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Research Article

A Clinical Audit: Assessing the Diagnostic Delay of Primary Brain Tumors, Glioblastoma IDH-Wild Type and Primary CNS Lymphoma; Exploring the Use of CSF Liquid Biopsy

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Abstract

Background: Diagnostic delay is a critical issue in healthcare, often leading to delayed treatment and poor patient outcomes. Diagnostic errors contribute to about 10% of patient deaths annually. Delays are very prevalent in Glioblastoma and Primary CNS Lymphoma. Both have an aggressive nature and a short survival rate, which urges for early diagnosis to ensue treatment as quick as possible. The delayed diagnosis can be attributed to tumor-specific factors such as challenging differential diagnoses, inadequate methods in achieving optimal outcomes, and the presence of confounding factors like steroid usage.

Aims and Objectives: The objective of this clinical audit is to analyse patient records within an oncology unit. The primary goal is to assess the quality of practice by evaluating the standards of diagnosis and treatment. Additionally, the audit aims to pinpoint areas that require improvement at every stage of patient management, encompassing administration, symptom evaluation, and post-therapeutic measures. Based on these findings, a comprehensive plan of action will be proposed to address the identified issues effectively.

Standards and Methods: In this Clinical Audit, we assessed the adherence of 10 Glioblastoma and PCNSL patients in a neuro oncology unit, to standards and guidelines set by professional medical associations. Which include World Health Organisation (WHO), National Health Service England (NHS), and European Association of Neuro-oncology (EANO).

Results: Our cohort showed long diagnostic delays, minimum and maximum values of 30 and 1825 days. The sample did not show significantly longer delays compared to other PCNSL or HGG patients, all collected from the literature, with p values of 0.174 and 0.637, respectively.

Conclusions: In Conclusion, Diagnostic Delay attributed to PCNSL and Glioblastoma can be pinpointed to the inadequacy of existing standards in relying solely on stereotactic biopsies for definite diagnosis when it may not be ideal for all patients at all tumor stages. A plan of action encouraging large clinical trials of CSF Liquid Biopsy is recommended with an employment of recent advances and focusing on detecting circular tumor DNA for the diagnostic insight it provides. As well as exploring its use during or post therapy to monitor the lesion and pathology. To also adhere strictly to the discouragement of steroid use for the negative prognostic factors it causes.

***Keywords:** CNS Lymphoma; Brain Tumors; Glioblastoma IDH-Wild Type; Clinical Audit*

Introduction

Primary CNS Lymphoma and Glioblastoma – IDH Wild Type are two types of primary brain tumors, with a very aggressive nature. They present very similarly clinically and radiologically. In addition to a poor prognosis and an average survival rate of 14 months for Glioblastoma [1] and a 5 year relative survival rate of 30% for PCNSL Patients [2].

There is exceeding importance in exploring tools that may have a positive effect on the overall survival, and better prognosis. To do this, gaps that may have a negative impact need to be identified and addressed. This is done by assessing guidelines that have been set to ensure the best possible outcome using the available tools.

This Clinical Audit aims to achieve an understanding of how well implemented are the guidelines, and to also compare findings with published studies in the same area, to assess and identify any shortcomings and their attributed cause. One of the main problems that faces PCNSL and Glioblastoma – IDH Wild Type is diagnostic delay [3]. This is expected to be found in our sample, and a plan of action is to be proposed.

Glioblastoma- IDH Wild Type

The 2021 World Health Organization (WHO) classifies Grade 4 Glioblastoma- IDH wild type as what was previously known as Glioblastoma multiforme (GBM) [4] . They are malignant brain tumors and are very aggressive cancers that invade brain tissue rapidly by mitotic characteristics causing necrosis and hemorrhage[5]. It is challenging to diagnose due to its differential diagnoses and the multiforme genetical and molecular characteristics. These characteristics manifest grossly in a multitude of forms that challenge a singular form of treatment.[6]

Glioblastoma is the most common adult brain tumor and has a high incidence (0.59 to 5 per 100,000) that is rising due to different factors[7]. It is incurable and has a high relapsing probability, along with a low life expectancy; this increases the importance of early diagnosis for an immediate therapy start.

Clinical Presentation:

Most cases are sporadic, although patient's history is important to locate any high risks. Which may include an increased familial risk, or exogenous factors such as a highlighted exposure to radiation [8]. Symptoms include focal neurological deficits, seizures, and symptoms of increased intracranial pressure. Unspecific symptoms like headaches and fatigue are very prevalent which may play a part in an overall diagnostic delay. [9]

Biomarkers:

Glioblastoma- IDH wild type is diagnosed and distinguished with an IDH mutation, confirmed status of TERT (Telomerase Reverse Transcriptase) promotor mutation, EGFR (epidermal growth factor receptor) amplification, or a gain of chromosome 7 or a loss of chromosome 10[9]

Management:

If symptoms are severe; dexamethasone, a corticosteroid is to be given at a low starting dose and should be stopped once symptoms start to improve, to minimize the adversity of its effects.[10] Radiography is done to locate the tumor and ideally, a stereotactic biopsy would follow to confirm immunophenotypes and genetical variants.

Treatment:

Maximal surgical resection of the mass is the current therapeutic approach, followed by concomitant radiotherapy and chemotherapy with or without temozolomide (TMZ) [11]. Glioblastoma has distinct subclasses (classical, mesenchymal, proneural) that cause different pathological and clinical presentations which respond differently to therapy. In addition to its proliferative, heterogenous and chemo resistant nature that compromises therapy outcomes[12]

Primary CNS Lymphoma

PCNSL is a non-Hodgkin tumor that derives from lymphoid cells and are 95% B-cell cases (DLBCL) and rarely, T-cells. It classifies as intraparenchymal as it arises in the brain and spinal cord. it may also involve the eyes (ocular PCNSL)[13]. PCNSL cells may enter the cerebrospinal fluid. A small number of cells and exosomes enter the bloodstream. PCNSL is more common in people who are immunocompromised. Presenting symptoms include focal neurological deficits, cognitive deficits, seizures and increased intracranial pressure that often lead to symptoms of headaches and nausea, all of which can overlap with glioblastoma patients.

