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Photodynamic Diagnosis Using 5-Aminolevulinic Acid in 41 Biopsies for Primary Central Nervous System Lymphoma


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ABSTRACT

We evaluated the feasibility of 5-aminolevulinic acid (5-ALA)-mediated photodynamic diagnosis (PDD) in the biopsy for primary central nervous system lymphoma (PCNSL). 5-ALA (20 mg/kg) was administered orally 4 hours preoperatively. Forty-one biopsies obtained under PDD in 47 consecutive biopsies (46 patients) that were finally pathologically diagnosed as PCNSL were evaluated. Positive fluorescence was observed in 34 of those 41 biopsies (82.9%). An intraoperative pathological diagnosis (IOD) of suspected PCNSL was made in 21 of the biopsies with positive fluorescence (61.8%). However, the 8 IODs in the remaining 13 biopsies (23.5%) were not correct (atypical cell, 4; high-grade glioma, 1; gliosis, 1; unremarkable, 2). In those 8 biopsies, PCNSL was confirmed by the final pathological diagnosis. There was no difference in the mean Mib-1 labeling index between the biopsies with positive fluorescence (86.5%) and those without (90.0%). IOD was not performed in 6 biopsies; however, 5 of those biopsies (83.3%) showed positive fluorescence and were finally pathologically diagnosed as PCNSL. Use of PDD in biopsies for patients with suspected PCNSL is a reliable way of obtaining specimens of adequate quality for the final pathological diagnosis and may lead to improved diagnostic yield in the biopsy of PCNSL.

Keywords: primary central nervous system lymphoma, 5-aminolevulinic acid, endoscopic biopsy, photodynamic diagnosis
Introduction

Photodynamic diagnosis (PDD) in neurosurgery for several types of brain tumors was first described by Moore and colleagues [1]. While the PDD in their study was predominantly mediated by fluorescein, 5-aminolevulinic acid (5-ALA) has been successfully applied for fluorescence-guided tumor resections, leading to the widespread use of this technique in the neurosurgical field worldwide. In 5-ALA-mediated PDD, fluorescence is obtained on the basis of accumulation of protoporphyrin IX (PpIX) in malignant tumor cells following oral administration of 5-ALA as a precursor of heme biosynthesis. Proof of the benefit of 5-ALA-mediated PDD for glioblastoma was obtained by a randomized controlled multicenter phase-III trial conducted by Stummer and colleagues, which showed statistically significant improvement in the extent of tumor removal and 6-month progression-free survival after surgery, although the overall survival was not prolonged [2].

PDD using 5-ALA has also been applied in surgery for brain tumors other than glioblastomas, such as meningiomas, pituitary adenomas, and metastatic tumors [3-5]. For instance, 94.0% (31 of 33) of meningiomas showed positive fluorescence [3]. The sensitivity of 5-ALA-mediated PDD for pituitary adenoma was 80.8% (21/26) in endoscopy with photodiagnostic filters and 95.5% (21/22) in the PpIX spectroscopy optical biopsy system [4]. Fifty-two of 78 pediatric brain tumors (66.7%) showed positive fluorescence; they included high-grade gliomas (HGG), ependymomas, primitive neuroectodermal tumors (PNETs), gangliogliomas, medulloblastomas, and pilocytic astrocytomas [6].

Biopsy and subsequent pathologic confirmation are undertaken as the standard of care for PCNSL such as systemic
chemotherapy and radiotherapy. However, only a few PCNSL cases have previously been reported (a portion of a patient cohort who received 5-ALA-mediated PDD), and the role and impact of PDD for PCNSL have not been established.

Minimizing the extent of surgery is essential for reducing the risks during brain tumor biopsies, while samples that are adequate in number and quality are required to ensure the final pathological diagnosis. To evaluate the feasibility of PDD during the biopsy strategy for PCNSL, we studied the rate of positive fluorescence and the relation between PDD and the pathologic diagnosis of PCNSL.

