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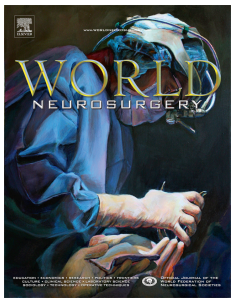

Safety and Technical Efficacy of Pediatric Brainstem Biopsies: An Updated Meta-Analysis of 1000+ Children

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Previous Presentations: Preliminary results were presented by Dr V Recinos at the annual meeting of the American Society of Pediatric Neurosurgeons in January 2024. Abstract has also been submitted for consideration to the annual meetings of the American Association of Neurological Surgery (AANS) 2024 and International Symposium of Pediatric Neuro-Oncology 2024.

Abstract

Background: Brainstem tumors represent ~10% of pediatric brain tumors, ~80% of these are diffuse midline glioma (DMG). Given invariably poor prognosis in DMG, there continues to be immense variation worldwide in performing biopsy of these lesions. Several contemporary studies in recent years have provided new data to elucidate the safety profile of biopsy and an updated meta-analysis is thus indicated.

Methods: We found 29 studies of pediatric brainstem biopsy in the last 20 years (2003-2023, 1002 children). We applied meta-analysis of proportions using a random-effects model to generate point estimates, confidence intervals, and measures of heterogeneity.

Results: 87% of procedures were stereotactic needle biopsies (of these, 62% with a frame, 14% without frame, and 24% robotic.) Biopsy resulted in a histological diagnosis ("technical yield") in 96.8% of cases (95% CI 95.4-98.2). Temporary complications were seen in 6% (95 CI 4-8), with the most common neurological complications being 1) cranial nerve dysfunction, 2) worsening or new ataxia, and 3) limb weakness. Permanent complications (excluding death) were seen in 1% (95% CI 0.5-2), most commonly including cranial nerve dysfunction and limb weakness. 5 deaths were reported in the entire pooled cohort of 1002 children (0.5%).

Conclusions: When counseling families on the merits of brainstem biopsy in children, it is reasonable to state that permanent morbidity is rare (<2%). If biopsy is performed specifically to facilitate enrollment in clinical trials requiring a molecular diagnosis, the risks of biopsy outlined here should be weighed against potential benefits of trial enrollment.

Introduction

Brainstem tumors compromise 10-20% of childhood brain tumors [1, 2]. Diffuse midline gliomas (formerly diffuse intrinsic pontine gliomas or DIPG) are the most frequently encountered brainstem tumor in children [3] and are associated with a universally poor prognosis, with a median overall survival of less than 12 months in a large contemporary DIPG registry [4].

The role of brainstem biopsies in the management of DMGs has been the subject of sustained controversy for the last three decades. Brainstem biopsies were being performed in the 1980s in both adults and children [5]. With the advent of MRI and the realization that a radiographic diagnosis of DMG could be made on the basis of hallmark findings in many cases, the following question arose: *even if the risks of biopsy are low, can they be justified if the prognosis of the disease is invariably poor?* In a seminal article in 1993, Albright and colleagues presented the findings of the Children's Cancer Group [6]. They argued that "MR scans provide images that are virtually diagnostic of brain stem gliomas and yield prognostic information equivalent to that obtainable from biopsies." Notably, they acknowledged that biopsies may indeed be indicated in select scenarios where diagnosis may not be clear (e.g. presence of a focal, enhancing mass or a dorsally exophytic tumor protruding into the 4th ventricle). They also left open the possibility that biopsy may be indicated if it could "alter the treatment for a patient in a prospective clinical trial." They concluded that biopsies outside of these relatively uncommon indications at the time were likely unwarranted, noting that "as yet no one has demonstrated that modifications in therapy based on the biopsy results contribute to improved outcome." In the thirty years since the Albright paper, elements of the case against widespread utilization of brainstem biopsy have been scrutinized. Several studies have called into question the notion of DMG as a radiographic diagnosis by noting issues of inter-observer variability [7] and low specificity [8].

Importantly, our knowledge around the disease has grown meaningfully over the last decade. We now recognize that though DMG shares the histological hallmarks of high grade astrocytomas in adults, it does not respond to similar therapeutic approaches [9, 10]. Furthermore, we now know that one of the defining features of DMG is epigenetic dysregulation. In seminal studies in 2012, the histone 3 mutation *H3K27M* was identified as a pathognomonic feature of DMG present in almost 80% of cases. This molecular feature was key to defining the new entity 'diffuse midline glioma H3 K27M-mutant' in the 2016 and 2021 WHO CNS tumor classification schemes with the vital recognition that tumors bearing this mutation are grade IV irrespective of histological features [11-14]. Other less frequent but nonetheless notable molecular features have been identified which are potentially druggable targets including ACVR1 [15, 16], PDGFRA [17], FGFR1, and PP2A [18]. Notably, a targetable surface antigen B7-H3 has also been identified [19]. In the context of these important developments in our understanding of the disease and the emergence of potentially viable therapeutic targets, there has been increasing interest in a more widespread utilization of brainstem biopsy in children [20-23].

In most high-volume academic centers, the question of whether or not to offer a brainstem biopsy will ultimately boil down to whether or not the patient may become a candidate for enrollment in a clinical trial on the basis of a histological diagnosis. In such a scenario, surgeons and families must perform a patient-specific risk-benefit analysis weighing the potential morbidity of a biopsy against the potential merits of trial enrollment. Through this meta-analysis, we aim to provide an updated set of estimates of the safety and technical efficacy of performing a biopsy in this area in children that may help surgeons and families make more fully informed decisions.

We are not the first to apply a meta-analysis lens to this question. Other authors have published meta-analyses with more limited scope or with mixed populations of adults and children [24-27]. To our knowledge, there is only one high-quality large-scale meta-analysis on this question in the pediatric population [28]. We believe an updated analysis is indicated because 1) A third of the studies included in the meta-analysis were from the 1980s and 1990s and thus would not be expected to reflect contemporary surgical workflows and outcomes, 2) even the most contemporary series included in the prior meta-analysis are now almost ten years old, 3) multiple large, multi-center prospective studies and registries have since been undertaken (most importantly, DIPG-BATS and INFORM) which should be included in an updated analysis.

Methods

Study Selection

The study was undertaken in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [29]. Studies were selected from major online databases (Google Scholar and PubMed) using an initial automated search-term based filtering approach followed by manual selection and exclusion approach (Figure 1 shows workflow for Google Scholar, same approach was used for PubMed). Databases were queried on 1st October, 2023. Notably, only contemporary studies from the last twenty years (2003-2023) were included. Where studies included both pediatric and adult cases, the study was included only if pediatric data could be disaggregated from the adult cases. Some centers have published multiple reports at different timepoints based on a single cohort. In such cases, only the most recent publication (usually with the largest overall cohort being reported) was included in the meta-analysis to ensure patients were not double counted. We did not exclude studies on the basis of sample size or language of publication. Study selection was performed by SS and VR.