Biomarkers:

Molecular Markers include recurrent mutations of MYD88 and CD79B and Frequent copy number gains of 9P24.1. High expressions of CD20 (B-cell surface marker) identify the presence of neoplastic cells[14]. Histologically, cells are highly proliferative; they appear to infiltrate the brain parenchyma and exhibit an angiocentric growth pattern. [15]

Management:

The gold standard for diagnosing PCNSL is surgical biopsy [16]. Steroids should be avoided prior to stereotactic biopsy for suspected PCNSL [17]. Stereotactic biopsies are carried out if the lesion is intracranial. Vitreous biopsies are otherwise carried out if the lesion is in the eyes. [2]

Treatment:

Treatment is over two stages, Induction and Consolidation. Induction stage targets tumor cells to eradicate cancerous cells using a high dose of methotrexate (HD-MTX) that is combined with rituximab and other cytostatic drugs that penetrate the BBB 18. Consolidation strategies depend on how well the patient responds to the chosen induction therapy and aims to maintain the remission phase. It may include myeloablative chemotherapy with autologous stem cell transplant (ASCT), or a high dose of non-myeloablative chemotherapy, or radiation [18]. Sometimes it would only necessitate general medical management and observation. [17]

Radiology's role in diagnosis

Magnetic Resonance Imaging (MRIs), Computerized topography (CT-Scans), Magnetic Resonance Spectroscopy (MRS), and Single Photon emission computerized topography (SPECT) are all used to visualize or showcase different characteristics of a tumor. They are important to localize the lesion, to give a clear trajectory to extract a biopsy of the mass, or to perform surgery. [19]

They can be very useful in revealing information about the tumor. Albeit is not enough to diagnose and differentiate PCNSL nor Glioblastoma. They overlap in radiological presentation of peritumoral edema, mass effect [20] and contrast enhancing lesions where neoplastic cells extend beyond the area of enhancement [21].

MRS can be used advantageously by classifying levels of metabolites in the brain to differentiate between PCNSL and Glioblastoma; an example of apparent diffusion coefficient (ADC) which is comparatively lower in PCNSL than in Glioblastoma [22]. Other common metabolites include Choline, Creatinine, Lactate, and lipids [23]. Yet, despite the support some imaging findings have in distinguishing or diagnosing, it is not established or relied upon due to the insignificant findings and figures that are showcased by different studies [24].

Alternative diagnostic methods can provide valuable information that complement brain biopsies in their outcome. They include a range of tests, some of which are reliable and essential for diagnostic direction prior to undertaking any surgical action. And others that are more innovative, and less relied on which include MR spectroscopy, PET scans, Blood, and CSF Analysis.

Stereotactic Biopsies

Importance:

Brain Stereotactic biopsies are done to acquire tissue from the lesion or mass itself. The biopsy is stained so that the tumor can be graded histopathologically. The tumor cells undergo molecular profiling to locate mutated genes or the protein products of mutated genes [25]. A neuropathological diagnosis of a brain tumor is a combination of the molecular markers and histopathological grade. It is applied using different techniques, such as Flow cytometry (FCM) and immunohistochemistry. It is a reliable technique, with a high diagnostic yield and is a valid source for rapid identification and accurate FCM categorization of CNS lymphomas.

Advantages:

It holds an advantage over microsurgical resection, for its less invasive nature and its safer approach. Recent advances have increased its sensitivity and diagnostic yield; A sensitive analysis of a panel of different molecular markers can be obtained even from small specimens, which was not viable until the introduction of contemporary refined technologies of molecular biology. [25]

Disadvantages:

While the sampling and examination of different areas of a heterogeneously composed tumor is being trialed with image guided tools, it is still challenging. along with its contraindication with bleeding disorders and whom are unfit for the procedure [26], which may eliminate or exclude many of the patients in need of a diagnosis confirmation to proceed to therapy. It also provides one snapshot of the disease at the time the biopsy is taken, which is often used to dictate therapeutic options later on [27]. This is not ideal as the tumor changes during the course of the disease and would compromise the effectiveness of chosen therapy and overall survival.

Steroids effect on the trajectory of Diagnosis

Steroids are given to manage severe symptoms, by reducing the increased intracranial pressure and increasing blood vessel permeability, reducing inflammation and edema. Leading to improved symptomatology and pain relief [28]. Dexamethasone, is a well-known and commonly used steroid for its antineoplastic effects against hematologic malignancies [29].

Despite its advantages, Steroids are not well recommended. Imaging can be compromised by the use of steroids; as they signal to apoptosis by binding to ligands [30]. Tumor lysis occurs and the lesion disappears radiographically, preventing an extraction of a biopsy for the lack of radiographic evidence, or leads to an inconclusive biopsy. This leads to a re-scan after a plan to wean off of steroids [31]. The lesion is also highly prone to return in an aggressive manner after a halt in their administration [32]. And if treatment is not initiated then death could occur.

According to West Midlands Cancer Alliance protocol for steroid use in PCNSL patients [33]; Steroids are to be avoided or stopped if diagnosis is suspected and can be commenced in regulation if diagnosis is confirmed.

As for High grade gliomas or glioblastomas, Steroids have shown to have a negative effect on the prognosis of the cancer, and its treatment [29]. Thus, is not given if patient is asymptomatic or slightly symptomatic and is tapered to the lowest dose possible for essential symptomatic relief and following surgery.

Methods

Retrospective review of the diagnostic process for glioma mimic patients was carried out. 12 patients of a nonspecific age group, ranging from 26 to 76 years old and nonspecific to gender; 6 males and 6 females were included. Data were collected of patients in a regional tertiary neuro-oncology unit and included new and follow-up patients.

The data included comorbidities, dates of first signs and symptoms, dates of referral and the dates of investigations or surgeries being carried out. Also includes histology results, description of MRI and CT scans and when needed, other investigations. Suspected diagnoses were noted with a follow up plan by multidisciplinary teams and the current working diagnosis for additional discernment.