Patients and methods

This was a retrospective study on a portion of the data obtained from an institutional review board-approved prospective study on PDD for intraparenchymal brain tumors (no. 89). The data collection included the preoperative neuroradiologic diagnosis, PDD, intraoperative pathologic diagnosis (IOD), and final pathologic diagnosis. Forty-six consecutive patients with a final pathologic diagnosis of PCNSL received 47 biopsies (open biopsy, 27; navigation-guided endoscopic biopsy, 14; stereotactic biopsy, 6) at the University of Tsukuba Hospital between July 2003 and December 2013. Of those 47 biopsies, 41 were obtained under PDD and were included in this study. In the other 6 biopsies, PDD was omitted owing to the need for emergency surgery. In all 47 biopsies, the preoperative neuroradiologic diagnosis was PCNSL.

>Figures 1 and 2<
5-ALA (20 mg/kg; Cosmo Bio, Tokyo, Japan) was administered orally 4 hours preoperatively. After induction of general anesthesia, the patient’s head was fixed using a Mayfield or Sugita frame, and the navigation system (StealthStation; Medtronic, USA.) was set up. For the endoscopic biopsies, a transparent sheath (7 or 10 mm in diameter) with a removable inner tube (Neuroport; Olympus, Tokyo, Japan) was inserted through the burr hole until the front of the target lesion was under the control of the navigation system; the lesion was further observed with a rigid endoscope (EndoArm; Olympus). In the endoscopic biopsies, the lesion was gradually removed [7]. Open biopsy was also performed under navigation-guided planning. After the skin incision and small craniotomy, a 12-Fr catheter tube was inserted from the brain surface towards the target, and a 1.5- to 2-cm corticotomy, performed. In the stereotactic biopsies, a Komai stereotactic frame or frameless neuronavigation was used, and the first target was set at the central or near-central region of the enhanced mass, and 4 or more samples were collected around the first target and periphery of the tumor for further pathologic diagnosis (Figs. 1 and 2). Samples were collected from at least 2 different biopsy targets, and those with positive fluorescence were sent to the pathology department for IOD. If suspected PCNSL was diagnosed, the remaining samples were kept for the final pathologic diagnosis. If IOD did not point to a diagnosis of suspected PCNSL or if there were no samples with positive fluorescence, additional sample collection was decided upon by each surgeon according to the potential risk in each case. The rates of positive fluorescence (strong or weak according to the macroscopic observations) and the relation between PDD and the pathologic diagnosis were analyzed.

Results
The final pathologic diagnosis was PCNSL (diffuse large B-cell lymphoma) for all biopsies except for 1 failed endoscopic biopsy after which the patient underwent an open biopsy and received a diagnosis of PCNSL. In 1 patient who underwent repeated biopsies and who received corticosteroids during a previous hospitalization at another hospital, the specimen obtained at the first biopsy showed weak fluorescence in the tissue suspected at the IOD as being lymphoma cells. However, the final pathologic diagnosis after the first biopsy could not definitely confirm that it was lymphoma tissue despite the presence of a few lymphocytes. Two weeks later, at the second biopsy, the surgical specimen was strongly fluorescent, and PCNSL was pathologically diagnosed.

>Figures 3 and 4<

Positive fluorescence was observed in 34 of the 41 biopsies conducted under PDD (82.9%; Fig. 3). Strong fluorescence was observed in 23 of those biopsies (56.1%), and weak fluorescence, in 11 of them (26.8%; Fig. 4). In 21 of the 34 biopsies (61.8%), an IOD of suspected PCNSL was made. In the remaining 13 biopsies, however, the IOD was either incorrect (atypical cell, 4; high-grade glioma, 1; gliosis, 1; unremarkable, 2) or not performed (5). Thus, for all 34 cases, PCNSL was diagnosed at the final pathologic examination. Intraoperative diagnosis was not conducted in 6 of the 41 biopsies obtained under PDD. Five of them (83.3%) showed positive fluorescence and were diagnosed as PCNSL at the final pathologic diagnosis. Fluorescence was not observed in 7 of the 41 biopsies conducted under PDD (17.1%). In 3 of those (42.9%), an IOD of suspected PCNSL was made. In the remaining 4 biopsies (57.1%), the IOD was either incorrect (atypical cell, 1; high-grade glioma, 1; necrosis, 1) or not performed (1). There was no difference in the mean
Mib-1 labeling index between the tumor cells with positive fluorescence (86.5%) and those without (90.0%).