Data Extraction

Datapoints of interest were extracted from the primary literature by the first author. A large random selection of datapoints were cross-checked by the senior author; no discrepancies were found. For each study the following datapoints were extracted about the study: first author name, year of publication, sample size, country of publication and name of hospital, age of patients (mean/median as well as measure of variance), accrual period of patients. The following datapoints were extracted about technical yield: proportion of cases resulting in histological diagnosis, histological diagnoses, method of biopsy (open vs stereotactic vs endoscopic, frame vs frameless vs robotic). The following data were extracted regarding complications: incidence and qualitative description of temporary complications, incidence and qualitative description of permanent complications, incidence and details related to perioperative mortality.

Outcome Definitions

A biopsy was considered to be technically efficacious if it resulted in a histological diagnosis. We considered a biopsy to be non-efficacious wherever study authors mentioned that the procedure was non-diagnostic, diagnosis was uncertain even after biopsy, or a repeat biopsy was necessary. We considered an event to be a temporary postoperative complication if the patient suffered a neurological deficit attributable to the surgery but with an explicit end date. We considered an event to be a permanent complication when the patient experienced a new neurological deficit attributable to the surgery without an explicit end date.

Details of any mortalities were individually captured and not compounded within the category of 'permanent complications'.

Statistical Analyses

Statistical analyses were performed using the Open MetaAnalyst platform [30]. This extensively validated software package provides inbuilt functionality for meta-analysis of proportions. We generated point estimates as well as 95% confidence intervals. A random-effects model was implemented. We also obtained the I^2 statistic to assess study heterogeneity (in general, values of 25%, 50%, and 75% correspond with mild, moderate, and severe heterogeneity.)

Quality and Bias Assessments

To assess publication bias, we generated funnel plots with 95% confidence intervals around mean effect size. Asymmetric accumulation outside of these confidence intervals especially in the regions of the plot signifying low precision and high deviation from mean effect size (i.e. lower left and right quadrants) would raise concern for publication bias. Funnel plots were generated in Python using the matplotlib library.

To evaluate the overall quality of pooled evidence, we used the GRADE approach[31]. This approach, which is extensively utilized in the academic literature[32-34] and is recommended in the Cochrane Handbook[35], allows investigators to systematically evaluate the quality of meta-analyzed evidence on the basis of multiple merits (superior study design type, higher effect sizes, demonstration of a dose-response relationship) and demerits (publication bias, lack of consistency in defining/measuring outcome, imprecise effect estimates etc.)

Ethics Considerations and Human Subjects Protections

No institutional review board (IRB) approval or registration was required for this retrospective review of published academic literature.

Results

After study selection process was applied, 29 studies were included in the meta-analysis as shown in Table 1 [8, 36-63]. Studies from across the globe were included; 20% of studies were from the United States, 45% were from Europe, 24% were from Asia, 10% were from Latin America. The median number of patients in each study was 21 with a wide spread (range 5-130).

Surgery Type

Surgery type was available for 913 cases. Of these 87% were stereotactic and the remainder were open (12.7%) or endoscopic (0.3%). Of the stereotactic cases for which methodology was available (n=625), 62% used a frame-based method, 14% were frameless, and 24% were robotic (with or without frame). A time-dependent change in the type of stereotactic surgery was discernable. In studies from 2003-2013, 90% of stereotactic brainstem biopsies were frame-based. In studies from 2013-2023, only 37% of stereotactic brainstem biopsies were frame-based, with a greater reliance on frameless stereotactic methods (21%) or robot-assisted methods (with or without frame, 37%).

Diagnostic Yield

We considered a biopsy to be technically efficacious if it was able to yield a histological diagnosis. Figure 2 shows the forest plot describing the meta-analysis for diagnostic yield. Our meta-analysis suggests that 96.8% of brainstem biopsies in children are technically efficacious, 95% CI 95.4-98.2%. I^2 statistic for this measure was 24% (p=0.1).

Reporting on histological subtypes in the context of a study spanning twenty years is greatly complicated by two factors: 1) there was variability in how much disaggregated histological information was presented in different studies, 2) multiple iterations of the WHO classification of CNS tumors were published over the years spanning this meta-analysis and it is hard to discern which classification system was being used by a particular study at the time of reporting as rates of adoption of new classification systems vary. Nonetheless, we note that gliomas represented 92% of the reported tumors. Of the gliomas for which grading was provided (n=484), 68% were histologically high grade (WHO Grade III/IV), though *H3K27M* status was not described for the majority of cases.

Temporary Complications

Figure 3 shows the forest plot describing the meta-analysis for temporary complications. We found that 5.9% of patients had a temporary complication (95% CI 4.1-7.8%). Inter-study heterogeneity for this measure was low (I^2 =29%, p=0.08). The most common temporary neurological complications were 1) cranial nerve dysfunction, 2) new or worsening ataxia, and 3) limb weakness. Notably, nine studies mentioned post-operative radiographic findings (hemorrhage or pneumocephalus) as a "complication" (n=22 children) but did not consistently state whether these findings were associated with a temporary neurological deficit or prolonged hospitalization[36, 38, 40, 41, 46, 52, 56, 61]. 2 studies referred to 'delayed awakening' (n=4 children) as a temporary complication but did not clarify how this diagnosis was made in the post-anesthesia setting [38, 41]. Pneumonia was the most common non-neurological temporary complication, only noted in 3 cases[41].

Permanent Complications

Figure 4 shows the forest plot for the meta-analysis on permanent complications. We found that 1.1% of patients suffered a permanent complication (95% CI 0.5-1.8%). Inter-study heterogeneity of this measure was low (I^2=1%, p=0.45). The most common permanent complications (not including death) were 1) cranial nerve dysfunction and 2) limb weakness.

Perioperative Mortality

In the cumulative series of 1002 cases, 5 deaths were reported (0.5%) [55, 61]. Given exceptionally low incidence, formal meta-analysis would be of limited utility but details of each case are presented in Table 2.

Bias Assessment

Funnel plots were generated to evaluate publication bias (Supplementary Figures 1-3). Visual inspection of the funnel plots revealed no gross asymmetries that would suggest a disproportionate failure to publish high-morbidity case series. The funnel plot for temporary complication rate showed that a handful of high-morbidity case series (with lower precision due to smaller sample sizes) were likely responsible for increasing the mean effect size. In the funnel plot for temporary complication rate, six studies fell outside the 95% confidence intervals of mean effect size; four of these had markedly low

incidence rate for temporary complications (range 0-3%) [8, 43, 47, 58] while 2 had markedly high rates (range 24-35%)[36, 53]. Funnel plot analysis was used as a component of publication bias assessment; studies were not removed post-hoc from the analysis on the basis of the funnel plot analysis.

Quality Assessment

Based on GRADE criteria, the baseline quality of evidence was low due to the fact that source studies were observational case-series as opposed to randomized controlled trials (Table 3). The quality of the pooled evidence is further decreased due to an inconsistent definition scheme for complications, imprecision in determining incidence of complications that is inherent in small cohorts, and a concern for publication bias. The quality of the pooled evidence is improved by the fact that effect sizes are high. On balance, the overall quality of the pooled evidence is low.

