



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

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

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13 **ABSTRACT**

14 We evaluated the feasibility of 5-aminolevulinic acid (5-ALA)-mediated photodynamic diagnosis (PDD) in the biopsy
15 for primary central nervous system lymphoma (PCNSL). 5-ALA (20 mg/kg) was administered orally 4 hours
16 preoperatively. Forty-one biopsies obtained under PDD in 47 consecutive biopsies (46 patients) that were finally
17 pathologically diagnosed as PCNSL were evaluated. Positive fluorescence was observed in 34 of those 41 biopsies
18 (82.9%). An intraoperative pathological diagnosis (IOD) of suspected PCNSL was made in 21 of the biopsies with
19 positive fluorescence (61.8%). However, the 8 IODs in the remaining 13 biopsies (23.5%) were not correct (atypical cell,
20 4; high-grade glioma, 1; gliosis, 1; unremarkable, 2). In those 8 biopsies, PCNSL was confirmed by the final pathological
21 diagnosis. There was no difference in the mean Mib-1 labeling index between the biopsies with positive fluorescence
22 (86.5%) and those without (90.0%). IOD was not performed in 6 biopsies; however, 5 of those biopsies (83.3%) showed
23 positive fluorescence and were finally pathologically diagnosed as PCNSL. Use of PDD in biopsies for patients with
24 suspected PCNSL is a reliable way of obtaining specimens of adequate quality for the final pathological diagnosis and
25 may lead to improved diagnostic yield in the biopsy of PCNSL.

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28

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

31

32 **Introduction**

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

42

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

49

50 Biopsy and subsequent pathologic confirmation are undertaken as the standard of care for PCNSL such as systemic

51 chemotherapy and radiotherapy. However, only a few PCNSL cases have previously been reported (a portion of a patient
52 cohort who received 5-ALA-mediated PDD), and the role and impact of PDD for PCNSL have not been established.
53 Minimizing the extent of surgery is essential for reducing the risks during brain tumor biopsies, while samples that are
54 adequate in number and quality are required to ensure the final pathological diagnosis. To evaluate the feasibility of PDD
55 during the biopsy strategy for PCNSL, we studied the rate of positive fluorescence and the relation between PDD and the
56 pathologic diagnosis of PCNSL.

57

58 **Patients and methods**

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

67

68 >Figures 1 and 2<

69

70

71 5-ALA (20 mg/kg; Cosmo Bio, Tokyo, Japan) was administered orally 4 hours preoperatively. After induction of
72 general anesthesia, the patient's head was fixed using a Mayfield or Sugita frame, and the navigation system
73 (StealthStation; Medtronic, USA.) was set up. For the endoscopic biopsies, a transparent sheath (7 or 10 mm in diameter)
74 with a removable inner tube (Neuroport; Olympus, Tokyo, Japan) was inserted through the burr hole until the front of the
75 target lesion was under the control of the navigation system; the lesion was further observed with a rigid endoscope
76 (EndoArm; Olympus). In the endoscopic biopsies, the lesion was gradually removed [7]. Open biopsy was also
77 performed under navigation-guided planning. After the skin incision and small craniotomy, a 12-Fr catheter tube was
78 inserted from the brain surface towards the target, and a 1.5- to 2-cm corticotomy, performed. In the stereotactic biopsies,
79 a Komai stereotactic frame or frameless neuronavigation was used, and the first target was set at the central or
80 near-central region of the enhanced mass, and 4 or more samples were collected around the first target and periphery of
81 the tumor for further pathologic diagnosis (Figs. 1 and 2). Samples were collected from at least 2 different biopsy targets,
82 and those with positive fluorescence were sent to the pathology department for IOD. If suspected PCNSL was diagnosed,
83 the remaining samples were kept for the final pathologic diagnosis. If IOD did not point to a diagnosis of suspected
84 PCNSL or if there were no samples with positive fluorescence, additional sample collection was decided upon by each
85 surgeon according to the potential risk in each case. The rates of positive fluorescence (strong or weak according to the
86 macroscopic observations) and the relation between PDD and the pathologic diagnosis were analyzed.

