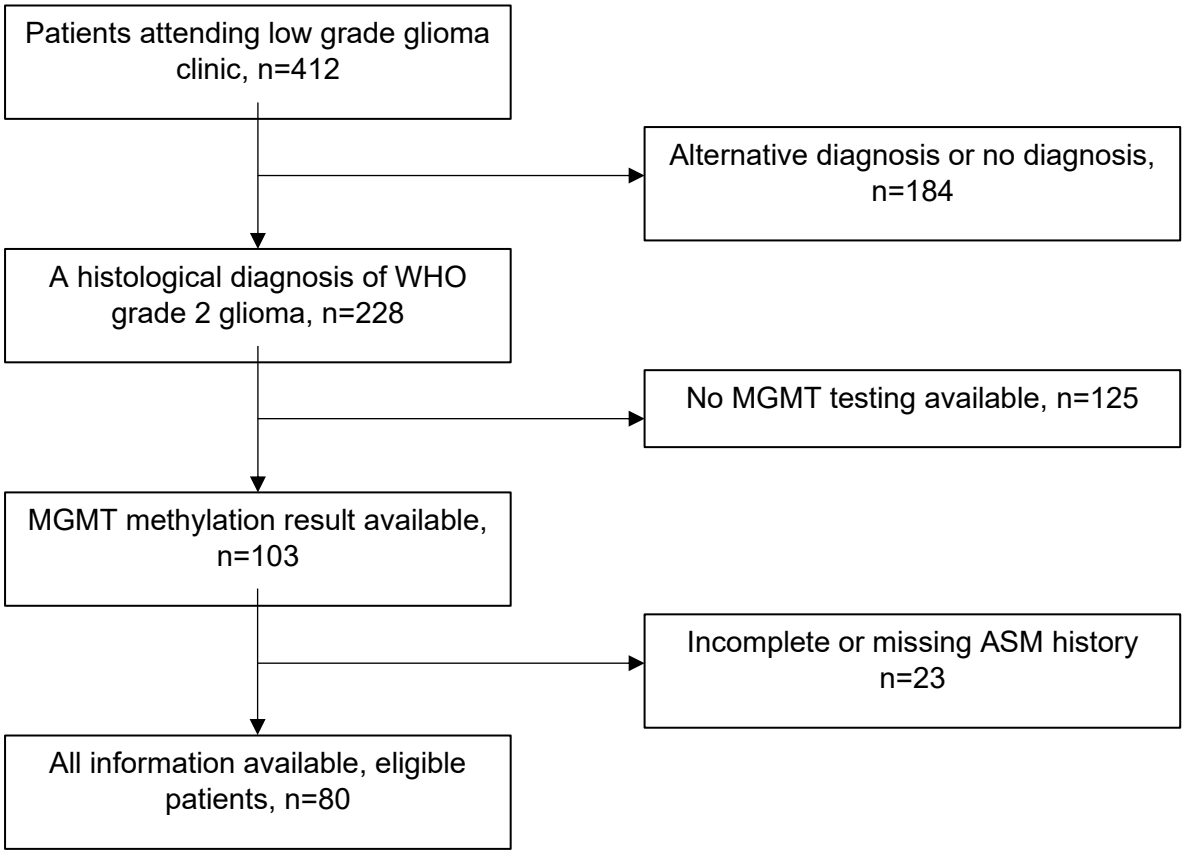

Figure 1- Flow chart depicting patient selection and eligibility

Data collection and study design: This was a retrospective observational study that examined the electronic medical records of patients between January 1997 and

December 2021. Data collected included: patient age at diagnosis, an integrated tissue/molecular diagnosis (according to WHO 2021 Tumours of the CNS criteria), MGMTp methylation percentage and ASM at the time of surgery/biopsy. The MGMTp methylation % was often reported as an average of the CpG sites 72-79. Where available, we also collected the individual methylation percentages for each of the number sites. For the sub-analysis in which individual CpG sites were analysed, 7 patients had missing data and were excluded.

Statistical analysis and variable selection: Study data was anonymised at collection. It was then coded to allow analysis using the statistical software 'R' (available via https://www.r-project.org/, version 4.3.2). Most analysis was performed using 'Base R'. Statistical testing was undertaken using a simple t-test as well as multiple linear and logistical regression modelling. Several variables were assessed for significance in relation to the mean MGMT methylation percentage value in addition to the use of levetiracetam. These were 'age at diagnosis', 'patient sex', 'histology (oligodendroglioma vs diffuse astrocytoma)' and 'if diagnosed with epilepsy'. Patient sex and the presence of epilepsy were not found to be significantly correlated with MGMTp methylation. The final multiple linear regression model to assess the effect of levetiracetam use on methylation percentage included 'age at diagnosis' and 'tumour histology' as co-variables.

Results

Using the multiple linear regression model, patients taking levetiracetam at the time of their surgery did not have a higher mean MGMTp methylation percentage than those taking an alternative ASM or no ASM (p=0.72, analysed as a single group). A

simple t-test (not including co-variables) returns a similar p-value of 0.76. The mean MGMTp methylation in the levetiracetam group was 24.0% compared with a mean of 25.1% in the no levetiracetam group. None of the CpG sites were significantly influenced by levetiracetam when analysed individually.

Removing levetiracetam status from the model, leaving only histology and age as predictors of methylation percentage, it was found that both remaining variables had a significant effect on mean MGMT methylation percentage. Having oligodendroglioma histology resulted in an increase in mean methylation of 8.2% (p=0.02). For each year increase of the age of diagnosis variable, average methylation increased by 0.34% (p=0.02). A scatter plot displaying age at diagnosis versus average methylation value is seen below (Figure 2).