Discussion

The recent neurosurgical literature is ripe with calls for consideration of judicious but more widespread utilization of brainstem biopsies in children, all motivated by the promise of emerging therapeutic targets which can only be tested in a trial setting with requisite tissue diagnosis [23]. In situations where trial enrollment is feasible, the discussion between surgeon and family will center around a risk-benefit analysis wherein the risks of biopsy will be weighed against the possible benefits of trial enrollment. For neurosurgeons engaging in these discussions, evidence-based estimates of safety and technical efficacy are indispensable. In this study, we present data to support the notion that brainstem biopsies in children are likely safe (risk of permanent morbidity <2%) and efficacious (diagnostic yield ~ 94%).

In 2020, the Neurosurgery Working Group (NWG) of the SIOP-Europe Brain Tumor Group (BTG) published results of a survey with 73 neurosurgeon respondents [64]. 86% of surgeons felt that biopsy was not needed to diagnose DIPG in every case and 57% would only offer biopsy in the context of a prospective clinical trial. Perhaps unsurprisingly, 93% agreed to biopsy if molecular targets identified would guide treatment. Notably however, 65% stated that biopsy was justified if molecular targets were being investigated *even if* these findings were *not* used as treatment targets for the patient in question. The results of the NWG survey highlighted the variability in how surgeons approach this challenging but not infrequently encountered clinical scenario. We outline one possible approach in Figure 5 based on our own experience.

An important finding from our study is that the incidence of temporary complications (resulting in prolonged hospitalization or temporary neurological deficit) is not trivial i.e. 5.9%, 95%CI 4.1-7.8%. Though temporary, these complications still carry a heavy weight in light of the limited overall survival of patients with DMG (the most frequently encountered diagnosis of these biopsies). On the opposite end of the spectrum, we found that perioperative mortality in the context of brainstem biopsy was exceptionally rare. Notably, all reported mortalities (n=5) were from only 2 series, both published within the last five years. The latter observation raises concern for publication bias which we endeavored to investigate and quantify though funnel-plot analysis. Though the funnel plots did not show gross asymmetries which would suggest that high-morbidity studies were failing to get published, we felt there was persistent concern that a publication bias was likely and thus incorporated this concern into our quality-of-evidence assessment.

It is useful to situate our results in the context of prior meta-analyses conducted on this topic. In their analysis of a large (n=1480) mixed (adult and pediatric) cohort, Kickingereder et al reported 96.2% diagnostic success, 7.8% overall morbidity (95% CI 5.6-10.2), 1.7% permanent morbidity (95% CI 0.9-2.7),

and 0.9% mortality (95% CI 0.5-1.4). [24] In their 2017 analysis of 735 children, Hamisch et al reported 96.1 diagnostic success, 6.7% overall morbidity (95% CI 4.2-9.6), 0.6% permanent morbidity (95% CI 0.2-1.4), and 0.6% mortality (95% CI 0.2-1.3). Our estimates, which are based on a purely pediatric population within a more contemporary context, are grossly stable compared to these earlier studies. Notably, we have provided data for the specific temporary and permanent complications encountered which were missing in prior studies. Recently, Fu et al have published a meta-analysis specifically on children undergoing brainstem biopsy wherein a radiographic diagnosis of DMG has already been made. In this highly selective study (pooled cohort N = 381), they reported an overall morbidity rate of 11% (95% CI 4.8 – 18.9%), 0.3% permanent morbidity (95% CI 0-2.2), and 3 mortalities in the total cohort (0.8%). It should be noted that the Fu study (which deals specifically with presumed DMG in children) reports a higher temporary complication rate (upper limit of 95% confidence interval is ~19%, compared to <10% in our and other prior studies). It is possible that children with DMG have a higher risk of developing temporary complications after brainstem biopsy compared to those with non-DMG brainstem lesions.

In our experience, it is not uncommon to witness some degree of nihilism when faced with the question of establishing tissue diagnosis in cases where the combination of radiographic findings and clinical course leave little room for differential diagnosis. Refrains dismissing potential benefits of trial enrollment on the grounds that countless previous trials have been unable to improve overall survival are not unheard of. We suggest that such a dismissal of the potential merits of trial enrollment neglects the substantial leaps that have occurred in 1) our understanding of the pathophysiology of DMG and 2) our ability to identify therapeutic targets (a pre-requisite for drug discovery and development). Importantly, when effective therapies for DMG do emerge, it is likely that the first patients to benefit may be those in a trial setting and in the vast majority of cases a tissue diagnosis will likely be a pre-requisite for enrollment.

Limitations

The most significant limitation of this report, which is common to the majority of meta-analyses in the neurosurgical literature, is that the source studies included in the analysis are retrospective observational case series and thus the overall quality of the pooled evidence will always be limited compared to those obtained from large trials. Unfortunately, there is no remedy to the problem of low-quality source literature. Nonetheless, we have followed best practices by evaluating bias and performing quality assessment of the pooled estimates. We suggest that, not withstanding this important limitation, clinicians may find the pooled estimates of such a large patient population informative.

The issue of inconsistent outcome definition was an important limitation of this study. Most notably, the definition of 'temporary complications' was inconsistent across the source literature. Before embarking on data extraction, we established an *'a priori'* definition of temporary complications which included any post-surgical events that the authors of the source studies explicitly classified as such. Nine studies referred to radiographic evidence of postoperative blood or air as a temporary complication, and thus we included these events in our counts. We note, however, that the mere presence of blood or air in the post biopsy setting would not generally be considered a complication unless there was an attributable deficit associated with the finding or the imaging results prolonged hospitalization. Several studies also referred to 'delayed awakening' as a temporary complication, but there was no consistent description of how this diagnosis was made in the post-anesthesia setting. This degree of inconsistency is perhaps to be expected given that our report utilizes data from over two dozen neurosurgical departments spread across 4

continents. Nonetheless, the inconsistency of definitions is undesirable and was factored in our quality assessment.

Finally, the present study is limited in scope as it only seeks to elucidate the risk profile of biopsies and does not simultaneously consider the benefits of the information that may be gained from biopsy. As such, this review only addresses one half of the risk versus benefit debate. In future studies, it will be worthwhile to evaluate pooled evidence of the benefits that have accrued to patients who have undergone biopsy in terms of changing treatment strategy and clinical outcome. To this end, multiple avenues are being investigated to utilize the molecular information obtained from biopsies to inform treatment schema. In the DIPG-BATS trial (cited above in relation to the important safety data it provided), patients were segregated into treatment groups (varying combinations of bevacizumab, erlotinib, and temozolomide) on the basis of EGFR and MGMT status [52]. Recent results from the INFORM study included 21 DIPG biopsies in which 16 were found to have 'potential targetable alterations'. [53] In 5 of these cases, investigators based initial therapy on molecular information (e.g. 2 patients had PDGFRA alterations so tyrosine kinase inhibitors were added to the standard treatment at their center i.e. radiation and temozolomide). In much the same vein, Del Baldo et al have recently published a series of 25 DIPG biopsies [65]. All patients received a standardized initial treatment plan (radiation, nomotuzumab and vinorelbine). At progression, they attempted to tailor treatment based on molecular markers wherever targeted therapies were available (e.g. patients with mTOR pathway alterations got everolimus). Notably, they reported that median overall survival for patients for whom targeted therapies could be used at progression was longer (22 months, n = 9) compared to those for whom no targeted therapy was available. These studies are representative of a new wave of literature wherein treatment schema for DMG will be rationally based on molecular features. Evidence that this paradigm shift in clinical decision making (as opposed to empirical treatment on the basis of radiographic diagnosis) is likely to improve outcomes is still emerging but this is undoubtedly a necessary field of inquiry within a clinical context where survival outcomes have not improved for many decades.