Data was refined and summarized to fit a diagnosis of HGG or PCNSL for the aim of this audit. 2 subjects were excluded from the analysis due to their unrelated confirmed diagnoses. No HIV positive patients were reported. 4 subjects do not have a confirmed diagnosis, but highly suspected gliomas or lymphomas. Biopsies have not been carried out for these 4 subjects for underlying factors that will be discussed and have caused a significant delay.

Different time spans were extracted, the data is not normally distributed, so the medians and interquartile ranges are reported. “First symptoms to referral”, is to assess how long it takes for the patient to report their symptoms, and for the symptoms to be reported to a neuro oncology unit. “Referral to Diagnosis” mostly reflects the patient’s condition to undergo a biopsy. Delays should be short following referral, if the patient’s condition allows to continue with protocol set by multidisciplinary teams.

For the lack of a consensus in the literature regarding the definition of a delay, or what is deemed to be an early diagnosis for PCNSL or Glioblastoma; figures were compared to the median of collected and grouped studies. PubMed and Google Scholar search bars were used to look up all studies under “Gliomas/Glioblastoma/Lymphoma/PCNSL/DLBCL diagnostic delay” and also using other phrases such as “diagnostic time”, “patient delay”, “doctor delay”, and “symptom to diagnosis” before or ahead of the words.

Figures adapted excluded lower grade gliomas, and all lymphomas that were not classified as PCNSL, DLBCL, or T-Cell Lymphoma. AIDS patients were excluded, as well as studies that were affected by Covid -19 pandemic. Data collected did include patients admitted to the emergency department, outpatients and rapid diagnostic clinics. Literature Data across different Diagnostic timespans are found in Tables 1,2 and 3.

Symptom to Diagnosis		Median / days
Stensjøen, A. L., Berntsen, E. M., et al [34]	Glioblastoma	330
Aggarwal, A., Herz, N., et al [35]	HGG	8 (A&E) 26 (OP)
Velasco, R., Mercadal, S., et al [36]	PCNSL	47, 64 with steroids, 40 w/o
Zurko, J.C., Wade, R.C. et al [37]	DLBCL	152.1
Haldorsen, I. S., Espeland, A., et al [38]	PCNSL	75
Nixon, S., Bezverbnaya, K., et al [39]	Lymphoma	16, 28
Bosch, X., Sanclemente-Ansó, C., et al [40]	Lymphomas	51
Cerqua, R., Balestrini, S., et al [41]	PCNSL	39
Cerqua, R., Balestrini, S., et al [41]	Glioblastoma	24.5
Patel, V., Mcninch, NL., et al [42]	CNS tumors	42
Rask, O., Nilsson F., et al [43]	HGG	186.2, 38.5, 37.8
Maaz, A. U.R., Yousif, T., et al [44]	HGG	15
Kukal, K., Dobrovoljac, M., et al [45]	HGG	49
Nikonova, A., Guirguis, H.R., et al [46]	DLBCL	56
Jennings, C., Livingstone, K.,et al [47]	DLBCL	24
Castro, A.B., Seoane, J., et al [48]	DLBCL	59
Howell, D.A., Smith, A.G., et al [49]	DLBCL	124
Howell, D.A., Smith, A.G., et al [50]	DLBCL	98
Howell, D.A., Smith, A.G., et al [50]	T- cell lymphoma	175

Table 1. Symptom to Diagnosis.

Symptom to Referral		Median / days
Kukal, K., Dobrovoljac, M., et al [45]	**HGG**	**13**
Rask, O., Nilsson F., et al [43]	**HGG**	**11.9**
Nikonova, A., Guirguis, H.R., et al [46]	**DLBCL**	**28**
Aggarwal, A., Herz, N., et al [35]	**HGG**	**10**
Howell, D.A., Smith, A.G., et al [49]	**DLBCL**	**71**
Howell, D.A., Smith, A.G., et al [50]	**DLBCL**	**9**
Howell, D.A., Smith, A.G., et al [50]	**T-cell lymphoma**	**18.5**

Table 2. Symptom to Referral.

Referral to Diagnosis		Median / days
Howell, D.A., Smith, A.G., et al [49]	**DLBCL**	**46**
Aggarwal, A., Herz, N., et al [35]	**HGG**	**7**
Howell, D.A., Smith, A.G., et al [50]	**DLBCL**	**69**
Howell, D.A., Smith, A.G., et al [50]	**T- Cell Lymphoma**	**71**

Table 3. Referral to Diagnosis.

Statistical Analysis

Independent Samples Kruskal Wallis Test was computed to test the null hypotheses between our sample and the literature, and between Glioblastoma and PCNSL. This test was chosen because the samples are continuous, independent, not normally distributed, have a small sample size and unequal sample sizes are to be compared. This conforms to the test's assumptions.

Diagnostic Delay Sample vs. Literature

Our sample will be compared against the literature medians, to assess whether a delay is due to a unit's lacking ability to live up to set standards. Or due to a weakness in the protocol that is set, because of an underperformance in the tools used.

The null hypotheses state that there is no difference in the median, between the diagnostic timespan taken from "Symptom to Diagnosis", "Symptom to Referral", and "Referral to Diagnosis" when comparing our observed data, to grouped data from the literature.

Literature Data was grouped to a PCNSL group, which mostly included DLBCL. A 'High Grade Gliomas' group, which mostly included Glioblastoma- IDH Wild Type. In addition to a group inclusive to both groups mentioned along with patients who are highly suspected of HGG or PCNSL but are yet to be diagnosed.

Diagnostic Delay PCNSL vs. Glioblastoma

There is a consensus that PCNSL patients deal with a longer delay than other types of tumors, and Glioblastoma in particular [23]. This hypothesis will be tested across different timespans to see if there is a noted difference in our sample, and at what stage the difference is bigger.