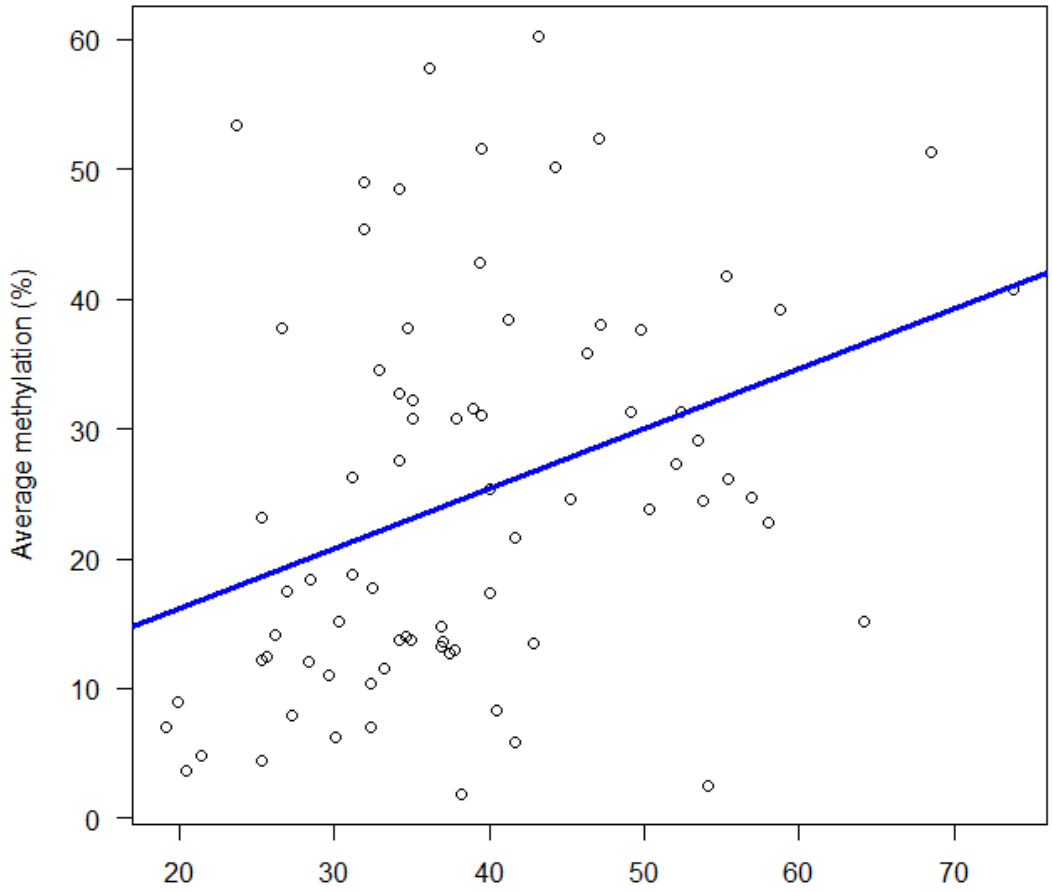

Figure 2- Scatterplot depicting age at diagnosis versus average methylation values

Despite these significant findings, the multiple R-squared statistic for the model was 0.19, suggesting high variance and that the variables within the model predict only a fraction of mean methylation percentage.

Discussion

Our results do not suggest that taking levetiracetam alters the average MGMTp methylation percentage in grade 2 glioma. Many of the previous studies regarding the ability of levetiracetam to downregulate the expression of MGMT protein have been conducted in vitro. Our study adds to the existing literature by using a clinical cohort and, although retrospective, attempts to answer whether levetiracetam may influence MGMT expression via methylation of its promoter region.

We found that mean MGMT methylation percentages increased with age of the patient at diagnosis. As methylation of the promoter region is thought to be irreversible, it may be that this is simply a function of cumulative methylation over time, be it mediated by p53 or other pathways. Patients who are older at diagnosis may have less aggressive tumours that have been present for a longer period of time compared with younger patients. It may be that age should not be considered a poor prognostic factor in IDH-1 mutant grade 2 gliomas.

Several factors could have influenced our study with regards to levetiracetam. One is that our laboratory does not routinely test for p53 mutations in glioma. For this reason, it is possible that by chance our levetiracetam group contained a larger number of mutated p53 tumours than would perhaps be normally expected. As discussed previously, this lack of presence of intact p53 protein could potentially derail the ability of levetiracetam to increase methylation levels.

Another possibility is that the levetiracetam is being given too late to ‘catch’ intact p53 protein. It is possible that the methylation percentage of a glioma is a function of when the p53 protein becomes mutated in the tumour’s existence. Early mutation of p53 may result in a relatively small window of opportunity for p53 protein to facilitate methylation of the MGMTp. Even if the mutation of p53 occurs relatively late, if levetiracetam is not given before this point it would still be unable to upregulate the process of methylation. Even if the p53 mutation status was known, we are unable to know when exactly in a tumour’s life cycle, the p53 mutation occurred and where this was in relation to the initiation of levetiracetam.

In the study by Bobustuc et al., 4 patients underwent biopsy in a short period of time with levetiracetam being initiated prior to the second operation. Although it is tempting to assign the increase in MGMT methylation percentages seen in all participants to the medication, other differences are likely to exist between the two biopsy events. Primarily, following the first surgery, there will likely be local post-surgical changes causing an inflammatory response. It is possible that any functional p53 protein is upregulated by this neuronal injury by other mechanisms and that this upregulation leads to an increase in MGMT methylation in tumour at the site of surgery. All of the patients in our study had grade 2 gliomas. It may be that the effect of levetiracetam is only seen in high-grade gliomas. A similar study could easily be performed in a glioblastoma population, and likely with a larger sample size due to its relatively higher incidence, in order to assess this.

Conclusion

Using a clinical cohort of WHO grade 2 glioma patients, we did not find that levetiracetam influenced the mean MGMT promoter methylation percentage. This contradicts many of the previously conducted in vitro studies, whereby levetiracetam was considered beneficial in glioma outcome. Further study of other commonly available ASMs may be required in order to understand how they may influence the natural history of glioma beyond that simply of seizure control.

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Critical discussion

The importance of seizures in grade 2 glioma

Seizure incidence across all grades of glioma presents an apparent paradox by which the lower the glioma grade, the more likely the occurrence of tumour related epilepsy. This is starkly demonstrated in the grade 2 glioma population. In the patient cohort studied above (Paper 2) we found that of 228 patients with grade 2 glioma, 86.4% received a diagnosis of epilepsy at some point during follow up. Furthermore, seizures appear not only to occur frequently, but also early in the disease course. Of the 197 patients who were classified as having epilepsy at last follow-up in our study, 180 patients (91.4%) had seizures listed as their initial presenting complaint. Being the heralding symptom of many grade 2 gliomas, seizures therefore present an opportunity to better understand glioma neurophysiology and development as well as serving as an important clinical indicator. As discussed in the introduction chapter, theories around why seizures are so prevalent in grade 2 glioma vary; from perhaps inferring particular genetic mutations (such as the presence of an IDH-1 mutation) to potential dysfunction of neurotransmitter homeostasis. For patients, improving our understanding of brain tumour related seizure generation may allow us to identify the optimal treatment strategies for reducing seizure burden and improving quality of life. Epilepsy often has a profound impact on a person's day to day functioning be it regarding their ability to work, drive or their psychological wellbeing.