Conclusion

Within the limitations of meta-analysis of observational studies, the pooled evidence suggests that brainstem biopsies in children are likely safe (permanent complication rate <2%). This favorable risk profile should be taken into account when considering brainstem biopsy in children especially when assessing the risks and benefits of trial enrollment for DMG.

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Figure Legends

Figure 1: PRISMA Flowchart showing selection criteria for studies included in meta-analysis.

Figure 2: Forest plot showing meta-analysis of incidence of diagnostic biopsy.

Figure 3: Forest plot showing meta-analysis of incidence of temporary complications.

Figure 4: Forest plot showing meta-analysis of incidence of permanent complications.

Figure 5: Schematic summarizing one feasible approach to brainstem biopsy based on safety data analyzed in the present study

Table 1 – List of studies included in meta-analysis after application of inclusion and exclusion criteria (n=29).

	First Author	N	Country	Center (Hospital if specified, otherwise University)	Age - Mean (Range) (unless specified)	Accrual Period
1	Chico Ponce de Leon et al., 2003[36]	50	Mexico	Hospital Infantil de Mexico Federico Gomes, Mexico City	Median 7, (6 mo - 15)	1989-2002
2	Pincus et al., 2006[37]	8	USA	University of Florida, Gainesville	11 (3-17)	Unclear
3	Pirotte et al., 2007[38]	20	Belgium	Erasme Hospital, Brussels	8.2 (3-13)	1995-2006
4	Schumacher et al., 2007[8]	126	Germany	Multicenter, 8 sites	6.9 (all <18)	Unclear
5	Patel et al., 2009[39]	24	India	Apollo Specialty Hospital, Chennai	8, (2 -13)	2004-2007
6	Perez Gomez et al., 2010[40]	20	Mexico	Instituto Nacional de Pediatria, Mexico City	7.95 (2-13)	2000-2008
7	Rajshekar et al., 2010[41]	106	India	Christian Medical College, Tamil Nadu	8.2 (2-18)	1987-2008
8	Haegelen et al., 2010[42]	5	France	Lille University Hospital, Lille	10.3 (6-17)	2004-2006
9	Dellaretti et al., 2011[43]	44	France	Roger Salengro Hospital, Lille	6	1988-2007
10	Cage et al., 2013[44]	9	USA	University of California, San Francisco	5.7 (8mo - 10)	2000-2011
11	Ogiwara et al., 2013[45]	7	Japan	National Center for Child Health and Development	6.2 (6-12)	2008-2012
12	Manoj et al., 2014[46]	41	India	National Institute of Mental Health ad Neuroscienes, Bangalore	Median 9	1994-2009
13	Puget et al., 2015[47]	130	France	Necker Enfants Malades Hospital, Paris	Media 6.7, (1-16)	2002-2015
14	Wang et al., 2015[48]	15	USA	Children's Hospital of Michigan, Detroit	Range 1-16	2001-2012

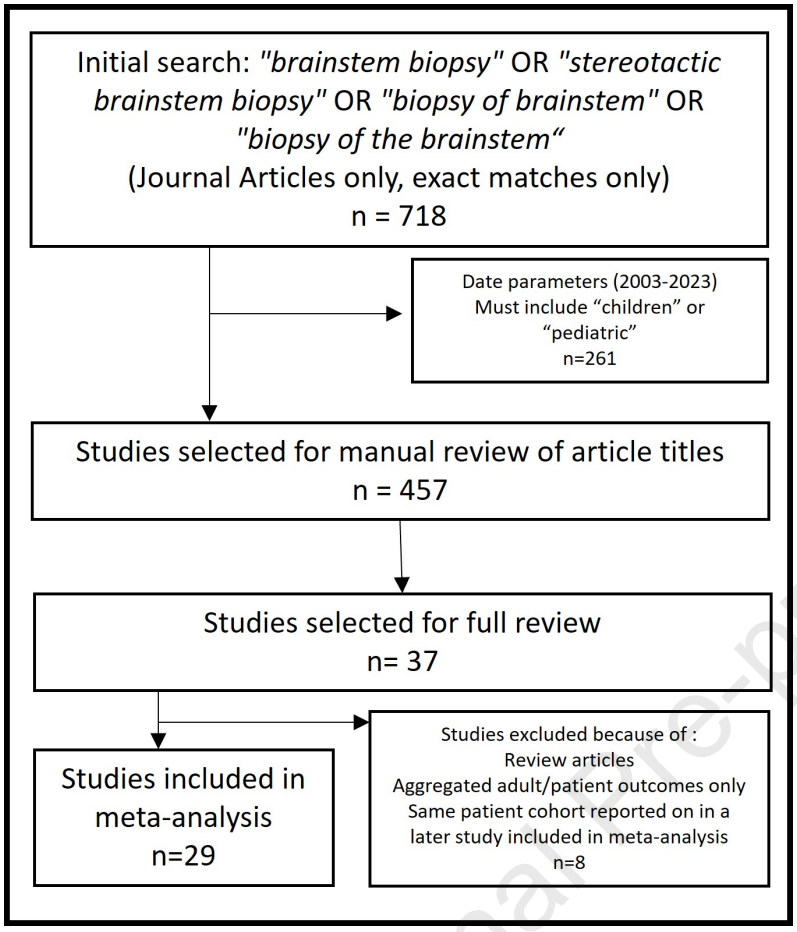
15	Coca et al., 2016[49]	5	France	CHU Hautepierre, Srasbourg	8.6 (5-13)	2012-2015
16	Quick Weller et al., 2017[50]	5	Germany	University Hospital, Frankfurt	7.5 (< 12)	2015-2016
17	Carai et al., 2017[51]	7	Italy	Bambina Gesu Children's Hospital, Rome	8, (6-13)	2015-2017
18	Gupta et al., 2018[52] (DIPG-BATS)	50	USA	Multicenter, 23 sites	Median 6.4, (3-17)	2011 - 2015
19	Pfaff et at., 2019[53] (INFORM)	21	Germany	Multicenter, 12 sites	7.5 (3-15)	2015-2018
20	Dawes et al., 2019[54]	11	UK	Great Ormond Street Hospital, London	Median 10 (2-15)	2015-2017
21	Cheng et al., 2020[55]	37	China	PLA General Hospital, Beijing	≤18	2015-2017
22	Gupta et al., 2020[51]	20	USA	Rady Children's Hospital, San Diego	Median 9.1 (IQR 7.3 - 11.2)	2015-2020
23	Morais et al., 2020[57]	26	Brazil	Clinic's Hospital of the University of Sao Paulo, Sao Paulo	8.8 (3-17)	2008-2018
24	Hersch et al., 2020[58]	58	USA	Unclear Hosptal site/sites	Median 7.5 (IQR 3.9 - 14.1)	2011-2019
25	Machetanz et al., 2020[59]	7	Germany	Eberhard Karls University, Tuebingen	11 (5-16)	Unclear
26	Peciu-Florianu et al., 2020[60]	31	France	CHU Lille, Lille	<16	2001-2017
27	Wang et al., 2022[61]	71	China	Children's Hospital of Fudan University, Shanghai	6.4 (1-13)	2016-2021
28	Lim et al., 2020[62]	21	Singapore	KK Women's and Children's Hospital, Singapore	Median 5, all <14	2006-2021
29	Fruh et al., 2023[63]	27	Germany	Charite-Universitatsmedizinin, Berlin	5 (3-10)	2009-2022
	Sum	**1002**				