Compliance

Compliance is the percentage that indicates how well a set standard is being conformed to. It is important to showcase how well the standard is being followed but also how well can the standard/protocol be implemented in an uncontrolled situation, with multiple factors intervening. Compliance is calculated for alternative testing used, CSF Liquid Biopsy in particular for the interest of this audit.

Key performance Indicator is to assess how well the clinical practice conforms to set standards, by assessing the data. Assessing the management of steroids is a priority for the threat they pose on early diagnosis, treatment, and better overall survival. Each case was individually assessed, and compliance was calculated by using the NHS foundation trust guide [51]. Number of patients who meet the standard, divided by the subtraction of number of patients to whom standard applies from the number of patients who meet any listed exceptions, and multiplied by a 100.

Results

Descriptive Statistics

Upon analysing the recorded data, symptoms encompassed a range of specific manifestations such as seizures, visual disturbances, gait issues, among others. These particular symptoms exhibited a lower frequency in comparison to more general symptoms like headaches. As illustrated in Figure 1.

A selective summary of the data that is found to be relevant is displayed in Table 4. Symptom to Diagnosis interval ranged from 30 to 1825 Days, 50% of the patients had steroid administered. Cytological analysis of cerebrospinal fluid (CSF) was conducted on a total of three patients using conventional methods. Different timespan intervals are displayed in Table 5, quantifying the delay in median average and interquartile range.

Symptoms to Diagnosis is longer in Lymphomas than in HGG, with an average difference of 106.8 days. A Bar Chart in Figure 2 is displayed for visual representation. "Referral to biopsy" had a longer timespan than "Symptoms to Referral" with an average difference of 17 days in Lymphoma patients, and 7 days in high grade gliomas.

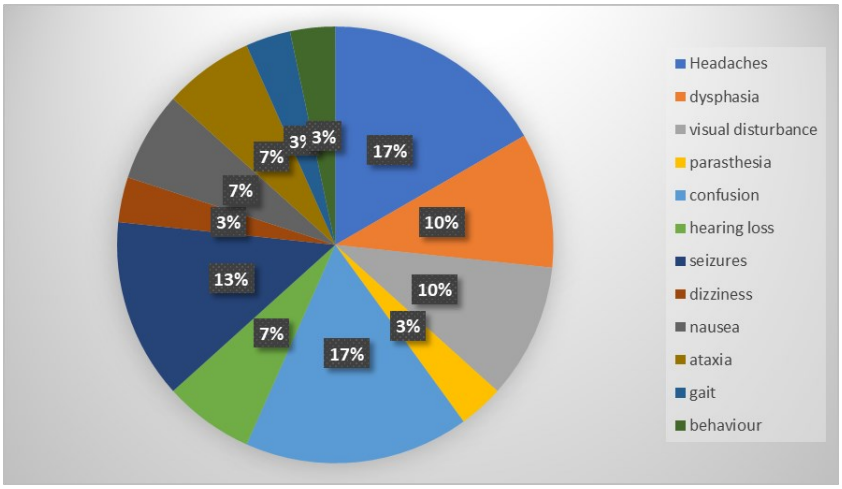

Figure 1. *Pie chart of symptoms detected in our sample.*

Type of Tumor – Age in Years	Symptom to Diagnosis / median in days	Steroid use	CSF analysis
Lymphomas			
72F	**275**	**Yes**	**No**
76M	**30**	**No**	**No**
65F	**60**	**Yes**	**Yes**
53M	**652**	**Yes**	**No**
High Grade Gliomas			
69F	**90**	**No**	**No**
49M	**30**	**No**	**No**
Suspected HGG/PCNSL			
55M	**365**	**No**	**Yes**
26M	**90**	**Yes**	**No**
40F (not documented well)	**1826**	**No**	**Not documented**
42M	**335**	**Yes**	**Yes**

Table 4. Selective Summary of Patient Data.

Time Span from (days)	Lymphomas (n=4)		HGG (n=2)		Either / Both suspected (n=4)		Total (n=10)	
	IQR	Median	IQR	Median	IQR	Median	IQR	Median
First symptom to Referral / days	6 - 545	84	14 -50	32	55 - 255	155	6 - 545	55
Referral to Biopsy /days	49 - 143	101	34 -44	39	null	null	34 - 143	74
Symptom to Diagnosis /days	31 - 652	168	31 - 92	61	90 -1825	345	30 - 1825	183

Table 5. Medians and IQRs of Diagnostic Delays in different types of tumors across different timespans.

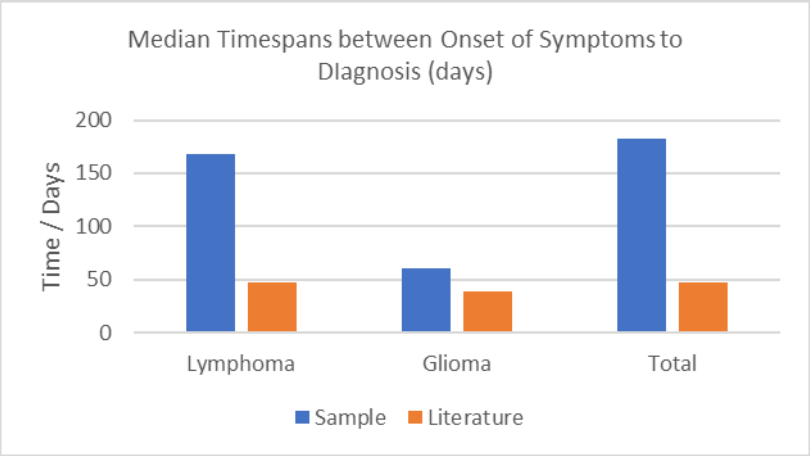

Figure 2. *Bar Chart Displaying Lymphomas and HGGs Diagnostic Delay in Both, the Sample and the Literature.*

A timely diagnosis is essential, this standard was tested using Independent Samples Kruskal Wallis to test the null hypothesis, with a 95% confidence α= 0.05. Onset symptom to diagnostic timespan was not significantly worse compared to findings of different samples. The null hypothesis was retained for lymphomas alone and gliomas alone: P=0.174 and P=0.63, respectively. However, the null hypothesis failed to be accepted, when all cases were compared against the corresponding medians from different studies, with a difference of 135.5 days and a P value of 0.007, which is less than 0.05. The difference is significantly longer than the average seen across different studies.