Whether antiseizure medications are able to alter the prognosis or course of a tumour remains open to debate. Studies are yet to demonstrate an added benefit of improved survival in patients taking antiseizure medications, despite multiple possible mechanisms being proposed. Our study (Paper 3), exploring the possibility

that levetiracetam could alter MGMT promoter methylation, continues this trend. Though studies including ours have proved negative to date, it remains an interesting avenue of research. A tumour does not exist in a vacuum, it remains a part of the brain and influences its structural and chemical makeup. It would seem logical that seizures influence or shape tumour development in some way, be it for better or worse we are yet to discover.

Predictors of tumour related epilepsy

Factors that predispose some patients more than others to develop tumour related epilepsy are reported in the literature. These include tumour location, presence of an IDH-1 mutation, tumour histology and patient age. We investigated and/or included some of these known factors when constructing our statistical models in *Paper 2*.

Tumour location: In the preliminary stages of the project, gliomas were assigned 1-2 tags depending on their location and involvement of particular brain regions (either frontal, parietal, occipital, temporal, insula, brainstem or cerebellar). Basic frequency tables were drawn up and did not show any significant difference in epilepsy prevalence between any of the supratentorial locations, with all showing frequent co-morbid seizures (>80% in all regions). Brainstem or cerebellar regions showed a lower incidence of epilepsy. This is similar to other authors, though it has previously been suggested in the existing literature that tumours involving the occipital lobe are also less likely to cause seizures than other supratentorial areas. In our cohort 76% of patients with occipital lobe tumours experienced seizures, similar to the other supratentorial areas (range 70.7-82.7%). It may be that in other cohorts these seizures are under recognised, ignored or mistaken for other pathology, such as

migraine. Insular tumours resulted in the highest rate of epilepsy (82.7%) in our patient group.

Despite some concordance with other authors, with regards to supratentorial areas in particular, it was felt that the approach outlined above, with a heavy focus on tumour location, was insufficient. In grade 2 glioma, deciding whether or not there is involvement of a particular brain region on routine MRI alone can be difficult. These are often large tumours spanning multiple regions and so involvement of several lobes simultaneously is not uncommon. In addition, it is thought that they possess far reaching, spindle-like projections and invade diffusely and widely early on in their development. This occurs beyond the visible margin seen on neuroimaging, hence the lack of curative surgical treatment. When assigning location tags to the tumours during the data collection phase the choice of which tags were the best fit for each tumour often felt slightly arbitrary and unscientific. Not only does the use of location tags in this way poorly reflect the position of the tumour, but it also manages to ignore the most commonly held theory that the tumour margin is the critical region for seizure generation. If, for example, a tumour had its core sited approximately at the insula, but the peri-tumoural margin was predominantly based within the parietal and temporal lobes, it may be inaccurate to label the tumour as primarily insular from a seizure generation perspective. How exactly to get around this issue for ourselves and future researchers is not completely clear. It may be that in future work we would be able to map out the surface area of the tumour in 3D using neurosurgical guidance software. Delineating the tumour margin in this way still has downsides however, as the approach is one of proximity to structures rather than connectivity to important region. Perhaps the use of tractography to delineate tumour proximity to major networks of neurons is another future avenue. For all of the above reasons,

despite the frequency with which it is reported in the literature, it was decided not to include tumour location as a significant factor in the projects.

Tumour genetics: Multiple studies have shown than IDH-1 mutated gliomas are more likely to be frontal in location and it may be that their association with seizures is a result of proximity/connectivity to structures prone to epileptogenesis in the frontal lobe. The updated WHO classification in 2021 narrowed down the definition of grade 2 glioma by essentially removing the category of IDH wild-type grade 2 tumours. These tumours are now considered grade 4, on par with glioblastoma. This update meant that only IDH mutant tumours could be included in the studies, further limiting our cohort numbers. For this reason, there was no need to control for IDH 1 mutation status as a variable, as is often required in older work in the literature.

Tumour histology: It has been previously reported that oligodendrogliomas are more likely to cause epilepsy than diffuse astrocytomas. This was not the case in our cohort following analysis with Chi square testing ($p=0.42$). It is possible that this has arisen from older studies including less epileptogenic tumours in the astrocytoma cohort that by modern classification would not be considered grade 2 gliomas.

Patient age and sex: The age at diagnosis was compared between those patients that presented with epilepsy to those that did not as it has been suggested that older patients are less likely to develop seizures. Using a t-test, no significant difference between the groups ($p=0.17$, mean age in ‘seizures at presentation’ group = 42.0 years, mean age in ‘alternate presentation’ group = 39.2 years) was found, with the seizures at presentation group in fact being older. Paper 3 demonstrates that mean MGMT promoter methylation appears to increase with the ‘age at diagnosis’ variable.

It may be that patients who are older at diagnosis have less aggressive tumours which in turn may be less likely to cause seizures.

Epilepsy may be more common in men with grade 2 glioma. In our cohort (Paper 2) males were more likely than females to be diagnosed with epilepsy (91.1% vs 77.1%, p=0.003).

Predictors of seizure freedom

Key finding – Patients with predominantly focal to bilateral tonic clonic seizures are more likely to achieve seizure freedom at any point within 10 years of diagnosis compared to those with purely focal seizures (paper 1).

Seizure freedom in grade 2 glioma may be influenced by multiple factors. Seizure type, time since epilepsy onset and tumour location. Surgery is also a major factor in terms of seizure outcomes and should be considered in itself as an antiseizure treatment.

Seizure type and the role of surgery: We found that patients who experienced predominantly focal seizures were much more likely to have refractory epilepsy than those that experienced secondary generalisation. In our study (Paper 1) we classified seizure onset type as either focal or focal to bilateral tonic-clonic based on the ILAE classification. We treated all tumour related epilepsy as focal in origin, as describing these seizures as having 'generalised' onset is not logical given their likely origins in the peri-tumoural margin. During data collection patients had surprisingly stereotyped semiology with very few being hard to classify retrospectively. In the Cox proportional hazards model of time to seizure freedom in Paper 1, whether or not a patient is prescribed levetiracetam is controlled for along with the seizure type. At

each time interval (2, 3, 5 and 10 years) a patient was between 39-42% less likely to attain seizure freedom if they had predominantly focal seizures without bilateral tonic-clonic spread (all $p<0.05$).