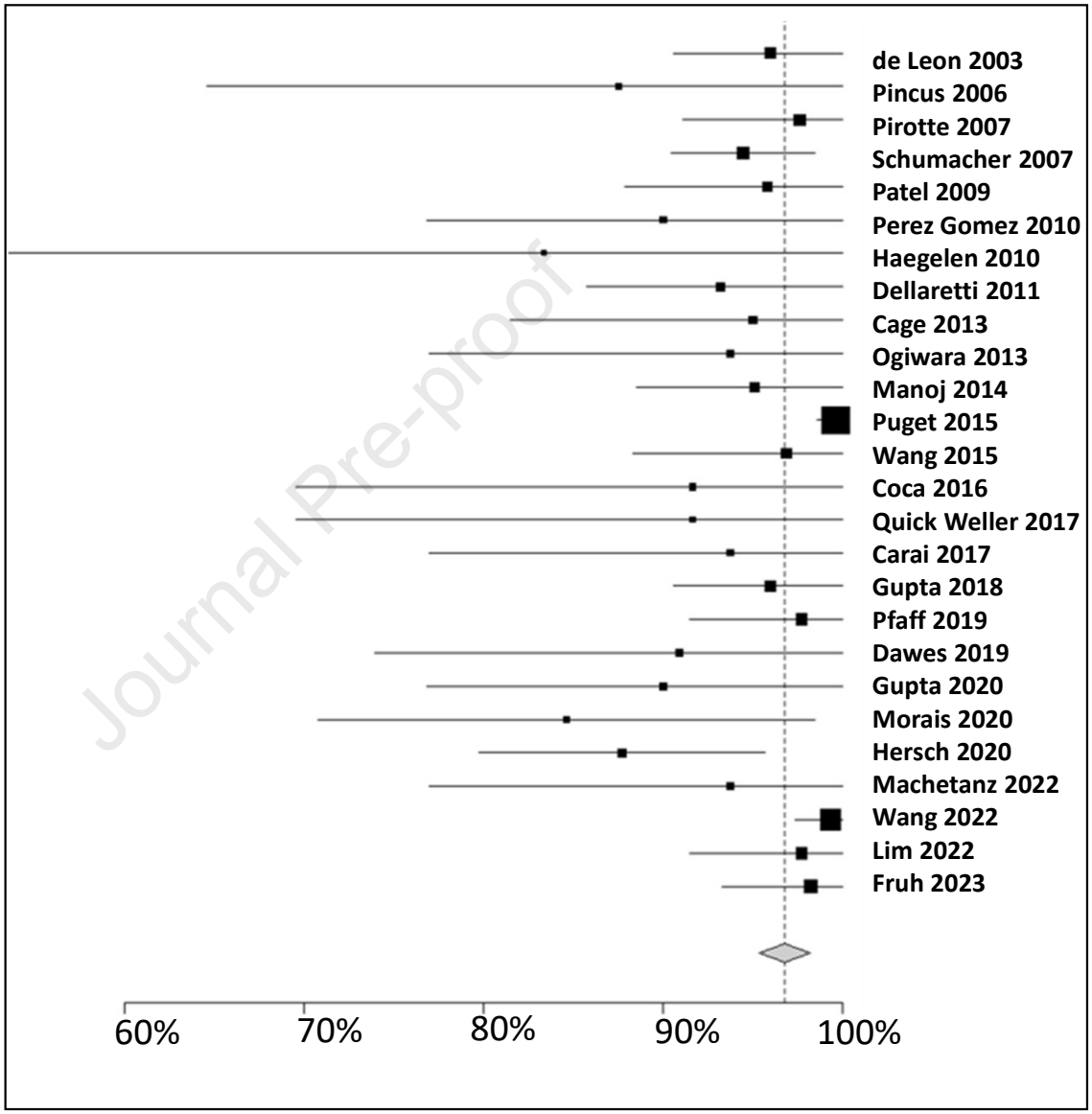
Table 2 – Details of the 5 mortalities that were documented in the reviewed studies