Discussion

5-ALA-mediated PDD has often been performed with tumor biopsy, and such a combination is advantageous in facilitating diagnostic yield by distinguishing tumor-containing samples by use of fluorescence [7]. Although several pathologic types of brain tumor were reported in the literature on fluorescence-assisted biopsy, it is difficult to estimate the rate of positive fluorescence in PDD of PCNSL because of the limited number of such published cases. To the best of our knowledge, only 11 PCNSL cases with biopsy or resection conducted under 5-ALA-mediated PDD have been reported, and all of those showed positive fluorescence [4, 8-10]. Moriuchi and colleagues reported stereotactic biopsy conducted under PDD for a PCNSL of the thalamus and for a pontine glioma to confirm the target tumor tissues [9]. Grossman and colleagues reported positive fluorescence in PCNSL of the fourth ventricular floor [8]. Eljamel and colleagues reported 2 PCNSL with positive fluorescence [11]. Widhalm and colleagues found that all 16 samples taken from 7 PCNSL cases showed positive fluorescence (strong, 14; weak, 2) [10]. In the present study, positive fluorescence was observed in 34 of the 41 biopsies (82.9%), which is consistent with the findings of our previous biopsy report on 59 intraaxial tumor cases, which showed that 9 of the 12 PCNSL cases (75%) showed positive fluorescence [7].

According to a recent review, fluorescence is observed in HGG patients with 91% sensitivity and 59% specificity [12]. To date, the rate of positive fluorescence and the diagnostic yield of PDD for PCNSL have not been established. This report is the first on a large series to confirm a relatively high (82.9%) fluorescence-positive rate in
intraoperative PDD for PCNSL. 5-ALA is metabolized intracellularly by tumor cells to form the fluorescent molecules of PpIX after passing through the intact blood-brain barrier [13]. Although the mechanism of fluorescence in lymphoma tissue is not fully understood, it has been reported that a proton-coupled folate transporter (the membrane transporter of the folate analog methotrexate in lymphoma cells) also acts as a transporter of 5-ALA [14, 15]. Some reports have indicated the correlation between the Mib-1-labeling index in glioma tissue and PDD fluorescence [16, 17]. However, more than 90% of the tumors showed a high (≥80%) Mib-1 labeling index in this series, and no relation was found between the labeling index and positive fluorescence in PDD.

In biopsies of intraparenchymal tumors with a preoperative neuroradiologic diagnosis of suspected PCNSL, the number of samples obtainable per operation is limited to avoid postoperative complications. Therefore, it is useful that the positive fluorescence in PDD suggests a high probability of lymphoma tissue in the samples collected. Given that IOD is not routinely recommended, the application of 5-ALA PDD in brain tumor biopsies may be used to select cases that require IOD; ie, those in which only vague or no fluorescence is observed [10]. However, we observed fluorescence in some specimens that did not contain or contained a very limited
number of CD20-positive lymphoma cells (Fig. 5). It is known that the glial response, macrophages, and reactive lymphocytic infiltrates are commonly found in brain tissue adjacent to the periphery of PCNSL [18]. Importantly, in clinical and laboratory investigations, positive fluorescence was related not only to the tumor tissue but also to the inflammatory cells, reactive astrocytes, gliosis, or the interstitial bulk flow of PpIX from the fluorescence-positive tissue [19, 20]. The limitation of our report is the lack of a comparison with fluorescence-negative specimens, owing to the risk of postoperative neurologic deficits that might result from collecting fluorescence-negative tissue, especially in the tumor border or in normal tissue. According to the diagnostic reliability of positive and negative fluorescence of PDD in this series, the true-positive and false-negative rate was 34/41 (82.9%) and 7/41 (17.1%), respectively. Although random biopsy specimens from healthy brain tissue as well as from tumor tissue are required to determine true specificity and sensitivity in conjunction with PDD, we did not collect such specimens in this study. In PDD, a positive fluorescence rate is highly influenced by the diagnostic systems used, ie, photosensitizers, light source, charge-coupled device (CCD) camera, measurement devices, and filters. The fluorescence-positive rate in the non-fluorescence specimens in this series might have increased if the specimens had been spectroscopically analyzed.