The fact that this difference between seizure types persists for up to a decade suggests that not only might ASM therapy be less effective against FwP seizures but that debulking surgery may also have more antiseizure effect against seizures that generalise to involve bilateral tonic-clonic activity vs those that do not. All patients in our cohort had surgery, though the extent was variable and had to be controlled for in the models. As a debulking procedure is likely to cause widespread disruption to cortical networks, it is possible to see how bilateral tonic-clonic seizures may be more readily averted via a post-operative reduction in connectivity- even though not all tumour tissue can be resected due to their infiltrating nature. Equally, it may the case that seizures that would have generalised pre-operatively are commuted post-operatively to focal seizures due to the aforementioned reduction in connectivity. This would result in an apparent persistence of refractory focal seizures in the post-operative population.

Time since epilepsy onset: It is logical to expect that currently symptomatic patients with a longer time since seizure onset would be more likely to have refractory epilepsy, as they will no doubt have had ample time to trial various antiseizure treatments, which have presumably failed. For the majority of our cohort, the onset of seizures signified the unveiling of the glioma and none had significant seizure histories prior to the glioma being diagnosed.

Tumour location: As with the risk of ever developing epilepsy, tumour location has been linked with refractory seizures. For similar reasons as above regarding

accurate and reflective classification of tumour location, we did not assess for the impact of tumour location on the chance of seizure freedom.

Predictors of seizure type

Key finding – Men with grade 2 glioma are more likely than women to be diagnosed with epilepsy and are more likely to experience at least one focal to bilateral tonic-clonic seizure (paper 2).

Men with epilepsy were much more likely than women to experience focal to bilateral seizures in our patient cohort (p=0.05, 69.4% vs 54.1%). With men being more commonly diagnosed with tumour related epilepsy than women, the question arises whether there is a physiological reason for this discrepancy in the incidence of epilepsy or whether women may be being underdiagnosed. Seizures with secondary generalisation can be much more clinically obvious compared with focal seizures that can sometimes be subtle. It's possible that epilepsy is as common in both sexes and that the tendency for women to have seizures that could be purely sensory or involve phenomena such as deja-vu leads to them being given alternative diagnoses such as migraine, or the symptoms being underreported or ignored altogether. Whether this is the case or not isn't clear from our cohort as it relies on the diagnosis of the clinicians seeing the patients at the time. There does however seem to be a trend in the literature whereby the incidence of epilepsy in grade 2 glioma in older studies was reported to be around 60%, whereas newer studies often cite an incidence of 80% or more. This perhaps suggests that recognition of some of the more subtle forms of epilepsy is improving or, that due to the significant leaps

forward in genotyping, we are starting to analyse more homogenous brain tumour populations.

Tumour laterality and the risk of secondary generalisation

Key finding – Left hemisphere grade 2 gliomas were twice as likely to ever have resulted in a focal to bilateral tonic-clonic seizure compared with right hemisphere gliomas (paper 2).

A simple metric obtained during the data collection phase of this project was the laterality of the tumour. This was coded as either ‘left’, ‘right’ or ‘midline/bilateral’. In Paper 2 we report significant differences in the risk of ever experiencing a focal to bilateral tonic-clonic seizure based on tumour laterality. We found that patients with left sided tumours were much more likely to experience focal to bilateral tonic clonic seizures than those with a right sided tumour ($p=0.02$). To our knowledge this has never been previously reported in the glioma literature. We assume that in our study population, the majority of patients are left hemisphere dominant as would be found in the general population. This is not something that we were able to ascertain from the medical records or functional imaging retrospectively, though there seems no reason to doubt that this would be the case. We would therefore suggest that seizure activity in the dominant hemisphere is more likely to result in propagation across to the non-dominant hemisphere than the other way around. There were no obvious confounders seen on testing that could have influenced the risk of focal to bilateral seizures occurring more commonly from left hemisphere origins. Specifically, there was no difference seen in the laterality of tumours in males vs females (Fisher’s,

p=0.33). Tumour histology also did not influence the risk of ever experiencing a focal to bilateral seizure (Fisher's, p=0.22).

A clue as to why laterality may influence the evolution of focal seizures may be found in the general epilepsy literature. In a study by Seethaler et al. it was shown that left hemisphere origin focal seizures were likely to have a longer duration than those on the right.[110] It is reasonable to assume that the longer a seizure lasts in one hemisphere the greater the risk of the other becoming involved. Whether this is because regional connectivity in the dominant hemisphere is more permissive of seizure continuation, or that the non-dominant hemisphere is less able to resist involvement, is not clear. Reproduction of this result by other groups is required and it would be of interest to see whether this relationship holds for other types of lesional epilepsy or even other grades of glioma. It is therefore recommended that tumour laterality should be considered as a risk factor for the occurrence of tonic-clonic seizure activity in future studies.

Survival in grade 2 glioma

Key finding – Experiencing predominantly motor seizures inferred an increased chance of survival at 10 years post diagnosis compared with patients with predominantly non-motor seizure types (paper 2).

Tumour histology and genetics: Grade 2 gliomas have a relatively favourable survival time in comparison to their higher-grade counterparts. One of the primary attributes of grade 2 gliomas that influences survival is the tumour histology. Oligodendrogliomas consistently show a much more favourable prognosis than diffuse astrocytomas. In our cohort this was no different. Patients with a confirmed

oligodendroglioma were significantly more likely to be alive at 10 years than those with an astrocytoma ($p<0.001$, 1df) as shown in Paper 2. In the literature, some of this discrepancy may be down to misclassification of tumours prior to the advent of genotyping or the inclusion in previous classifications of IDH wild type astrocytomas and it may be, that in studies taking place post the 2021 classification, the gap starts to narrow. As our study shows however, even with up-to-date classification incorporating tumour genotyping, a significantly different prognosis between the two histological types exists. MGMT promoter methylation status is well established as a prognostic marker in terms of response to chemotherapy in high grade glioma, its significance in grade 2 glioma is less certain. Ideally, we would have liked to include MGMT status in the survival calculations in Paper 2. Unfortunately, our cohort contained too much missing data and including MGMT at the exclusion of a large proportion of patients would have significantly underpowered the analysis. Ki67 values was also frequently missing from our lab data.

Patient age: Some studies include either advancing age or lower Karnofsky performance status (KPS) scores as a predictor of poorer survival. We did not have access to KPS data for our patient group and so used age at presentation in our survival models. Instead of using age groups, we simply added stratification by the age variable to the Cox proportional hazards survival model in Paper 2. This method does not calculate hazard ratios for the stratified variables and so we were unable to estimate how risk of death changes per year of age. A likelihood ratio test suggested that including age did not significantly improve the model's predictive power and so it was removed for the final analysis. As noted in paper 2, even when included, the significance of the variables of interest did not change.

