

Prospective Collection of Tissue Samples at Autopsy in Children With Diffuse Intrinsic Pontine Glioma

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BACKGROUND: Brain tissue obtained at autopsy has been used in research for non-oncologic disorders. However, to the best of the authors' knowledge, this tool has never been systematically used in large investigational studies for cancer. A prospective, multicenter study was conducted to assess the feasibility of tissue collection at autopsy and its suitability for molecular analyses in children with diffuse intrinsic pontine glioma. **METHODS:** Tumor tissue was collected at the time of diagnosis, if clinically indicated, or at autopsy. Normal brain tissue was also collected at autopsy. The integrity of DNA and RNA was evaluated in all samples. Logistic data regarding autopsies were recorded. The feasibility of tissue collection at autopsy was assessed for patients treated at a single institution over a 43-month period. **RESULTS:** Tumor samples were collected at the time of diagnosis (n = 3) or at autopsy (n = 38) at 29 centers across the United States; samples were obtained at diagnosis and autopsy in 2 cases. The median interval from death to autopsy was 7.7 hours. DNA and RNA with minimal or partial degradation, which were suitable for genome-wide analysis, were obtained from 100% and 63% of tumor samples, respectively. At the coordinating institution, approximately 40% of parents consented to autopsy and 40% declined. During the study period, 12 autopsies were performed on patients who did not receive therapy at the coordinating center. **CONCLUSIONS:** Multicenter, biological studies based on tissue obtained at autopsy appear to be feasible in children with brain cancer. The current experience established a new paradigm for brain tissue collection, which may increase the potential for research studies in patients with cancer. *Cancer* 2010;000:000-000. © 2010 American Cancer Society.

KEYWORDS: autopsy, brain, children, diffuse intrinsic pontine glioma, molecular studies.

Brain tumors are the second leading cause of death among children with cancer.¹ Likewise, primary and metastatic brain cancer account for a disproportionately high share of the mortality among adults with cancer.^{1,2} After breakthroughs in the use of targeted therapies for other cancers in adults,^{3,4} there has been a huge effort to better understand the biology of aggressive brain cancers with the hope that it may lead to the design of more rational and better therapies. Recent studies have described detailed molecular analysis of glioblastoma (World Health Organization [WHO] grade 4), the most lethal brain cancer in adults.^{5,6}

Diffuse intrinsic pontine glioma (DIPG) is 1 of the deadliest brain tumors in children.⁷ Despite the use of radiotherapy (RT) with or without chemotherapy, the long-term survival of affected children has remained at <10% for the past 30 years.⁷ Radical (>90%) resection of DIPG is not feasible because the tumor is an infiltrative neoplasm arising within the pons. Although tumor biopsy can be safely obtained for histologic confirmation,⁸ it is rarely recommended because of a lack of benefit to patients. Likewise, autopsy is hardly ever performed in these patients. Histologic review of DIPG at the time of diagnosis or at autopsy invariably demonstrates a high-grade glioma, particularly glioblastoma.^{8,9}

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Because tumor tissue for research studies is seldom available, little is known regarding the molecular characteristics of DIPG.¹⁰⁻¹³ A recent single-institution study reported genome-wide DNA abnormalities in 11 children with DIPG; 9 of the tumor samples were collected at autopsy.¹⁴

Although autopsy was standard practice until the middle of the 20th century, its frequency among adults has declined steeply in Western countries.^{15,16} Autopsy rates among children have declined less dramatically compared with adults.^{17,18} Autopsy has been shown to be a valuable tool in obtaining brain tissue for research purposes in several devastating disorders in adults and children.¹⁹⁻²² However, no study to date that we are aware of has ever addressed the feasibility of systematic collection of brain tissue at autopsy and the suitability of this tissue for molecular studies focused on cancer.

Herein, we describe a successful prospective clinical trial in which tumor and normal tissue samples were collected from children with DIPG at the time of diagnosis, when clinically indicated, or at autopsy at multiple centers throughout the continental United States. Our primary goal was to increase the understanding of the molecular mechanisms of tumorigenesis in DIPG to provide the opportunity to target specific genetic aberrations within this cancer. Our experience established a new paradigm for brain tissue collection, which may increase the potential for research studies in patients with cancer.