87

88 **Results**

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

96

97 >Figures 3 and 4<

98

99 Positive fluorescence was observed in 34 of the 41 biopsies conducted under PDD (82.9%; Fig. 3). Strong
100 fluorescence was observed in 23 of those biopsies (56.1%), and weak fluorescence, in 11 of them (26.8%; Fig. 4). In 21
101 of the 34 biopsies (61.8%), an IOD of suspected PCNSL was made. In the remaining 13 biopsies, however, the IOD was
102 either incorrect (atypical cell, 4; high-grade glioma, 1; gliosis, 1; unremarkable, 2) or not performed (5). Thus, for all 34
103 cases, PCNSL was diagnosed at the final pathologic examination. Intraoperative diagnosis was not conducted in 6 of the
104 41 biopsies obtained under PDD. Five of them (83.3%) showed positive fluorescence and were diagnosed as PCNSL at
105 the final pathologic diagnosis. Fluorescence was not observed in 7 of the 41 biopsies conducted under PDD (17.1%). In 3
106 of those (42.9%), an IOD of suspected PCNSL was made. In the remaining 4 biopsies (57.1%), the IOD was either
107 incorrect (atypical cell, 1; high-grade glioma, 1; necrosis, 1) or not performed (1). There was no difference in the mean

108 Mib-1 labeling index between the tumor cells with positive fluorescence (86.5%) and those without (90.0%).

109

110 **Discussion**

111 5-ALA-mediated PDD has often been performed with tumor biopsy, and such a combination is advantageous
112 in facilitating diagnostic yield by distinguishing tumor-containing samples by use of fluorescence [7]. Although several
113 pathologic types of brain tumor were reported in the literature on fluorescence-assisted biopsy, it is difficult to estimate
114 the rate of positive fluorescence in PDD of PCNSL because of the limited number of such published cases. To the best of
115 our knowledge, only 11 PCNSL cases with biopsy or resection conducted under 5-ALA-mediated PDD have been
116 reported, and all of those showed positive fluorescence [4, 8-10]. Moriuchi and colleagues reported stereotactic biopsy
117 conducted under PDD for a PCNSL of the thalamus and for a pontine glioma to confirm the target tumor tissues [9].
118 Grossman and colleagues reported positive fluorescence in PCNSL of the fourth ventricular floor [8]. Eljamel and
119 colleagues reported 2 PCNSL with positive fluorescence [11]. Widhalm and colleagues found that all 16 samples taken
120 from 7 PCNSL cases showed positive fluorescence (strong, 14; weak, 2) [10]. In the present study, positive fluorescence
121 was observed in 34 of the 41 biopsies (82.9%), which is consistent with the findings of our previous biopsy report on 59
122 intraaxial tumor cases, which showed that 9 of the 12 PCNSL cases (75%) showed positive fluorescence [7].

123

124 According to a recent review, fluorescence is observed in HGG patients with 91% sensitivity and 59%
125 specificity [12]. To date, the rate of positive fluorescence and the diagnostic yield of PDD for PCNSL have not been
126 established. This report is the first on a large series to confirm a relatively high (82.9%) fluorescence-positive rate in

127 intraoperative PDD for PCNSL. 5-ALA is metabolized intracellularly by tumor cells to form the fluorescent molecules of
128 PpIX after passing through the intact blood-brain barrier [13]. Although the mechanism of fluorescence in lymphoma
129 tissue is not fully understood, it has been reported that a proton-coupled folate transporter (the membrane transporter of
130 the folate analog methotrexate in lymphoma cells) also acts as a transporter of 5-ALA [14, 15]. Some reports have
131 indicated the correlation between the Mib-1-labeling index in glioma tissue and PDD fluorescence [16, 17]. However,
132 more than 90% of the tumors showed a high ($\geq 80\%$) Mib-1 labeling index in this series, and no relation was found
133 between the labeling index and positive fluorescence in PDD.

134

135 >Figure 5<

136 >Figure 6<

137

138

139 In biopsies of intraparenchymal tumors with a preoperative neuroradiologic diagnosis of suspected PCNSL,
140 the number of samples obtainable per operation is limited to avoid postoperative complications. Therefore, it is useful
141 that the positive fluorescence in PDD suggests a high probability of lymphoma tissue in the samples collected. Given that
142 IOD is not routinely recommended, the application of 5-ALA PDD in brain tumor biopsies may be used to select cases
143 that require IOD; ie, those in which only vague or no fluorescence is observed [10].