Extent of resection and time to surgery: It was important to ensure that the extent of tumour resection was accounted for in the survival model, as earlier, more aggressive surgical debulking has led to improved outcomes in many centres managing grade 2 glioma in the last decade. The extent of tumour resection was judged using the post operative MRI scan (usually within 72hrs). The four categories used were ‘gross total’ (apparent complete resection), ‘near total’ (<3mm rim residual only), ‘subtotal’ (nodular residual) or ‘biopsy/gross residual’. The amount of residual tumour was usually reported by the neuroradiologists in their scan report, however, in cases where this was not immediately obvious the opinion of the neuro-oncology multidisciplinary team (MDT) was taken. There are some who advocate ‘supra-marginal’ resection i.e. going beyond the visible tumour margin. This is not something that is routine practice or commonly reported in radiology reports in our centre. It is possible that some patients had a supra-marginal resection though likely they would have been labelled as a gross total resection. As earlier surgery may be a factor that influences survival, the time to debulking surgery was accounted for in the survival models. We did not count patients who underwent a biopsy only as having significant surgery at that time point and, in these patients, if they went on to have subsequent debulking, the time to this second surgery was used. Both of the extent of resection and time to surgery variables were stratified in order to allow inclusion in the model. This is due to the fact that they were found to violate proportional hazard assumptions on model testing but were felt too likely to be confounders if left out entirely.

Presence of epilepsy: The presence or absence of epilepsy in grade 2 glioma and whether or not this influences survival is controversial. One possible confounder in the existing literature is the influence of IDH-1 mutations on the likelihood of epilepsy.

Though some studies report the presence of epilepsy at diagnosis to be a positive prognostic factor, it may be that this is simply a reflection of the IDH-1 mutation status, and that those without the mutation are in fact grade 4 tumours that are less likely to experience seizures. We did not find that either having seizures at presentation (Chi-sq, p=0.6) or ever developing epilepsy (Chi-sq, p=0.5) impacted overall survival. Our sub-analysis in paper 2 used the synthetic minority oversampling technique (SMOTE) with the aim of trying to negate the fact that the non-epileptic group in our cohort is small. Despite this additional work, the difference between the groups remained non-significant.

Mechanisms by which seizures could influence survival are not well understood, however, we have outlined some potential mechanisms around vascular supply in the initial review section (above) and also outline potential future studies that may address this question (below). Further study with larger cohorts may also be warranted.

Do motor seizures infer longer overall survival?

Experiencing predominantly motor seizures appeared to confer a more favourable overall survival time compared with those patients experiencing predominantly non-motor seizures. At 10 years after initial diagnosis, those with motor seizures had a 65% reduction in the risk of death (HR=0.35, p=0.02). This is despite accounting for previously described prognostic markers including tumour histology/genetics, time from diagnosis to surgery and the extent of tumour resection. That these patients are more likely to survive raises the question as to whether the motor activity marks out

a particular physiological attribute of a tumour or whether it leads to change in their management.

Tumour location and resection extent: Tumour location is possible explanation of why this relationship exists. As we have previously noted, oligodendrogliomas are more likely to occur in frontal location, however, we would expect this to be controlled for in our model as all patients had histology results. It may be the case that a frontal tumour is more amenable to a more radical resection with a greater margin due to the tumour being away from more eloquent areas controlling movements, speech and language. We again controlled for this to a degree by including the extent of resection variable. There was no significant difference in the extent of resection variable between the motor seizure vs non-motor seizure groups (p=0.85) with chi-square testing ('biopsy' 15.4% vs 16.7%, 'subtotal' 47.7% vs 52.8%, 'near-total' 23.5% vs 16.7% and 'gross total' 13.4% vs 13.9% respectively). It may be that there are differences in resection extent between the groups that are not visible in the way we have classified the variable and that more granular data would reveal this as a confounder. This seems unlikely however as the seizure types attributed to the patients are often present both pre- and post- operatively, and the motor seizure patients are not those whom have simply undergone greater debulking and developed epilepsy following radical surgery. If anything, seizures were much more likely to improve post operatively compared with before surgery.

Tumourigenesis and time to presentation: An alternative hypothesis may come from the differing timeframes with which patients are likely to present with motor vs non-motor seizures. Some of the non-motor seizure patients in our cohort experienced intermittent sensory symptoms or emotional/behavioural phenomena. Both of these presentations could easily be labelled as an alternative diagnosis for a significant

period of time in this relatively young patient cohort such as migrainous aura or psychiatric complaints. Frank motor seizures on the other hand are likely to be a greater cause for concern of neurological disease amongst both patients and the clinicians they initially present to, therefore resulting in urgent neuroimaging and a comparatively earlier diagnosis.

Whilst we know that seizures are a very frequent occurrence, and often the first symptom to manifest clinically in grade 2 glioma, it is not clear how early they occur in tumourigenesis. It may be that whilst seizures are the first clinical manifestation, their development is still several years after the onset of tumourigenesis, negating the impact of perhaps a few months earlier discovery due to experiencing more explicit seizures. It doesn't appear from our modelling that experiencing the most clinically obvious focal to bilateral seizures either regularly or at least once influences overall survival (Chi-sq, p=0.6). It is not possible to retrospectively date the birth of a tumour on MR imaging at the time of diagnosis though there are research strands that aim to model tumour growth kinetics.[111] For this reason, we do not know truly when in a tumour's existence epilepsy is most likely to occur. Epilepsy in grade 2 glioma may be inevitable. If so, it would seem that the majority of the non-epileptic cohort are diagnosed incidentally (that is that the original reason for their neuroimaging isn't actually a symptom of their glioma). These patients may, if they had been left uninvestigated, have gone on inevitably to develop epilepsy but for the surgical debulking that followed diagnosis of their glioma, preventing this outcome. If this were the case, we would perhaps expect that not having epilepsy at diagnosis would be a positive prognostic marker as the tumour may have been picked up earlier. This is not seen in our cohort, as previously noted, as presenting with

seizures did not show significance with regards to overall survival times (Chi-sq, p=0.6).

It is possible that seizure activity type infers something more fundamental about a glioma's biology. A brain that is more permissive to the development of motor seizures may also be one that the tumour finds more difficult to exploit as easily. Delineating why exactly this should be the case requires further work and future researchers should consider seizure semiology as a potential marker of the environment in which a glioma is to operate.