MATERIALS AND METHODS

After approval by the Institutional Review Board (IRB) at St. Jude Children's Research Hospital in June 2006, we prospectively collected tumor and normal tissue samples from patients of any age with a clinical and radiologic diagnosis of DIPG, including secondary DIPG after RT. Patients with high-grade glioma originating outside the brainstem and non-high-grade glioma arising within the brainstem were excluded. At the time of IRB approval, we also obtained permission to include tumor samples collected at autopsy before activation of the study. Magnetic resonance imaging (MRI) of the brain at diagnosis or during therapy was centrally reviewed by 1 investigator (A.B.) to confirm the diagnosis of DIPG. Tumor samples underwent central histologic review by a senior neuropathologist at the coordinating center.

Flexibility was granted to physicians and other healthcare providers to discuss the issue of brain-only autopsy with parents at the time judged to be most

appropriate, either before or after death. The process of consent to autopsy was variable and followed local and institutional regulations. In addition, consent for this research was obtained from parents in all prospectively collected tissue samples.

Protocol guidelines for tissue collection at autopsy recommended a brain-only autopsy as soon after death as possible. Autopsies were performed at 29 centers in 18 of the continental United States. Selection of these centers was based on their proximity to the place of death and availability of infrastructure to perform autopsy. Only 4 of the autopsies were performed in the coordinating center. Tissue samples were to be collected from the brainstem tumor and from normal brain. The cerebellum (if no tumor involvement was suspected) or the frontal lobes were chosen as the source of normal brain tissue. When possible, approximately 100-mg fragments of tumor and normal brain samples were to be snap-frozen in liquid nitrogen and stored at temperatures of -70°C or lower. The remaining samples of normal brain and tumor were processed for routine histologic examination.

Clinical data and information regarding autopsy (place of death, interval between death and autopsy, location of autopsy, and processing of samples) were collected prospectively. The rate of consent to autopsy was investigated for all consecutive patients with DIPG treated at the coordinating institution who experienced disease progression during the study period.

Snap-frozen tissue samples were pulverized on dry ice. DNA was extracted as previously described.²³ RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The integrity of DNA was evaluated by agarose gel electrophoresis and by labeling and hybridization to Affymetrix SNP (single nucleotide polymorphism) 6.0 Gene Mapping arrays (Affymetrix, Santa Clara, CA). RNA integrity was evaluated by using the 2100 Bioanalyzer Lab-on-a-Chip system (Agilent Technologies, Santa Clara, CA), which produced an RNA integrity number (RIN). RNA was classified as minimally degraded (RIN >7.5), partially degraded (RIN of 5-7.4), or degraded (RIN >5). Logistic regression was used to investigate the association between RNA integrity and the interval from death to autopsy.

RESULTS

Tumor tissue was obtained from 38 patients with DIPG (median age at diagnosis, 6.1 years; range, 3-15.7 years) and from 1 patient with secondary DIPG. The secondary

Table 1. Clinical Characteristics of Patients and Yield of Tissue Collected at Autopsy

Characteristic	Value
Median age at diagnosis (range), y ^a	6.1 (3-15.7)
Race	
Caucasian	27 (69%)
African American	6 (15.5%)
Other ^b	6 (15.5%)
Median interval from death to autopsy (range), h	7.7 (2.25-23)
Histologic diagnosis	
Biopsy	Anaplastic astrocytoma (n=1) Glioblastoma (n=2)
Autopsy ^c	Glioblastoma (n=35)
Quality of DNA^d	
Tumor	Minimal degradation: 33 (87%) Partial degradation: 5 (13%)
Normal brain	Minimal degradation: 31 (94%) Partial degradation: 2 (6%)
Quality of RNA^d	
Tumor	Minimal degradation: 13 (34%) Partial degradation: 11 (29%) Degraded: 14 (37%)
Normal brain	Minimal degradation: 19 (58%) Partial degradation: 7 (21%) Degraded: 7 (21%)

^aOne 15-year-old patient with secondary diffuse intrinsic pontine glioma was excluded.