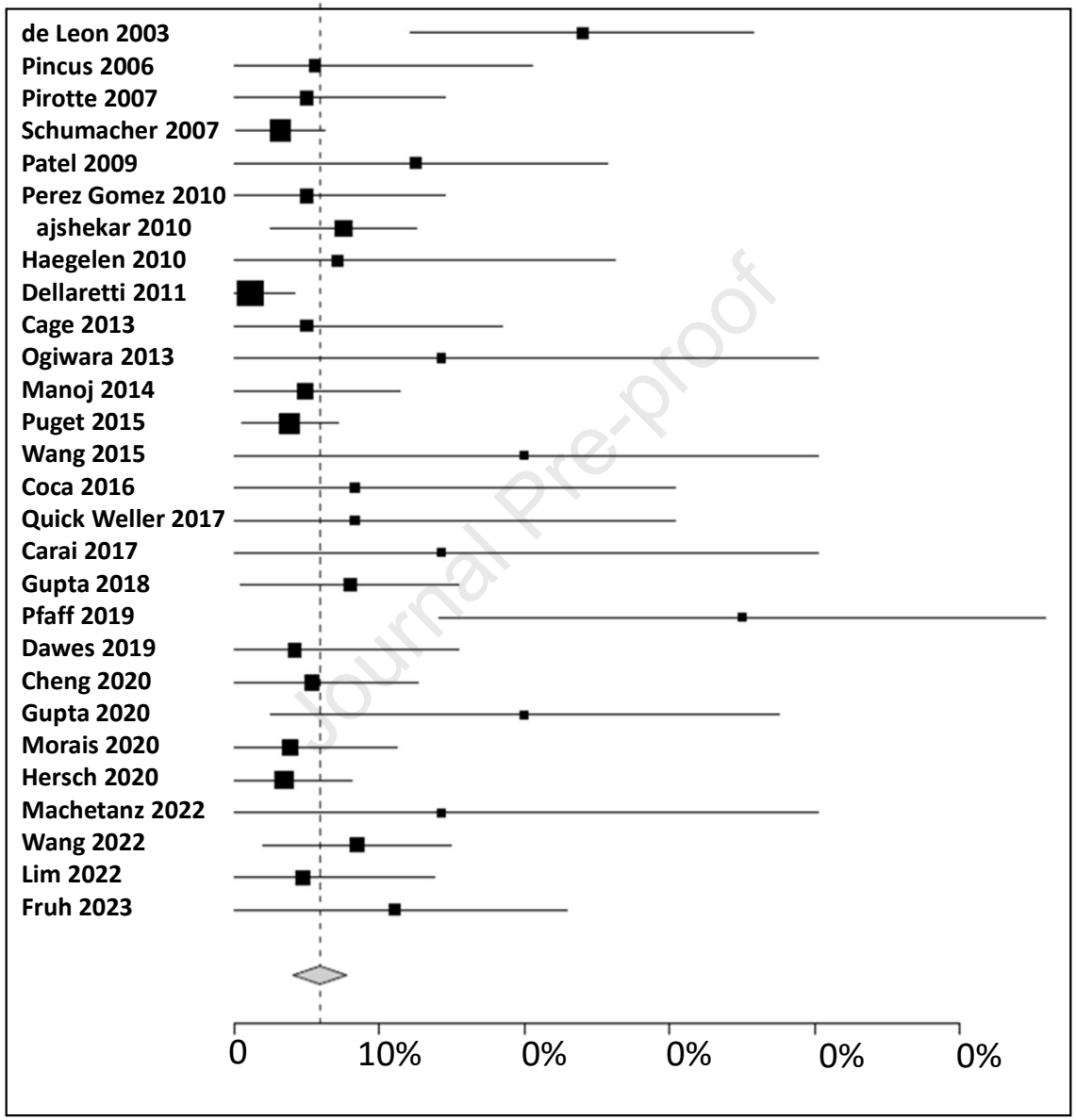
	Study	Patient Age/Gender	Notes
1	Cheng 2020	8 M	stereotactic pontine biopsy, anaplastic astrocytoma, progressive swelling in surgical bed, death on post-operative day 18
2	Cheng 2020	17 F	stereotactic pontine biopsy, anaplastic astrocytoma, cerebral herniation on post-operative day 3
3	Wang 2022	9 F	open microsurgical biopsy of pontine lesion, diffuse glioma, cerebellar swelling noted intraoperatively requiring removal of part of cerebellum, cardiac arrhythmia on post-operative day 2 with subsequent death due to cardiorespiratory arrest
4	Wang 2022	unclear	open microsurgical biopsy, death on post-operative day 5 in the context of cerebellar and tumor edema, increased hydrocephalus, secondary diabetes insipidus and electrolyte abnormalities
5	Wang 2022	unclear	open microsurgical biopsy, post-operative CNS infection, death on post-operative day 23

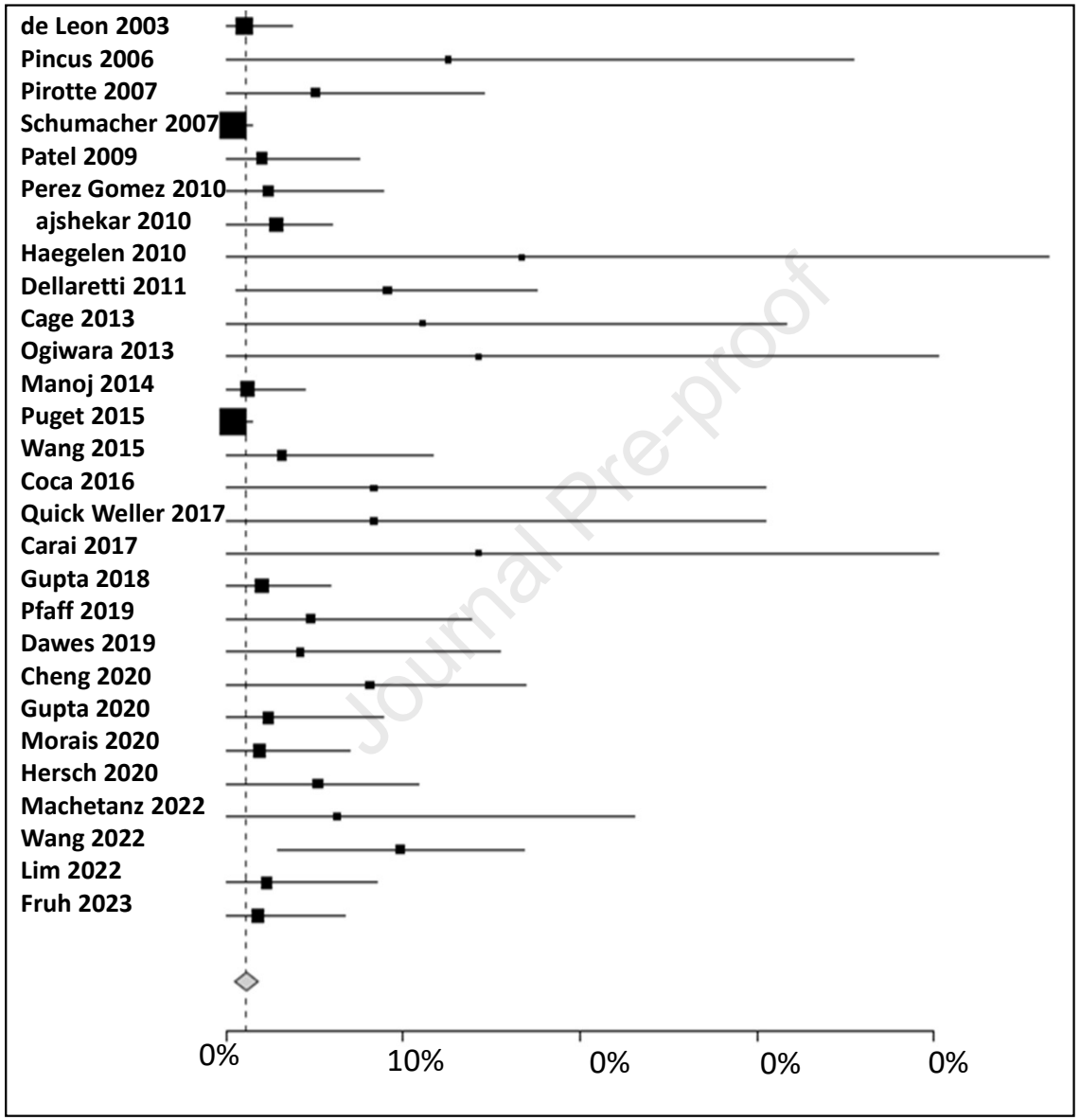
Table 3: Quality assessment of pooled evidence based on GRADE criteria