All other null hypotheses testing the difference of a tumor's diagnostic delay across different samples, were accepted (all p values shown in Table 6) and we can conclude that there is no significant longer delays.

Sample vs. Literature	P- value
Lymphoma Symptom - Diagnosis	.174
HGG Symptom - Diagnosis	.637
L+G Symptom - Diagnosis	.007
HGG Symptom - Referral	.083
Lymphoma Symptom - Referral	.773
L+G Symptom - Referral	.132
Lymphoma Referral - Diagnosis	.157
HGG Referral - Diagnosis	.221
L+G Referral – Diagnosis	.394

Table 6. P values of null hypothesis when comparing Diagnostic delay of Sample Vs. Literature. L+G: Lymphomas and High Grade Gliomas. (L+G : PCNSLs + HGG).

Using Independent Samples- Kruskal Wallis test, PCNSL and GBM were compared within our sample, to test if the difference is significant. The delay was longer for Lymphomas across all three different timespans consistently, with a difference of 106.8, 51.5, 61.5 days for S-D, S-R, and R-D Respectively. However, the delay is not significant. P values are equal to 0.481, 0.814, and 0.064.

Accurate staging and risk assessment is needed for treatment planning. Prognostic factors and distinctive neural precursor cells are identified and guide through different treatment options for tumors with different properties [52]. An example of this, is the DNA methylation status of MGMT promotor in Glioblastoma patients. It is determined by bisulfite modification and subsequent nested methylation- specific PCR (polymerase chain reaction) and sequencing analysis. Temozolomide's (TMZ) benefit is largely limited to patients with the methylated promotors [53]. Hypo fractionated radiotherapy alone would be given as the most suitable therapy for non-methylated MGMT promoters. The 2 HGG patients in our dataset, have not been treated accordingly. 1 patient with a negative methylation status, underwent TMZ therapy. While another patient had a positive methylation status and no record of TMZ being part of their management. This is not in line with treatment guidelines and protocols. [8]

One patient (patient 4 seen in figure 3) was not well-documented as opposed to the other patients and no mention of steroids was reported. Thus, it was excluded when calculating steroid compliance so it does not introduce unfairness, bias and potentially distort the overall perspective. 50% of the patients did not comply to the standards and received steroids despite being suspected for PCNSL. It is clear in Figure 4, that those who were on steroids.

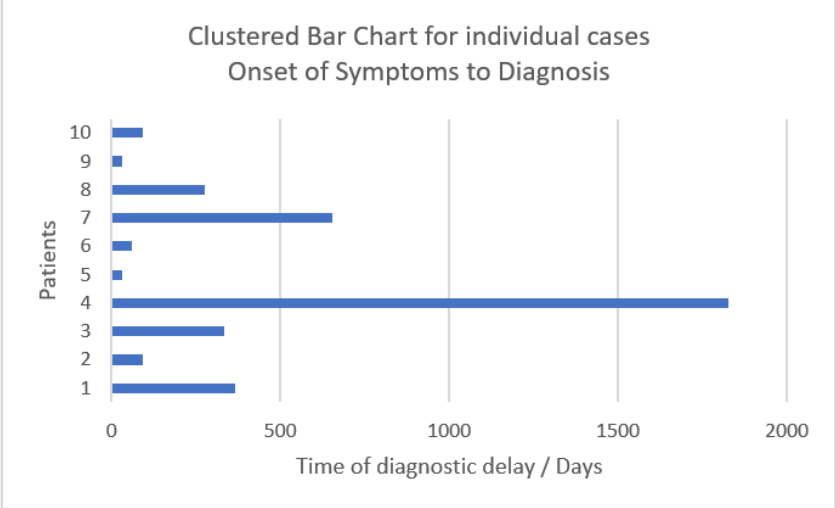

Figure 3. *Clustered Bar chart of all patients plotted against their individual diagnostic delay in days.*

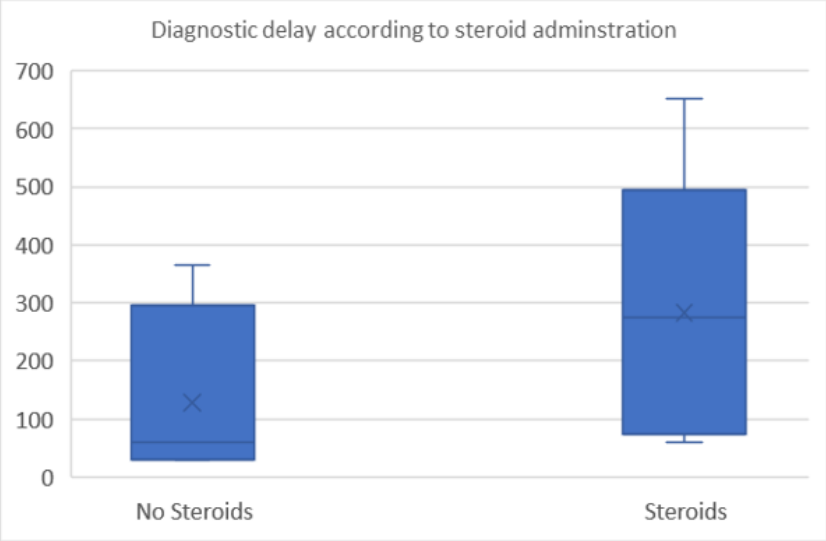

Figure 4. *Diagnostic Delay according to Steroid Use.*

In summary, the data showcased long diagnostic delays and a considerable number of patients with no definite diagnosis even after significant delays. This outcome can be attributed mainly to what is referred to in the literature as a "patient delay", However this doesn't entirely hold to be true, as it is evident that the "doctor delay" (stages after referral) is longer in every category.