144

145 However, we observed fluorescence in some specimens that did not contain or contained a very limited

146 number of CD20-positive lymphoma cells (Fig. 5). It is known that the glial response, macrophages, and reactive
147 lymphocytic infiltrates are commonly found in brain tissue adjacent to the periphery of PCNSL [18]. Importantly, in
148 clinical and laboratory investigations, positive fluorescence was related not only to the tumor tissue but also to the
149 inflammatory cells, reactive astrocytes, gliosis, or the interstitial bulk flow of PpIX from the fluorescence-positive tissue
150 [19, 20]. The limitation of our report is the lack of a comparison with fluorescence-negative specimens, owing to the risk
151 of postoperative neurologic deficits that might result from collecting fluorescence-negative tissue, especially in the tumor
152 border or in normal tissue. According to the diagnostic reliability of positive and negative fluorescence of PDD in this
153 series, the true-positive and false-negative rate was 34/41 (82.9%) and 7/41 (17.1%), respectively. Although random
154 biopsy specimens from healthy brain tissue as well as from tumor tissue are required to determine true specificity and
155 sensitivity in conjunction with PDD, we did not collect such specimens in this study. In PDD, a positive fluorescence rate
156 is highly influenced by the diagnostic systems used, ie, photosensitizers, light source, charge-coupled device (CCD)
157 camera, measurement devices, and filters. The fluorescence-positive rate in the non-fluorescence specimens in this series
158 might have increased if the specimens had been spectroscopically analyzed.

159

160 In contrast to the treatment for other brain tumors, biopsy and the subsequent pathologic confirmation is
161 undertaken as the standard of care for PCNSL and includes systemic chemotherapy with or without whole-brain
162 radiotherapy or intrathecal chemotherapy. Recently, a potential survival advantage after debulking surgery rather than
163 biopsy in patients with PCNSL has been reported [21]. In the German PCNSL Study Group-1 trial, a large randomized
164 phase-III study comprising 526 patients with PCNSL, the progression-free survival and overall survival were

165 significantly shorter in the biopsied patients than in the patients with subtotal or gross total resections [21]. Our results
166 show the potential utility of PDD in the biopsy procedure or surgical removal of PCNSL. Thus, attention should be paid
167 to the probability of positive fluorescence being unrelated to the tumor tissue in intraoperative PDD for PCNSL. There
168 have been reports of photodynamic therapy combined with PDD using second-generation photosensitizers such as
169 mTHPC and talaporfin sodium, those have superior quantum efficiency, phototoxicity, and depth of light penetration [22-
170 24].

171

172 **CONCLUSION**

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

177

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181

182 Informed patient consent

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

184 results.

185

186 Conflicts of interest

187 None.

188

189

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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.

266 *PDD* photodynamic diagnosis, *IOD* intraoperative pathological diagnosis, *NE* not evaluated.

267

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

271 *FL* fluorescence

272

273 **Figure 5** Representative microphotographs (x200).

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

279

280 **Figure 6** Representative photograph of fluorescence of the specimen from tumor tissue.