Antiseizure medication use in glioma

Key finding - The use of levetiracetam as a first line antiseizure medication results in roughly one-third of patients reaching seizure freedom at 12 months, with over two-thirds having achieved seizure freedom at 24 months (paper 1).

Key finding - Levetiracetam was more effective at inducing seizure freedom at 2 years (p=0.02) compared with a cohort taking an alternative ASM (paper 1).

There exists little consensus as to the optimal treatment strategy of seizures in grade 2 glioma. Most clinicians base treatment decisions on established strategies and common thinking already employed in the general epilepsy population. Many of the antiseizure medication studies in brain tumour related epilepsy mix tumours of all histology and grade. As seizures are such a cardinal feature of grade 2 gliomas and, as novel mechanisms of seizure generation are likely to exist, it seems an oversimplification to suggest that they should be treated in the same manner as tumours with likely much more conventional methods of epileptogenesis.

Prescribing in clinical practice: As we have discussed, epilepsy caused by brain tumours is invariably focal in nature. The highest quality evidence as to how we should treat focal epilepsy comes from the SANAD II trial, in which levetiracetam, lamotrigine and Zonisamide were pitted against each other in a randomised controlled trial measuring the time to seizure freedom from ASM initiation.[112] Lamotrigine was found to be more effective at inducing seizure remission than both levetiracetam and zonisamide as well as being more cost effective per QALY (quality adjusted life year). Although not conducted in a homogenous brain tumour population (though some patients in the study did have brain tumours), many physicians would extrapolate the results of SANAD II to imply that lamotrigine should be potentially the first line treatment in glioma related epilepsy. At our institution, levetiracetam was by far the most prescribed first ASM. In our analysis in paper 1, 101 patients were prescribed levetiracetam as a first ASM, with only 15 prescribed lamotrigine. There is no guidance locally to select levetiracetam first line in brain tumour related epilepsy. Several reasons likely exist that explain the high use of levetiracetam and the comparatively low use of lamotrigine. The most likely is that many of these patients have seizures at presentation and, in many cases, require treatment acutely in the emergency department. The current consensus in the management of first seizures denotes that whilst a first fit itself often does not necessitate initiation of an ASM, if neuroimaging reveals a potential lesional cause then the risk of seizure recurrence is much higher and an ASM may be required. Many patients with brain tumours may have a computed tomography (CT) scan of their head at first presentation that is abnormal, demonstrating a tumour, and hence an ASM is started. For clinicians in the emergency department, the reason to reach for levetiracetam ahead of all others is likely a combination of its favourable

administration route, rapid loading ability and general familiarity with dosing. Levetiracetam is available orally as well as in an intravenous preparation, whereas lamotrigine is oral route only. This means that if a patient is seizing or drowsy post-ictally, lamotrigine cannot be given unless they have parenteral access. The medium-term dosing schedule for levetiracetam is also more favourable as it can be quickly built up to a therapeutic dose over a matter of days, even quicker if a loading dose is used as is often the case in status epilepticus. Lamotrigine on the other hand has to be built up slowly due to the risk of Steven-Johnson syndrome and can take several weeks to get to a minimum therapeutic dose for most patients. It is likely that the reassurance given to clinicians that patients are likely to be taking an ASM at a therapeutic level by the time they are discharged from hospital (or very soon after) results in this bias towards levetiracetam. A helpful future trial would be to see whether the perceived ‘risky’ 6-8 weeks after starting lamotrigine, before it reaches a therapeutic dose, is actually as dangerous for seizure recurrence as physicians perhaps believe. Levetiracetam also has minimal to no interactions with other medications and specifically chemotherapy. Lamotrigine is also fairly benign with regards to interactions with commonly prescribed drugs, though can have its levels changed notably by oestrogen use/pregnancy.

Measuring seizure response to antiseizure medications: Measuring seizure response in a retrospective observational study is difficult as it relies on finding an easily measurable indicator of seizure freedom that can be reliably extracted from the clinical notes. We opted to mimic the measure used in the SANAD II study and to measure time to seizure freedom (defined as 12 months since a seizure). As we were reviewing mainly clinic letters, we found that, usually for reasons of driving eligibility, the date of a patients last seizure was often noted. This method has

imperfections as if a patient had a seizure relapse at one year and 1 day post their previous seizure this would not be picked up. Epilepsy is a relapsing-remitting disease and so there is no doubt that there will be a small number of patients with still quite problematic epilepsy that do not register as such in our study. However, seizures often beget seizures, and by 12 months of seizure freedom, at least in the general epilepsy population, patients can be reassured that relapse is less likely.[113]

Levetiracetam as a first choice ASM: In paper 1 we found that roughly one third of patients reached seizure freedom at 12 months, with approximately two thirds having reached seizure freedom at 24 months. Compared to a group of commonly prescribed alternative ASMs, those prescribed levetiracetam as a first ASM were 76% more likely to be seizure free at 2 years from ASM initiation (p=0.02, HR=1.76, CI [1.06 – 2.94]). Though levetiracetam is commonly perceived as being well tolerated it is not without side effects. These are primarily psychiatric in nature with irritability, somnolence and asthenia being the most common.[76] Use of levetiracetam as a first line ASM did not demonstrate a significant change to the risk of treatment failure (either inefficacy at treating seizures or side effects) when compared to alternative ASMs (p=0.82, HR=0.94, CI [0.53 – 1.66]).

The findings in Paper 1 suggest that levetiracetam is one of the more effective first line ASMs in patients with grade 2 glioma. We are unable to say as to whether it is the most effective, as the alternative ASM group contained a variety of agents, some of which may not be ones we would consider first line. In order to answer this question levetiracetam would have to be compared directly with another contender for the optimal ASM in this patient group. We would suggest that this other contender should be lamotrigine given the focal nature of seizures in this patient group and based on the SANAD II study. Unfortunately, we did not have the numbers in terms

of patients taking lamotrigine first line to be able to perform this direct head-to-head comparison. This is something we would to explore in future work and is discussed below in the future directions section. Including the time to treatment failure analysis in our study helps solidify the finding that levetiracetam is an effective choice by making unlikely the possibility that it causes high rates of treatment failure and that in fact whatever physicians were choosing locally as a second line agent was producing the beneficial effect. In the levetiracetam group 21.2% of those that failed treatment did so due to issues with side effects, with the other 78.8% simply not gaining a beneficial effect on seizure frequency. In the alternative ASM group 15.8% of those that failed did so due to adverse events. This difference was not significant ($p>0.05$).