^b"Other" category was comprised of mixed race or other minority groups.

^cOne autopsy demonstrated extensive tissue destruction and 1 focus compatible with glioma, and another autopsy revealed a diffuse infiltrative astrocytoma. Tumor tissue was not available for review in 1 case.

^dIncluding only samples obtained at autopsy.

DIPG was diagnosed at autopsy in a 15-year-old boy who had been treated with RT and chemotherapy for glioblastoma originating elsewhere in the central nervous system. Twenty-seven (69%) children were Caucasian, 6 (15.5%) were African American, and the remainder were of other minority groups or of mixed race/ethnicity (Table 1).

Three patients with DIPG underwent surgery at the time of diagnosis for histologic confirmation; 2 of them also underwent autopsy. Five autopsies had been performed as part of standard care before study activation. Thirty-three autopsies were performed between June 2006 and January 2010. Thirty-six autopsies occurred after the failure of treatment for DIPG, 1 was obtained shortly after a diagnosis of secondary DIPG, and 1 was obtained shortly after a diagnosis of DIPG in a child who died of rapid tumor progression before the initiation of treatment.

Thirty-two (84%) patients who underwent autopsy died at home. Six patients died in the hospital to which they were admitted to receive terminal care. The median interval from death to autopsy was 7.7 hours (range, 2.25 to 23 hours). Thirty-one (82%) autopsies were performed at academic centers, and the remainder were performed at

community hospitals or private institutions. Liquid nitrogen was used to snap-freeze tissue samples from 28 (74%) autopsies. Tissue samples were stored at temperatures of -70°C or lower in all cases except 1. Normal brain tissue from 33 (87%) autopsies was available for analysis.

Central histologic review of 3 tumor samples obtained at diagnosis revealed anaplastic astrocytoma (WHO grade 3; n = 1 sample) and glioblastoma (n = 2 samples). Glioblastoma was the histologic finding in 35 autopsies, including 2 obtained at the time of diagnosis. One autopsy disclosed extensive tissue destruction and a tiny focus compatible with glioma, and another autopsy revealed a diffuse infiltrative astrocytoma. Tissue for histologic review was not available for only 1 case.

Integrity of DNA and RNA Samples

DNA with minimal degradation was isolated from tumor and normal brain samples obtained in 33 (87%) and 31 (94%) autopsies, respectively (Table 1). The remaining samples yielded DNA with partial degradation (Fig. 1A). All DNA samples yielded call rates $>89\%$ when labeled and hybridized to Affymetrix SNP 6.0 Gene Mapping arrays, indicating outstanding quality for genome-wide copy number analysis.

Minimally degraded RNA was recovered from 13 (34%) tumors and 19 (58%) normal brain samples obtained at autopsy. Eleven (29%) tumors and 7 (21%) normal brain samples demonstrated partial RNA degradation but were considered suitable for Affymetrix Gene Expression array analysis on the basis of institutional experience (Fig. 1B). A shorter interval from death to autopsy was significantly associated with minimal degradation of RNA ($P = .042$). Whereas RNA with minimal degradation was obtained from 70% of the autopsies performed within 5 hours of death, the yield of good-quality RNA dropped to 21% beyond 5 hours from death.

Rate of Consent for Autopsy

During the study period, 53 children with DIPG (n = 51) or secondary DIPG (n = 2) who were treated at St. Jude Children's Research Hospital died after experiencing progressive disease. Consent for autopsy was not sought in 11 cases (20.8%) because of the primary physician's preference (n = 6), loss of follow-up (n = 2), or inability to reach the parents (n = 3). The parents declined autopsy in 21 (39.6%) cases. Brain-only autopsy was obtained in the remaining 21 (39.6%) cases.