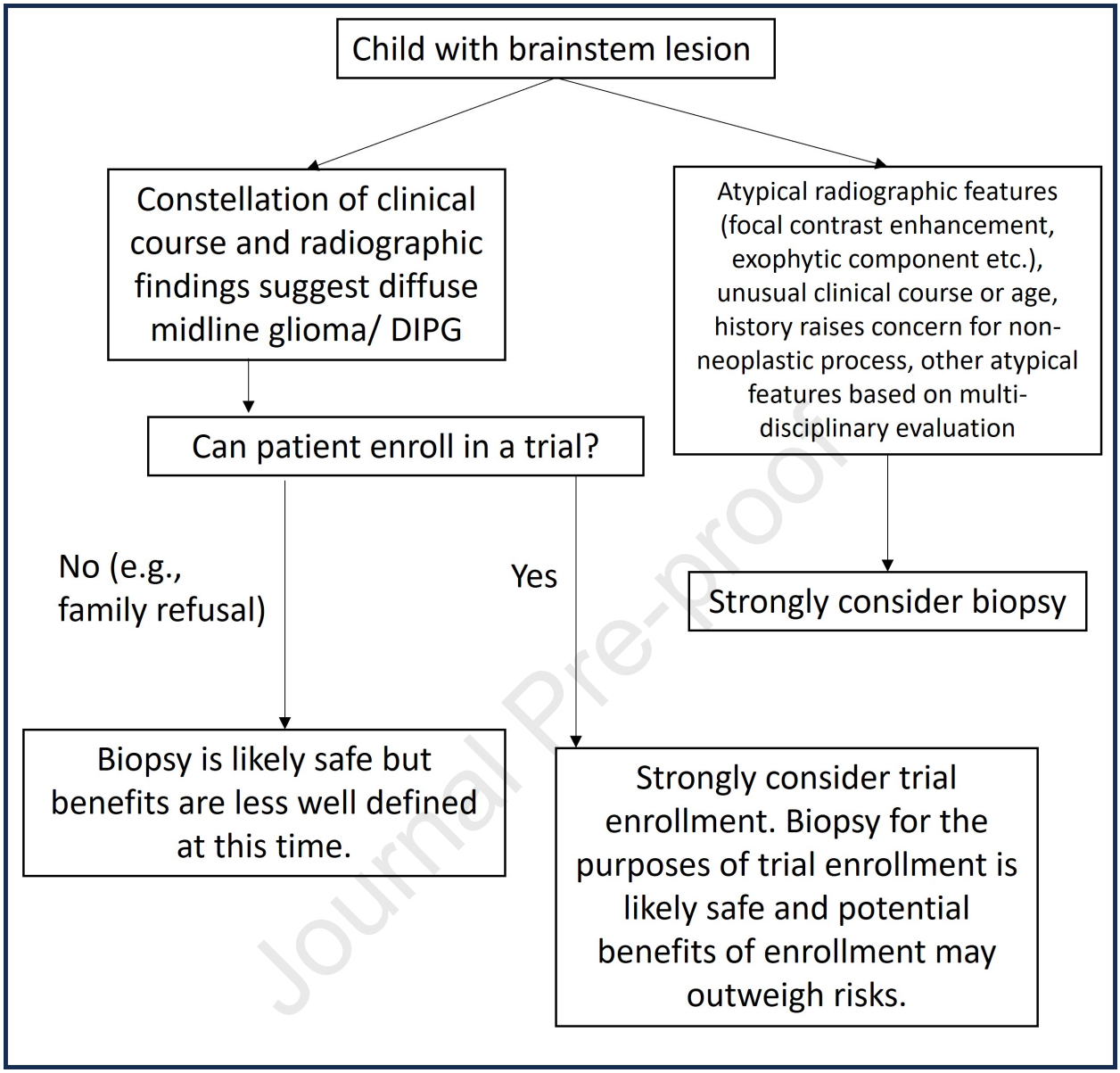
Outcome	Rate (%) (95% CI)	Number of Cohorts	Baseline quality of evidence based on study type (by default, RCT is 'High' and observational is 'Low')	Factors that would lower the quality of evidence by GRADE criteria				Factors that would improve the quality of evidence by GRADE criteria		
				Publication Bias (-1 for likely, -2 for very likely)	Inconsistency (-1 for serious, -2 for very serious)	Indirectness (-1 for serious, -2 for very serious)	Imprecision (-1 for serious, -2 for very serious)	Effect Size (+1 for significant/large effect, +2 for very significant/large effect)	+1 if all possible confounding would increase effect size	+1 if evidence supports a dose-response relationship
Rate of technically efficacious biopsy	94.2 (92-96.5)	26	Low	-1	-1	0	-1	+2	0	0
Rate of temporary complications	5.9 (4.1-7.8)	28	Low	-1	-2	0	-1	+2	0	0
Rate of permanent complications	1.1 (0.5-1.8)	28	Low	-1	-1	0	-1	+2	0	0