Standards were implemented, by following with an expert multidisciplinary team, as it is crucial for accurate diagnosis, deciding most fitting treatment and management and ongoing patient care.

Discussion

Based off the results, it's evident that our patients have experienced comparable differences when compared to other cohorts. The literature gathered spans had longer delays, and a much higher median.

Various countries, systems, and multidisciplinary teams, leading us to infer that these delays are likely attributed to factors beyond the control of patients or medical professionals.

Despite one null hypothesis being rejected where the total of Lymphoma and HGG patients were compared to the total in the literature. It is important to note that this alone does not provide enough grounds to draw a significant difference conclusion where the sample had significantly longer delays in comparison. This is justified by the collective within-group comparisons acceptance to the null hypotheses. As well as a difference in sample size between our cohort and all other patients grouped from different studies, specifically with the "Total" group.

There is no consensus in the literature on what defines an early diagnosis for HGG in general or Glioblastoma – IDH Wild Type specifically, and neither for PCNSL. This lack of agreement can lead to subjective interpretations and variations in defining diagnostic delay. However, all cases observed have exceeded 30 days, which is considerably a long time for a diagnosis. This demonstrates a pattern of delays across the cases, suggesting a need for improvement in the diagnostic process.

Within the overall cases, there are 4 instances where diagnosis confirmation has not been made despite very prolonged periods. These exceptional cases further underscore the need to address diagnostic delays, evaluate the reasons that cause it and provide an alternative for diagnosing, to start essential treatment.

Diagnostic Delay Impact on Health

Extended delays in diagnosis can result in various negative consequences, including the progression of symptoms, elevated intracranial pressure, and the emergence of more region-specific manifestations within the brain. Furthermore, these delays can contribute to end organ damage, such as visual impairment, hypopituitarism, or persistent motor and cognitive impairments. [54]

Following the above-mentioned consequences, it becomes more challenging for treatment to take its preferred course. Maximal surgical resection for glioblastoma tumors become more challenging or even impossible. Complete remission for PCNSL following chemotherapy and radiotherapy becomes more difficult to achieve. And as the symptoms worsen, the patient's ability to tolerate aggressive treatments becomes limited, and the need to rely on corticosteroids to manage the severity of the symptoms increases.

The emphasis on withholding corticosteroid therapy should be placed. Along with more attention directed towards treatment tailored to prognostic factors, which determine a preferable route of management.

A follow up plan and regular monitoring are essential, to adjust and facilitate monitoring and consolidation treatments. and while no timely schedules are noted, follow up plans are set for 40% of the patients, and 2 of whom have been referred to palliative care due to deterioration.

While these outcomes observed are marginally expected due to the severity and survival rate of these aggressive brain tumors, what is observed is unfavourable and warrant improvement, to avoid or lessen the negative implications. Therefore, there is a need for quicker diagnoses, more rigidity in following set standards, whether diagnostic or therapeutic. And efforts should be directed towards identifying and implementing strategies that can lead to more favourable outcomes, ensuring optimal outcomes are achieved and maximizing the well-being of patients involved.

Recommendations

Lack of education or awareness, and lack of healthcare recourses may act as confounding variables in causing diagnostic delay. Although these may be discussed and tackled through certain regimens to reduce the delay, they are not regarded as cornerstones of the primary issue but rather supplementary tools that if implemented can show better effects. This conclusion is derived from the fact that no significant differences were shown within cohorts from different countries, systems, qualities, and resources.

The main factors seem to be regarding steroid administration, and present diagnostic tools. Liquid Biopsy has been on the rise as a promising technique to identify genetic information early. Blood samples are more researched with cancers, compared to other types of liquid biopsies, yet they do not show abundancy of markers with Glioblastoma or PCNSL. This is due to the blood brain barrier and the prevention of the shedding into the bloodstream [55].

Markers in the blood are mostly present in the late stages of the cancers, when the BBB is severely compromised. This defies the main goal of early diagnosis, although could still be useful for monitoring purposes.

CSF has higher sensitivity and specificity than peripheral blood and a better qualitative source of nucleic acids when examining Glioblastoma and PCNSL [56]. Choosing the best and most suitable method of isolation and analysis will increase the likelihood of definitive diagnoses using CSF. This will be discussed along with methods that help increase diagnostic yield by increasing sensitivity and specificity.

CSF Liquid Biopsy

Cerebrospinal Fluid (CSF) Liquid Biopsy will be mainly proposed to mitigate delays, high costs, and act as a good long term monitoring technique. Liquid Biopsies are a promising technique for their non-invasive nature and the identification of cellular, molecular, and genetic characteristics.

Types of Liquid Biopsies include Blood, Urine, Saliva and CSF. CSF is considered a major route for brain tumors, as it is considered an extension of extra cellular compartments within the CNS.

CSF samples are extracted by performing a lumbar puncture. They can be used to detect and analyse cells, proteins, lipids and genetic material. CSF protein levels are measured when feasible to establish a prognostic score. This can be utilised to introduce methods which would be time and cost effective, as a lumbar puncture and CSF extraction are already established as a small part of diagnostic timeline.

Sequencing methods are used to isolate molecular markers for screening, such as digital droplet PCR and Next Generation Sequencing (NGS) or the combination of them. In addition to biomarker mediated platforms employing the use of magnetic beads, microfluidic chips and charge and size sensitive microfiltration.

CSF is not routinely extracted or analysed for Glioblastoma patients. However, there is so much potential in detecting markers and DNA, which can act as a quicker diagnostic tool than a biopsy and reducing overall risks for when surgical resection will need to follow, following a positive diagnostic outcome.