In contrast to the treatment for other brain tumors, biopsy and the subsequent pathologic confirmation is undertaken as the standard of care for PCNSL and includes systemic chemotherapy with or without whole-brain radiotherapy or intrathecal chemotherapy. Recently, a potential survival advantage after debulking surgery rather than biopsy in patients with PCNSL has been reported [21]. In the German PCNSL Study Group-1 trial, a large randomized phase-III study comprising 526 patients with PCNSL, the progression-free survival and overall survival were
significantly shorter in the biopsied patients than in the patients with subtotal or gross total resections [21]. Our results show the potential utility of PDD in the biopsy procedure or surgical removal of PCNSL. Thus, attention should be paid to the probability of positive fluorescence being unrelated to the tumor tissue in intraoperative PDD for PCNSL. There have been reports of photodynamic therapy combined with PDD using second-generation photosensitizers such as mTHPC and talaporfin sodium, those have superior quantum efficiency, phototoxicity, and depth of light penetration [22-24].

CONCLUSION

Positive fluorescence was observed in 34 of 41 biopsies (82.9%) obtained under photodynamic diagnosis for PCNSL. PDD offers a high probability of positive tumor tissue fluorescence in patients with PCNSL. PDD of biopsy samples in patients with suspected PCNSL is a feasible way to obtain accurate samples and may lead to improved diagnostic yield in the biopsy of PCNSL.

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Informed patient consent

The patients provided consent to the use of their medical records and specimens and to the publication of the study.
results.

Conflicts of interest

None.
References


249  photodynamic therapy with talaporfin sodium and semiconductor laser in patients with malignant brain tumors. J

251

252

253 Figure Legends

254 Figure 1 Magnetic resonance images showing the region of the biopsy.

255 The Gd-enhanced T1-weighted image showed a diffusely enhanced tumor mass in the subcortical white matter (left),

256 which appeared as the superficial portion of the high-intensity region on the FLAIR image (middle). Surgical specimens
257 were taken from the FLAIR high-intensity region without Gd-enhancement, the Gd-enhanced region, and the cerebral
258 cortex region without abnormal intensity on the MRI image (right).

259

260 Figure 2 Representative photographs of surgical specimens from the same patient as those shown in Figure 1.

261 Upper: Surgical specimens obtained from a 71-year-old man with left parietal PCNSL. The 2 specimens shown on the
262 right were obtained from the brain cortex, and the others, from the tumor and border regions. Lower: Positive
263 fluorescence was observed in all but 1 specimen, which was covered by a clot.

264

265 Figure 3 Profile of the 41 biopsies obtained under photodynamic diagnosis.
PDD photodynamic diagnosis, IOD intraoperative pathological diagnosis, NE not evaluated.

Figure 4 Positive fluorescence rates in 41 cases of primary central nervous system lymphoma (PCNSL). Positive fluorescence was observed in 34 of the 41 biopsies (82.9%). Strong fluorescence was found in 23 biopsies (56.1%), and weak fluorescence, in 11 biopsies (26.8%).

FL fluorescence

Figure 5 Representative microphotographs (x200).

Upper: Specimens with hematoxylin and eosin (H&E) staining (left) and immunohistochemical staining using CD20 antibody (right), showing CD20-negative lymphocytes with no nuclear atypia infiltrating the cerebral cortex. Note that this specimen with almost no B-cell lymphoma cells had positive fluorescence. Middle: Specimens from tumor tissue. H&E stain (left) and Ki-67 stain (right), showing the typical angiocentric infiltration pattern of PCNSL cells with a Mib-1 labeling index of 80%. Lower: Tumor cells expressed the pan-B-cell markers CD20 (left) and CD79a (right).

Figure 6 Representative photograph of fluorescence of the specimen from tumor tissue.
biopsies with PDD
N=41 (40 patients)

- PDD
  - positive fluorescence
    - 34 (82.9%)
  - negative fluorescence
    - 7 (17.1%)

- IOD
  - suspected PCNSL
    - 21 (61.8%)
  - others 13 (38.2%)
    - atypical cell 4
    - high grade glioma 1
    - gliosis 1
    - unremarkable 2
    - NE 5
  - suspected PCNSL
    - 3 (42.9%)
  - others 4 (57.1%)
    - atypical cell 1
    - high grade glioma 1
    - necrosis 1
    - NE 1

Final pathological diagnosis: PCNSL