During the same period, 12 (36%) autopsies were obtained from children treated at other institutions and

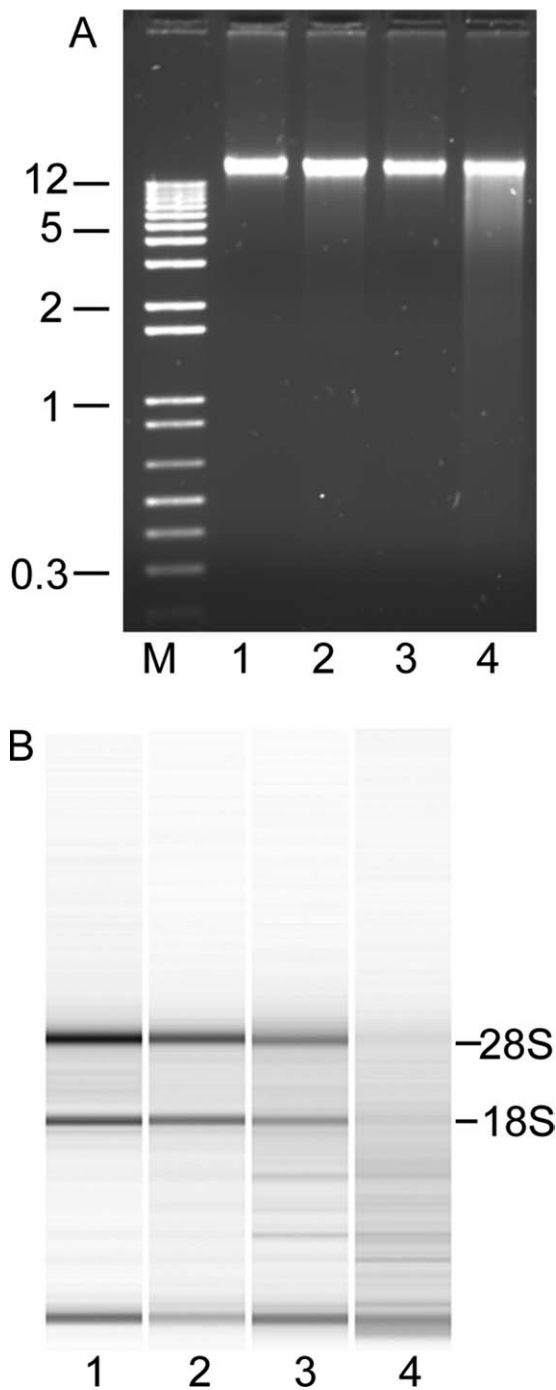


Figure 1. Representative DNA and RNA were extracted from tissue obtained at autopsy. (A) Agarose gel electrophoresis of DNA extracted from 2 paired sets of normal brain and tumor (Lanes 1-4) is shown. All lanes except Lane 4 demonstrated minimal DNA degradation. Lane 4 demonstrated partial DNA degradation. M indicates 1-kilobase ladder molecular weight marker. (B) RNA analysis of the same samples as shown in Panel A are shown. Lanes 1 and 2 demonstrate minimal degradation (RNA integrity number [RIN] 8.9 and 8, respectively), Lane 3 demonstrates partial degradation (RIN 6), and Lane 4 demonstrates degraded RNA (RIN 2.2). Migration of 28S and 18S ribosomal RNA is indicated.

tissue was submitted to this study. Two of the 12 children had been seen at the coordinating institution for consultation at the time of tumor progression. The process of tissue donation was initiated by either the parents or the patient's local oncologist in 11 (92%) of these 12 cases.

DISCUSSION

To the best of our knowledge, the current study is the first prospective study of the feasibility of tissue collection at autopsy from a relatively large number of patients with brain tumors. These findings demonstrate that the systematic collection of tissue samples at autopsy in children with DIPG is not only feasible but yields DNA and RNA suitable for genome-wide studies. All tumors and normal brain samples yielded DNA of sufficient quality for use in genome-wide SNP array studies. RNA in 63% and 79% of tumor and normal brain samples, respectively, was suitable for genome-wide expression studies. Samples obtained after a short interval between death and autopsy were most likely to yield the best quality RNA. However, RNA with minimal degradation was recovered from a sample collected >17 hours after death, indicating that samples suitable for genome-wide expression studies may be obtained even after longer intervals from death. Although approximately one-third of the cases yielded degraded RNA, these samples could still be useful for other molecular studies (eg, polymerase chain reaction amplification of smaller fragments to detect gene mutations).