Diagnostic Methodologies

1. Conventional Cytology

A CSF sample is collected, stained, and examined under the microscope, a diagnostic interpretation or conclusion is then reached. It includes protein detection levels of albumin, immunoglobulin, glucose, lactate, and red and white blood cells with differential antigen and antibody testing for infectious agents. This is an established method and is used with various diseases [57]. Although, it is relatively quick and cost-effective, it does have its limitations and is prone to error. It is subjective to input and variability in interpretation could occur. This is not helpful for reaching a definite diagnosis but provides support and insight [58]

2. Flow Cytometry (FCM)

Flow Cytometry is a powerful analytical technique that analyses cells based on their physical and chemical properties. The cells are suspended and followed through with a laser beam, the outcome is converted to numerical data that is then analyzed by computers. It locates the expression of markers. It also allows for multiparameter analysis, and can analyse a big number of cells in a short period of time [59].

3. Polymerase Chain Reaction (PCR)

It involves separating the DNA strands by subjecting it to high temperatures, followed by primers binding to complementary sequences and then the synthesis of new strands complementing the original template. After the amplification process, molecular diagnostic is one of its various applications. It detects genetic mutations and alterations. [60]

It is highly sensitive and a quick technique. However, a primer of the wanted mutation to be tested needs to be designed, which poses as a challenge or a limitation if the mutation is unknown[61]. In regards to CSF Liquid Biopsy, this can also be a problem as it is still a growing area in research and not all markers can be predicted. So known information is not always employable.

4. Digital Droplet PCR (ddPCR)

Digital Droplet PCR partitions a PCR reaction and acts on absolute target quantification without a need for a reference. It partitions the DNA or RNA into droplets, each acting as a separate reaction vessel. ddPCR quantifies how many target sequences are found in the sample with very accurate and quantitative results [62].

This method shows increased precision for samples where targets are minimal; which is ideal for tumor cells that shed into the CSF as it doesn't occur readily. It also allows for multiplexing where more than one target can be tested within the same sample. [63] It can detect copy number variations, rare mutation detection, viral detection, and gene expression analysis. [60]

5. Next Generation Sequencing (NGS)

NGS is emerging with ddPCR as increasingly promising analytical techniques for molecular profiling. This is due to the multiplexing analysis it carries out [56], as well as ability to detect the heterogeneity of a tumor tissue or sample. It is a highly sensitive technique and detects low-frequency variants. [64]

Detectable Components

CSF Liquid Biopsies can detect various components that provide valuable information and also has the potential to be definitely diagnostic for their specificness. CTCs, CtDNA (cfDNA), and Exosomes [65].

1. Circulating Tumor Cells (CTCs):

CTCs shed from a primary tumor and enter the CSF or the Bloodstream. They appear in low quantities in PCNSL and Glioblastoma, for the rare occurrence of the tumor metastasizing outside the CNS and spreading to bodily organs [66] [67]. Despite its rare occurrence, CTCs can be detected before a lesion can be seen on imaging. This is highly unlikely though in a routine setting. However, if a CSF analysis is routinely done as a monitoring technique post-therapy, it can be beneficial in monitoring progression.

In PCNSLs, PCR is used to detect clonal immunoglobulin gene rearrangements [68]. Epstein-Barr virus is tested for in the CSF, for immunocompromised patients (AIDS). An elevated white blood cell count, elevated protein concentrations and low glucose levels are mostly indicative of immunocompetent PCNSL. [69]

CTC's can be a distinguishable marker between PCNSL and Glioblastoma. High mesenchyma and a small amount of nerve signature is found in gliomas in comparison to PCNSL [70]. Most techniques rely on monitoring epithelial cells adhesion molecules (EpCAMs) as biomarkers, and more recent techniques do not, but nonetheless they could be a distinguishable feature between PCNSL and Glioblastoma, due to their lack of expression in gliomas [70].

2. External Vesicles (EV):

External Vesicles can also be classified as exosomes, micro vesicles, and apoptotic bodies. They are membrane bound vesicles released by tumor cells that signal between cancerous cells and the surrounding environment, promoting metastasis. They are found in fluid surrounding the brain and have the ability to cross the Blood Brain Barrier. EVs may contain lipids, proteins, and genetic material like microRNA and can promote tumor progression in suitable environments. [71]

EVs can be found in the CSF in brain tumor patients and can diagnose by the biomarkers they carry. They can be detected by using cell-surface markers[72]. Flow cytometry can detect their membrane associated proteins, which is cost-effective as FCM is also used to detect CTCs. [73]

Micro vesicles quantity is lower in patients with evident tumor progression, miRNA is diagnostic for PCNSL and Glioblastoma, but it also can be a distinguishable feature. There is an overexpression of miR-19,21 and 92a while glioblastoma has an upregulation of miR-21,222,124-3p, and 10b [16,74,75] Exosomes also transfer functional EGFRVIII deletion variant protein in gliomas, that is not transferred in PCNSL [76].

3. Proteins

Proteins maintain tight junction's integrity of the blood brain barrier. A mutation in these proteins supports the access of tumor associated macrophages and hypoxia, which are typical consequences of solid tumors, resulting in a compromised BBB. Proteins which are shed from tumor cells are detected by ELISA or whole-protein mass spectroscopy analyses.[77]

19 proteins have been associated with gliomas but has its own limitations by being attributed to other diseases and it might not always be easy to distinguish. A low CSF glucose level is attributed to PCNSL. CSF proteins are also found in PCNSLs, like Haemopexin, Antithrombin III, Apolipoprotein A1, and Transferrin with high sensitivities and specificities. [78]

4. Cell Free DNA (cfDNA)

cfDNA detection has a high potential for diagnosis. As it is very specific to each cancer and their mutations. CSF ctDNA reveals protein coding mutations, copy number alterations, promotor mutations and structural rearrangements [56]. Which all detect gliomas and PCNSL mutations and alterations specifically, and other types of cancers, generally.