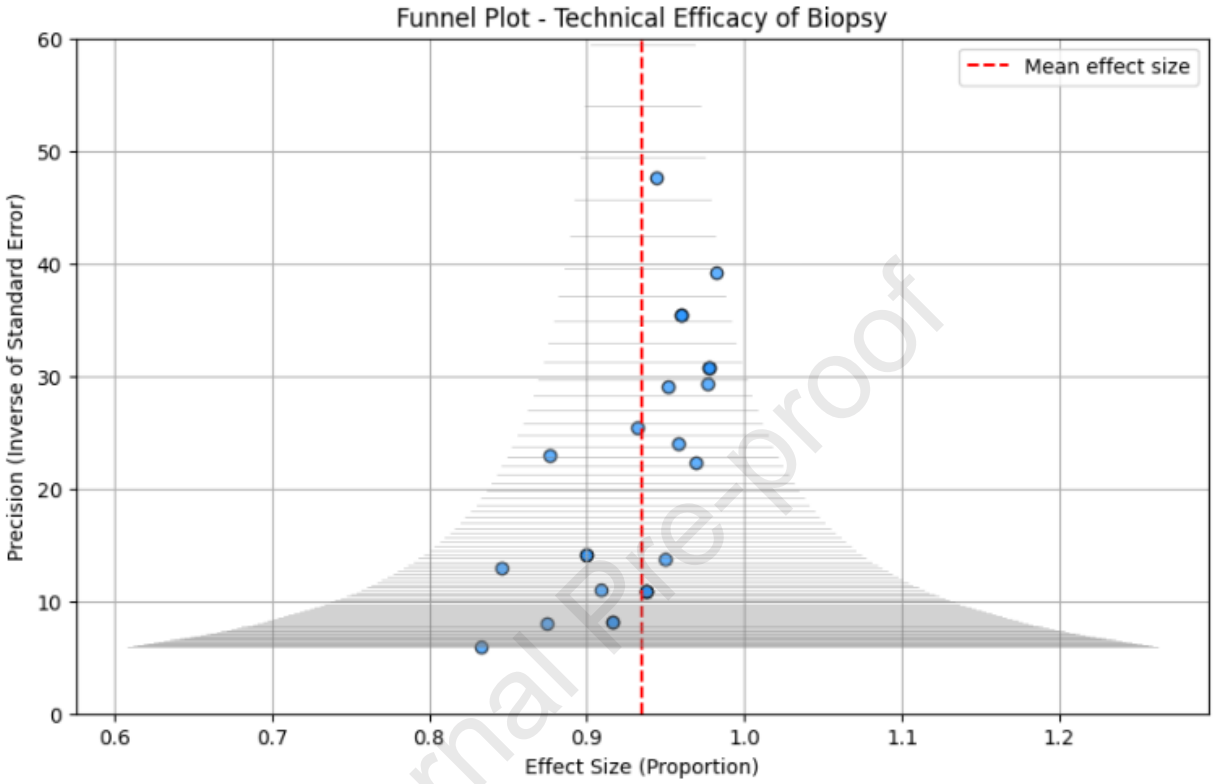




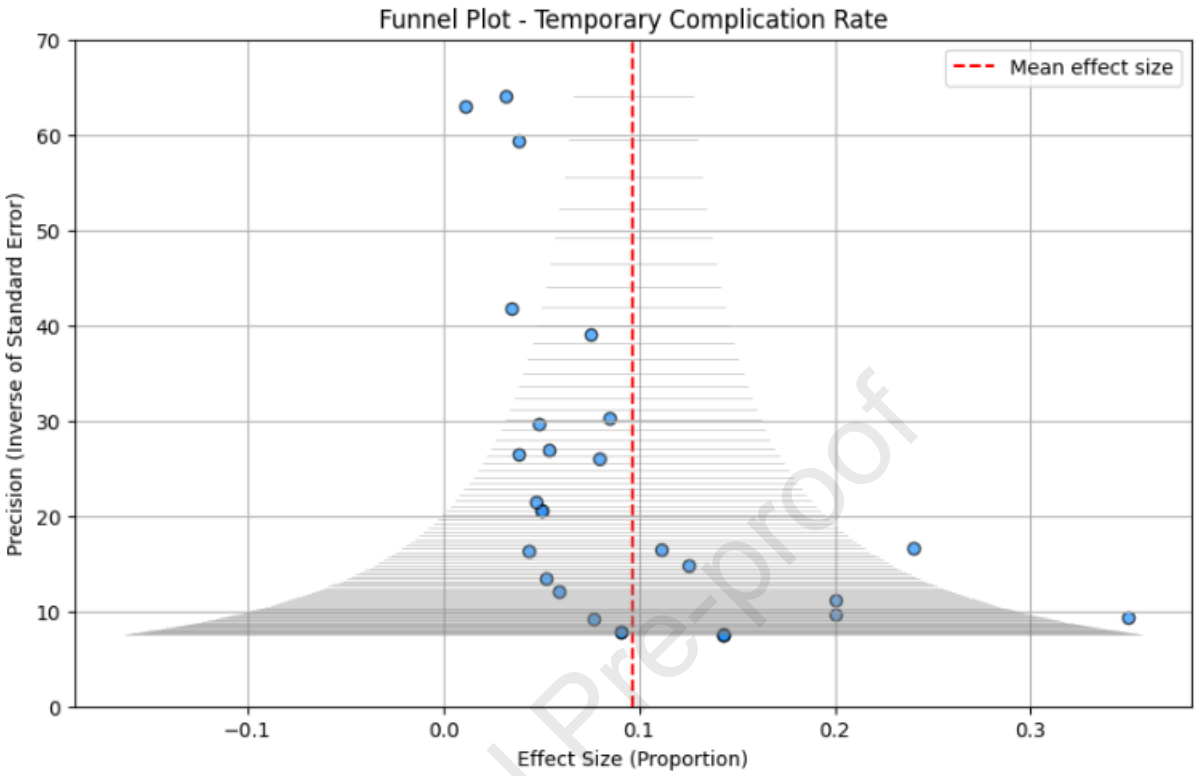




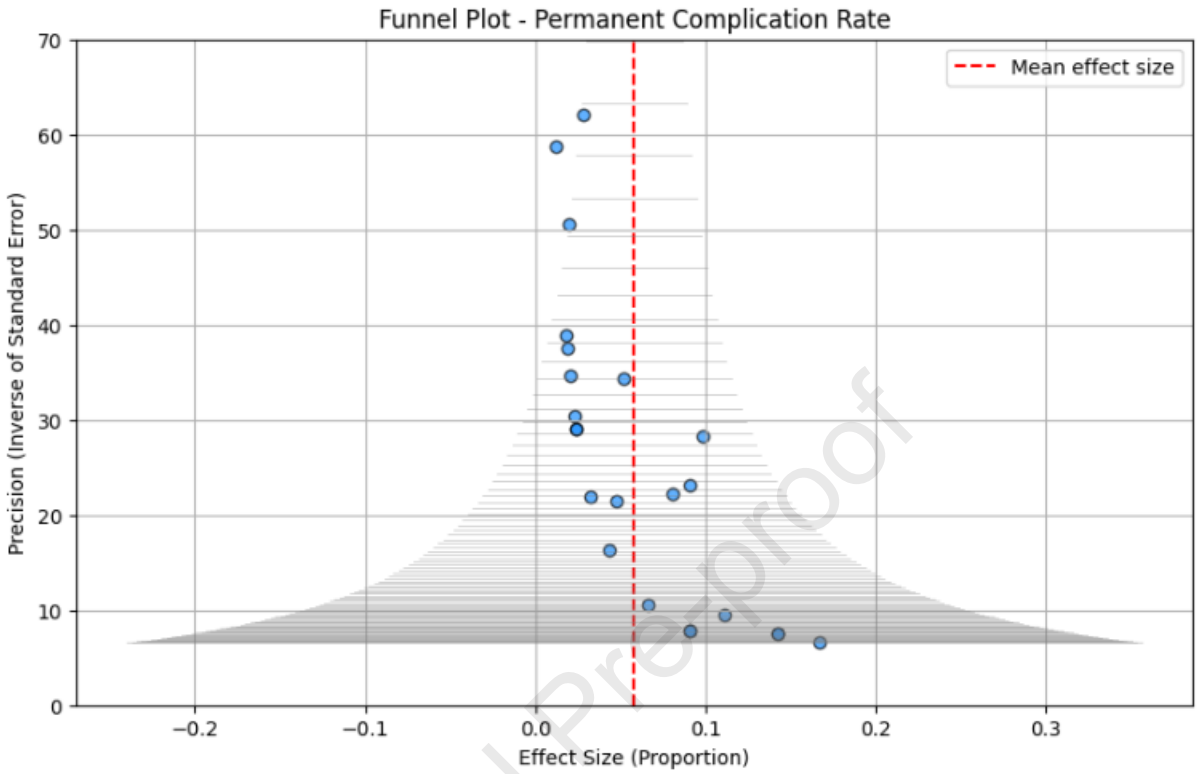
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



DIPG: Diffuse Intrinsic Pontine Glioma

DMG: Diffuse Midline Glioma

H3K27M: mutation causing substitution of lysine 27 to methionine in histone H3

DIPG-BATS: DIPG Biology and Treatment Study

INFORM: Individualized Therapy For Relapsed Malignancies in Childhood

The authors have no conflicts of interest to declare.