The fact that the treatment effect was observed in the Cox proportional hazards model at 2 years, but not at 3 and 5 years, is perhaps unsurprising. The likely explanation for this is that by this point patients are unlikely to still be in their original first ASM group if they have poor seizure control and treatment has failed. Take for example a patient prescribed levetiracetam first; should treatment fail and the patient not reach seizure freedom then it is very likely that by 2 and certainly 3 years the patient will have either transitioned to another ASM that then proves effective or had one added in as polytherapy. Equally, patients in the alternative ASM group may be switched to levetiracetam, hence the loss of significance at these time points. Polytherapy is another potential confounder in our study. Despite treatment failure rates being similar between the two groups, analysis of the average number of ‘ASMs prescribed per year of follow up’ data suggests that the levetiracetam group were prescribed an average of 0.31 ASMs/year of follow up versus 0.24 in the alternative group. This may suggest that levetiracetam could be more likely to be

continued alongside a newly introduced agent than one of the alternative ASMs which could be more likely to be simply stopped. This may be a reflection of local practices at our institution or reflective of a broader mindset that levetiracetam often has a favourable side effect profile meaning cessation is not felt beneficial. Our study does not suggest that levetiracetam is better tolerated than other ASMs though is not specifically designed to compare them like for like.

Antiseizure medication and its effect on glioma

Key finding - Concurrent use of levetiracetam at the time of histological sampling is not associated with an increase in the mean methylation percentage of the MGMT promoter region (paper 3).

In paper 3 we assessed whether the use of levetiracetam in grade 2 glioma had any significant impact upon the measured percentage of MGMT promoter methylation at biopsy. We found that there was no significant difference between the levetiracetam and no levetiracetam groups, and they were in fact almost identical when compared using linear regression models ($p=0.72$). As the methylation percentage that is assigned by the lab is an average of 8 different CpG sites we looked further at whether any of the CpG sites in particular were influenced by levetiracetam. Again, there was not found to be any significant difference between the groups. It's possible that this extra-ictal effect of levetiracetam only applies to grade 4 and not grade 2 glioma, after all, MGMT methylation is only uncontroversially a positive prognostic marker in glioblastoma and not low grades.

Other factors may explain this negative result. The original small case series of 4 patients (and multiple in vitro studies) on which this study was based, suggested that the presence of non-mutated p53 protein was an important factor, as it is via p53 that

lamotrigine may mediate its supposed effect on MGMT protein. Unfortunately, p53 mutation status is not routinely tested in our lab as, in current grade 2 glioma understanding, it holds no prognostic value and does not suggest a change to treatment/management. Another possibility is that the levetiracetam is being given too late to ‘catch’ intact p53 protein and thereby exert its effect. Early mutation of the p53 gene may result in a relatively small window of opportunity for p53 protein to facilitate methylation of the MGMTp. Even if the mutation of p53 occurs relatively late, if a patient is not exposed to levetiracetam before this point it would still be unable to upregulate the process of MGMTp methylation due to its supposed dependency on p53. Even if the p53 mutation status was known, we are unable to know when exactly in a tumour’s life cycle p53 mutation occurred and where this was in relation to the initiation of levetiracetam. Levetiracetam may have been given already prior to the p53 mutation. Although it is tempting to assign the increase in MGMTp methylation percentages seen in the 4 participants at second biopsy in the Bobustuc et al. study to the medication, other differences are likely to exist between the two biopsy events. Primarily, following the first surgery, there will likely be local post-surgical changes causing an inflammatory response. It is possible that any functional p53 protein is upregulated by this neuronal injury by other mechanisms and that this upregulation leads to an increase in MGMTp methylation in tumour at the site of surgery.

We found that mean methylation percentages increased with age of the patient at diagnosis. As methylation of the promoter region is thought to be irreversible, it may be that this is simply a function of cumulative methylation over time, be it mediated by p53 or alternative pathways. Patients who are older at diagnosis may have less aggressive tumours that have been present for a longer period of time compared

with younger patients. We also found that oligodendrogliomas are associated with a higher mean methylation percentage. As previously reported in the literature there is clearly a complex entanglement between 1p/19q co-deletions and MGMT methylation status that is difficult to unpick.

Future directions

Study 4: Efficacy of lamotrigine in brain tumour related epilepsy

The levetiracetam efficacy paper (Paper 1) showed that choosing levetiracetam as an initial ASM in grade 2 glioma related epilepsy is a reasonable decision. As levetiracetam outperformed the alternative ASM group it is likely that it is one of the better choices. However, we cannot say that it is the optimal choice and the best evidence for treatment of focal seizures in the general epilepsy population comes from the SANAD II trial, a trial in which lamotrigine was found to be superior to levetiracetam. Ideally, we would have been able to pit levetiracetam against lamotrigine in a head-to-head comparison. Unfortunately, a very small number of the grade 2 glioma cohort were prescribed lamotrigine first line and so this was not possible.

As can be seen in our database, there is a large amount of data that is unused in each of the papers with starting cohorts of over 400 patients. This is because the initial data collection was of a ‘low-grade glioma’ population and this included many other tumour types such as gangliocytomas, pilocytic astrocytomas and DNETs. Additionally, many patients who are suspected to have a grade 2 glioma were omitted as they only had radiological evidence of a tumour. For whatever reason, be it co-morbidities rendering them unsuitable for surgery or them refusing surgery they did not undergo biopsy. It would be reasonable to perform a retrospective analysis including all the low-grade patients in our cohort. Although we cannot be clear which low-grade tumour these patients have, from a clinical perspective, as the person prescribing the antiseizure medication, the main interest is in whether or not they respond from an epilepsy point of view. Including this larger cohort may allow us to

better assess seizure freedom and treatment failure rates at similar time intervals as in study 1. There is a need for studies such as this as, for reasons not entirely clear to the author, lamotrigine seems to have been largely ignored in the brain tumour related epilepsy research sphere. Researchers have often sought to investigate newer or more novel drugs in the treatment of brain tumour related seizures rather than those that are already commonplace in the general epilepsy population.

A reasonable target cohort size to aim for with this study would be 50-60 patients prescribed lamotrigine first line. It may be that further data collection is needed (the database was collected in 2020, over 3 years prior to this work) to identify patients whom have been treated more recently. A sub-analysis of purely grade 2 glioma patients may be possible, though, given the inability to conduct this analysis at this present time due to sample sizes, this would likely take significant additional data collection. Nevertheless, this study should be possible and would add much needed data regarding lamotrigine in brain tumour related epilepsy to the literature.