MGMT methylation is detected using methylation specific PCR [75]. DNA methylation profiling is used when the detection for homozygous CDNK2A/B deletion for grading is lacking. IDH1 and TERT promotor mutations are also detected in CSF Liquid Biopsy. MYD88L265P is detectable in the CSF, similarly to PCNSL tissue biopsies. Which is a positive indicator and a favorable finding for diagnostic and prognostic purposes. [25]

Advantages of CSF Liquid Biopsy

Obtaining a CSF Sample is considerably less invasive compared to a brain or a vitreous biopsy. This introduces less risks to all patients, but to comorbid patients, especially [60]. The easy access and less trauma caused also encourages continuous monitoring, where tumor progression and treatment response can be assessed. Accordingly, treatment strategies are adjusted. CSF analysis has the ability to distinguish between radiation-induced necrosis and tumor progression which is essential in treatment stages. [80]

The detection of CtDNA in the CSF may have additional informative mutations when compared to a solid biopsy [56,81]. This can be explained by the heterogenous nature of the tissue, while only a specific metastatic site is under analysis. CSF Liquid Biopsy also overcomes the limited success targeted therapy for glioblastomas, which is hypothesized to be because of the tumor's heterogeneity. [82]

CSF liquid biopsy is sometimes capable of early detection and provides prognostic information about tumor progression and survival rates. For example, a negative cfDNA with a negative IL-10 mutation, predicts a long time in maintenance post-therapy. On the other hand, a positive cfDNA marker with a negative IL-10, predicts a quick progression. [83]

Limitations of CSF Liquid Biopsy

Positive results obtained from CSF Samples may take longer than expected, which defies the point of early diagnosis. Although it can act as a substitute for brain biopsies if delayed due to steroid administration or high risk or, is not possible to investigate leptomeningeal involvement.

One of the main disadvantages of CSF liquid biopsy that has prevented the acceleration of this method in research and to the clinic, is the low yield it obtains, and low sensitivity and specificity figures. However, with a more detailed and targeted approach, better results seem to be achievable. [84]

Improving Diagnostic Yield

Increasing the diagnostic yield, by increasing true positives and negatives (sensitivity and specificity), and decreasing false positives and negatives is of much importance. To achieve this, diagnostic processes need to be continually refined. Sensitive and molecular assays enable enhancements and higher detectable rates. [85]

CSF Diagnostic yield may be improved by identification of Clonal IGH rearrangements. This refers to specific rearrangements of immunoglobulin heavy chain gene, they are produced by B-cells. and under lympho-proliferative environments, genetically identical arrangements can be detected [75]. This specific identification helps in identifying markers in a more sensitive way and will improve diagnostic yield of CSF analysis.

CSF Analysis is used in clinical practice, but is only limited to conventional cytology. [86] Making use of ctDNA in detecting cfDNA and RNA (like MYD88 [84], CD79B [84], CXCL-13 [87], B2M and Neoportin [88] in primary CNS lymphomas) has the potential to increase diagnostic yield greatly when combined with all markers that are observed in the CSF. Higher detection rates of cell-free DNA can be observed by ddPCR, targeted sequencing, and whole genome sequencing [89]. Obtaining CSF Samples closer to the lesion is also recommended to minimize the risk of false negatives. [90]

It is important to note a superior method in detecting wanted markers, detecting ctDNA using NGS and ddPCR offer several advantages over FCM and cytology. Exploring more of the technique for more suitable patients helps reach conclusions backed up with evidence on a large scale.

Plan of Action: A Prospective Clinical Trial

A Prospective Clinical Trial evaluating CSF Biopsy for the diagnosis and monitoring of suspected HGG and Primary CNS Lymphomas. This aims to test the efficacy of CSF Liquid Biopsies on bigger samples under more enhanced tools. This proposed trial seeks to explore the sensitivity and specificity of CSF liquid biopsy to evaluate the feasibility of integrating this diagnostic tool into clinical practice.

The aim of this trial is not to yield definitive diagnostic guidelines to replace existing standards. However to consider adding a method that is easy to carry out, with a diagnostic markup that is definitive if a yield is successful. To allow the transition to treatment phase as quick as possible.

It is important to assess ctDNA by using the recommended methods, as well as distinguishing it from available studies by including a large number of patients to avoid selection bias.

The implementation of this proposed trial does not disrupt the timeline of patient diagnosis, as a lumbar puncture is non- invasive and does not have many contraindications or risks involved. In addition to the fact that LPs are often carried out routinely to analyse cellular abundance; so an existing opportunity can be used. Intrathecal chemotherapy is another instance where a LP is done to help the reach of drugs to the brain and spinal cord through the fluid[2]. This would allow easy monitoring of CSF post treatment-start.

Conducting a large clinical trial not only provides more information and utility for PCNSL and Glioblastoma tumors, but will highly likely benefit a range of cancers for the concept CSF Liquid Biopsy provides in ease and reliability if outcomes have high yield.

Conclusion

In Conclusion, Our study revealed a prevalent diagnostic delay among the patients in our cohort, highlighting the need for improved diagnostic approaches in the field of medicine. Notably, stereotactic biopsies, while valuable in certain cases, exhibit limitations that may hinder their effectiveness in accurately diagnosing comorbid patients or a high risk situated tumor. The high incidence of diagnostic delay underscores the importance of developing innovative and efficient diagnostic methods to facilitate early and accurate detection of the lesion. Stereotactic biopsies, although widely used, may not always provide a comprehensive view due to high risks in deeply situated tumors, heterogeneous nature and the limited information gained from a single biopsy throughout the timeline of the cancer, in most cases. Therefore, it becomes crucial to explore alternative approaches that can overcome these limitations and improve diagnostic accuracy.

The introduction of CSF liquid biopsy, harnessing the power of ddPCR and NGS, holds promise for revolutionizing the diagnostic landscape. By detecting molecular signatures in the CSF, this non-invasive approach has the potential to offer a more comprehensive and sensitive analysis of disease-associated alterations. This method not only provides the opportunity for earlier detection but also has the potential to monitor disease progression and therapeutic response more effectively. The integration of CSF liquid biopsy with ddPCR and NGS technologies represents a promising avenue for future research and clinical implementation, offering the potential for more precise and timely diagnoses in a wide range of medical conditions. Further studies and advancements in this area will undoubtedly contribute to improving patient care and management.

Conflict of Interest

The author declares no conflict of interest.

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