It was also extremely gratifying to recover DNA and RNA of excellent quality from the majority of tumor and normal brain samples, particularly taking into account that the vast majority of our patients received end-of-life care and died at home.

Since the advent of MRI, DIPG has been diagnosed on the basis of its typical imaging characteristics.²⁴ Histologic confirmation is currently recommended to rule out other types of brainstem neoplasm only when the tumors demonstrate atypical imaging characteristics. Our central review confirmed the diagnosis of high-grade glioma in all but 2 of the evaluable samples. In the latter 2 cases, treatment effect and sampling error likely restricted our ability to make a correct diagnosis. Although DIPG and secondary DIPG are likely to arise through distinct mechanisms of tumorigenesis, we included the latter group in this study because it provided a unique opportunity to compare the molecular characteristics of the 2 neoplasms.

The extent to which parents consented to autopsy in the current study was of particular interest in view of recent controversy concerning pediatric autopsy and in view of concerns raised by parents concerning 2 incidents of undisclosed tissue retention in the United Kingdom.²⁵ Even more remarkable, in more than one-third of the autopsies performed during the study period, the patients had not been treated at the coordinating institution; the process of tissue donation was initiated by parents in at least 7 of 12 cases. We attribute this success partially to a growing awareness among the parents of children with DIPG that further collaboration with researchers is needed to improve the outcome of children with this lethal cancer. This growing awareness has been particularly fostered by networks of parents and support groups in the United States. We took particular care to obtain parents' consent for the retention of their child's tumor and brain tissue for molecular studies. We also made every effort within the context of a multicenter study to share the clinical information obtained at autopsy with the parents. It is interesting to note that expedited IRB approval outside the coordinating institution was only required in 1 autopsy because all other participating institutions considered this brain-only autopsy part of standard care.

We prospectively studied the rate of consent and decline to autopsy among parents of children treated at the coordinating institution. An equal proportion of parents consented to autopsy and declined permission to the procedure. For ethical reasons, we did not seek the reasons for decline to consent. The attending physicians opted not to raise the issue of autopsy in 11% of cases treated at the coordinating institution. We attribute this percentage largely to the fact that end-of-life care and death uniformly occurred at the family's home; therefore, the pediatric oncologists were not in close contact with the families near the time of death. However, we cannot rule out the possibility that the physicians were uncomfortable about discussing the issue of autopsy in some cases. We have begun to interview the parents who consented to autopsy, having first waited several months. Bereaved parents frequently seek ways to move forward in their bereavement and to find meaning in their child's death.²⁶ Our hope is that these interviews will reveal parents' reasons for participating in the current study and whether they have experienced any regret regarding their participation.

Although the end-of-life process in children is quite distinct from that in adults, we believe that our success in collecting brain samples in children with cancer may

encourage similar studies in adults with cancer and brain involvement. For example, recent studies have analyzed the molecular abnormalities in recurrent brain tumors in adults compared with samples obtained at diagnosis to better understand the mechanisms of tumor resistance.^{27,28} Brain tissue collection at autopsy represents a valuable source for such studies.

The strengths of the current study include its prospective nature, the conduct of autopsies in multiple centers across a large geographic area, the multicultural background of participating families, the relatively large number of autopsies obtained, and the high yield of DNA and RNA suitable for sophisticated genome-wide studies. These features allow these findings to be generalized to other clinical settings in which tissue obtained at autopsy could be used in research designed to benefit children. The current study remains open with the purpose of collecting a larger number of tissue samples for analysis. In the interim, we believe that our experience may encourage others to seek tissue for research in other lethal brain disorders.

CONFLICT OF INTEREST DISCLOSURES

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