Study 5: The effect of epilepsy status on ratio of cerebral blood volume measurements: a potential confounder?

and

Study 6: Do seizures influence chemoradiotherapy efficacy?

The ratio of cerebral blood volume (rCBV) has long been used as a measure to indicate the likelihood of a lesion being high or low grade. High grade tumours are associated with a higher rCBV, primarily due to their increased vascularity. For this reason, it is a parameter that is routinely measured using perfusion MRI both at initial staging scans and on routine follow up.

Seizures of any origin affect cerebral blood flow. They can cause profound hypoxia due to the intense metabolic stress which they induce. It seems logical that a tumour is subject to the same metabolic stress, if not more, than the rest of the brain. How this affects a tumours development is unknown. Some studies suggest that seizures are a positive prognostic sign, though why this may be, and the inconsistency with which this is finding is reproduced, is a clear issue in the existing literature. A hypothesis proposing a predominantly vascular mechanism of seizures modulating glioma growth seems logical. Following a seizure, changes in blood flow in affected brain regions lasting beyond the ictal phase have been observed, as seen on CT perfusion scanning in a study of stroke mimics.[71] We have one example in our cohort of a patient with a grade 2 glioma whom had a focal to bilateral seizure within 24hrs of perfusion MRI scanning. A significantly raised rCBV was subsequently reported. On follow up scanning, at which time the patient's seizures were controlled, this was never again demonstrated with the rCBV reverting to normal levels. It is possible that patients with ongoing seizures at the time of scanning are more likely to have a pseudo-raised rCBV which could in turn lead to a mistaken diagnosis of a tumour of

a higher grade. Epilepsy status would therefore be an important confounder of rCBV measurement.

A study could be designed in which rCBV measurements are taken in both patients with active epilepsy and those in seizure remission. It may be that this study would need to be prospective as working out the time since a last seizure from an MRI report, when often no clinical interview/history taking occurs in the days prior to scanning, would be difficult retrospectively. Patients could then go on to have a resection and the concordance between the histology and the rCBV prediction could be compared between the epilepsy group and the seizure remission group. As the changes in blood flow post seizure are transient there would have to be a definition of ‘active epilepsy’ that is not 12 months seizure free. Groups for analysis could be ‘seizure activity within the last week’ vs ‘remission for a month’ vs ‘never had a seizure’. This would rely on finding enough patients that were in the very active epilepsy group for the study to be adequately powered.

If seizures alter the blood flow to a tumour, another way in which they may alter the tumour course outside of radiological prognostication is during treatment with chemotherapy or radiotherapy. As chemotherapy for glioma is administered intravenously, greater blood flow to the tumour margin or other tumour affected brain regions at the time of treatment may influence drug delivery. This study would have to be undertaken prospectively, with patients’ current seizure status taken at the start of chemotherapy treatment. Volumetric response measurements to chemotherapy taken at the usual MRI scan post the third cycle of treatment could be compared between different seizure groups. Radiotherapy would also have to be taken into consideration as it too is affected by oxygen delivery. Radiotherapy requires the presence of oxygen to be effective and areas of hypoxia within tumours can lead to

treatment resistance in many tumour types. [108] It may be that either a) subclinical seizures at the time of radiotherapy cause hypoxia and thereby reduce its efficacy or that b) a history of seizures leads to increased angiogenesis/vascularisation of the tumour meaning that radiotherapy is more effective. Comparing radiotherapy outcomes between patient groups with a history of past seizures vs never having had seizures could also potentially be a prospective study in the future.

Conclusions

In the three papers detailed above, we explore the multidirectional relationships between grade 2 gliomas, tumour related epilepsy and commonly used antiseizure medications. We demonstrate that seizure semiology can infer an increased probability of seizure freedom (focal to bilateral tonic-clonic seizures) as well as the novel finding that motor activity seizures predict better overall survival. Conversely, tumour and patient characteristics such as sex and tumour laterality unexpectedly inform the risk of seizure generalisation. We show that one of the most commonly prescribed ASMs, levetiracetam, is an effective first choice agent in this patient cohort for seizure prevention as well as in terms of drug tolerability. Despite a promising previously published case series and a theoretical framework as to potential mechanisms, levetiracetam does not appear to alter MGMTp methylation in our patient cohort.

Being able to better gauge both overall survival and seizure trajectory by way of readily available clinical and patient factors improves the clinicians ability to prognosticate in addition to hinting towards underlying biology of these distinct tumours. With the uptake of tumour genetics in routine care, future patient cohorts should cease to be as heterogeneric as they have done historically. Additionally, future researchers should consider a wider range of factors, beyond purely surgical measures, that better describe how these tumours adapt and interact within the environment upon which they depend.

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Appendix 1 – Caldicott Letter

Enquiries to: Information Governance Team
Date: 28th September 2021
Our Ref: Low Grade Glioma

The Leeds Teaching Hospitals NHS Trust

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Re: Seizures in low grade glioma

Dear Dr Maguire

Thank you for your application for Leeds Teaching Hospitals NHS Trust regarding the seizures in low-grade glioma project.

Seizures represent a common symptom in low grade gliomas, when uncontrolled; they significantly contribute to patient morbidity and negatively impact quality of life. This patient population is under-studied and little is known as to the mechanisms which contribute to seizure generation in low-grade glioma.

The purpose of the project is to:

- Assess the current burden of seizures in the low-grade glioma population in West Yorkshire,
- Assess seizure type, frequency and control,
- See how the Trust currently treats these patients with antiepileptics,
- Identify common factors in determining prognosis, seizure control and first treatment failure rates.

A review of eligible patient records will take place, and data collected by members of the direct care team. All data will be de-identified and stored securely on the Trust network.

As no new or additional data is being collected, patient consent will not be required.

The results from this project will be published in a relevant medical journal and will also be used by Dr Sam Fairclough as part of her Master Dissertation write up. Data will be stored on the Trust's Neurosciences department for future reference and to help inform future studies.

Dr Maguire has demonstrated a clear understanding of the Data Protection legislation and Caldicott guidelines, understanding her duties to comply fully with the legislation during the collection and processing of Trust data/other organisations data.

I am happy to express my support for the seizures in low-grade glioma project and wish Dr Maguire well with her project.

Yours sincerely

J G. McElwaine

Dr John McElwaine
Deputy Caldicott Guardian
Leeds Teaching Hospitals NHS