Fig.1

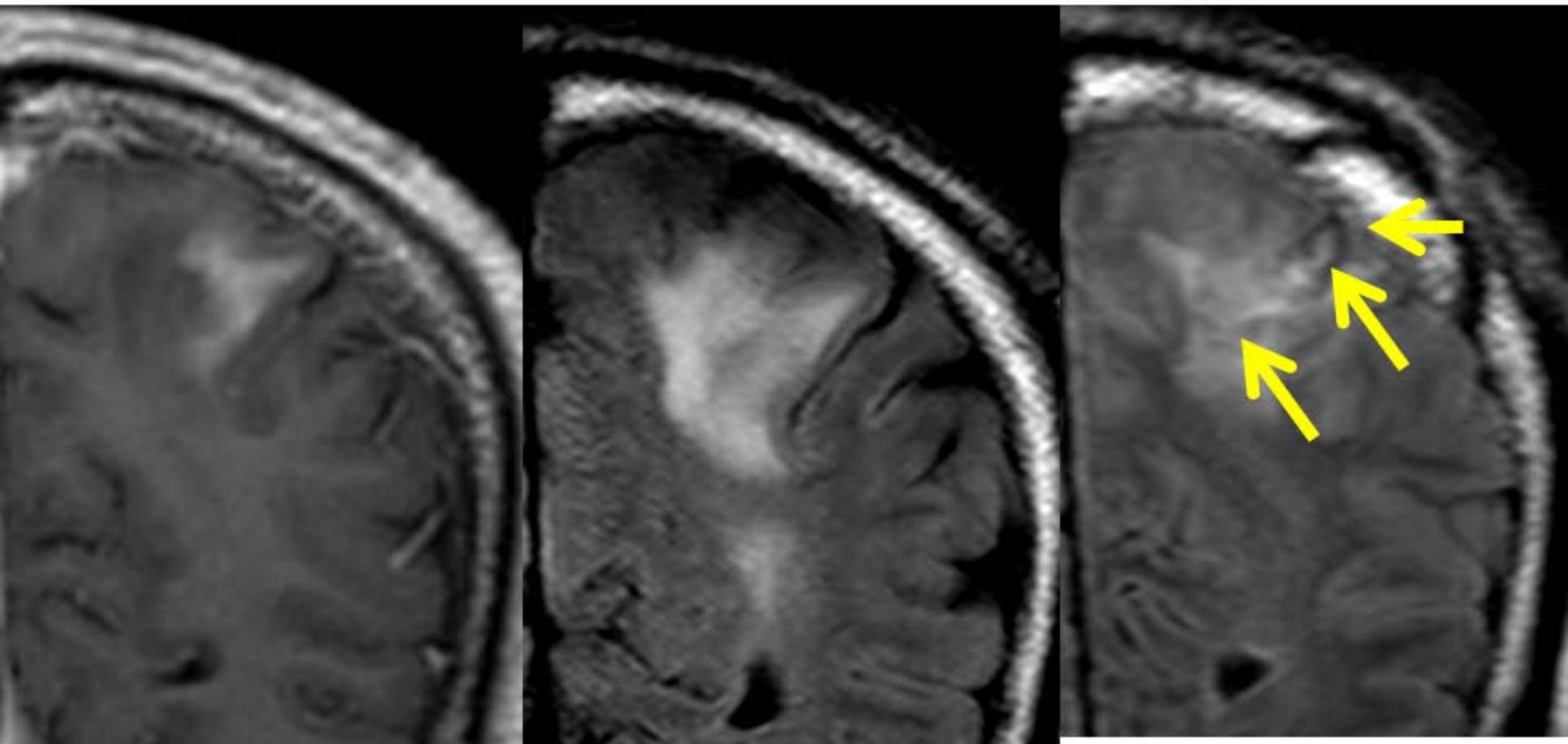
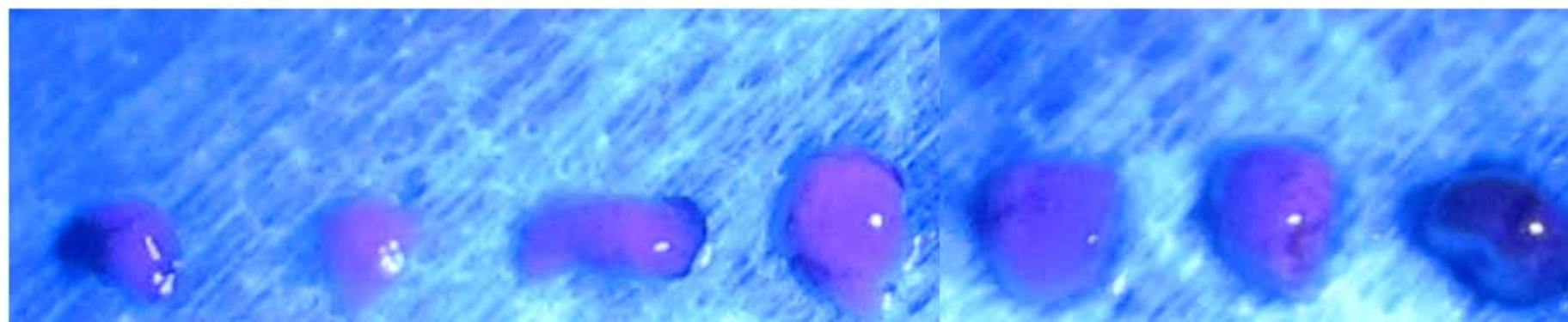


Fig.2



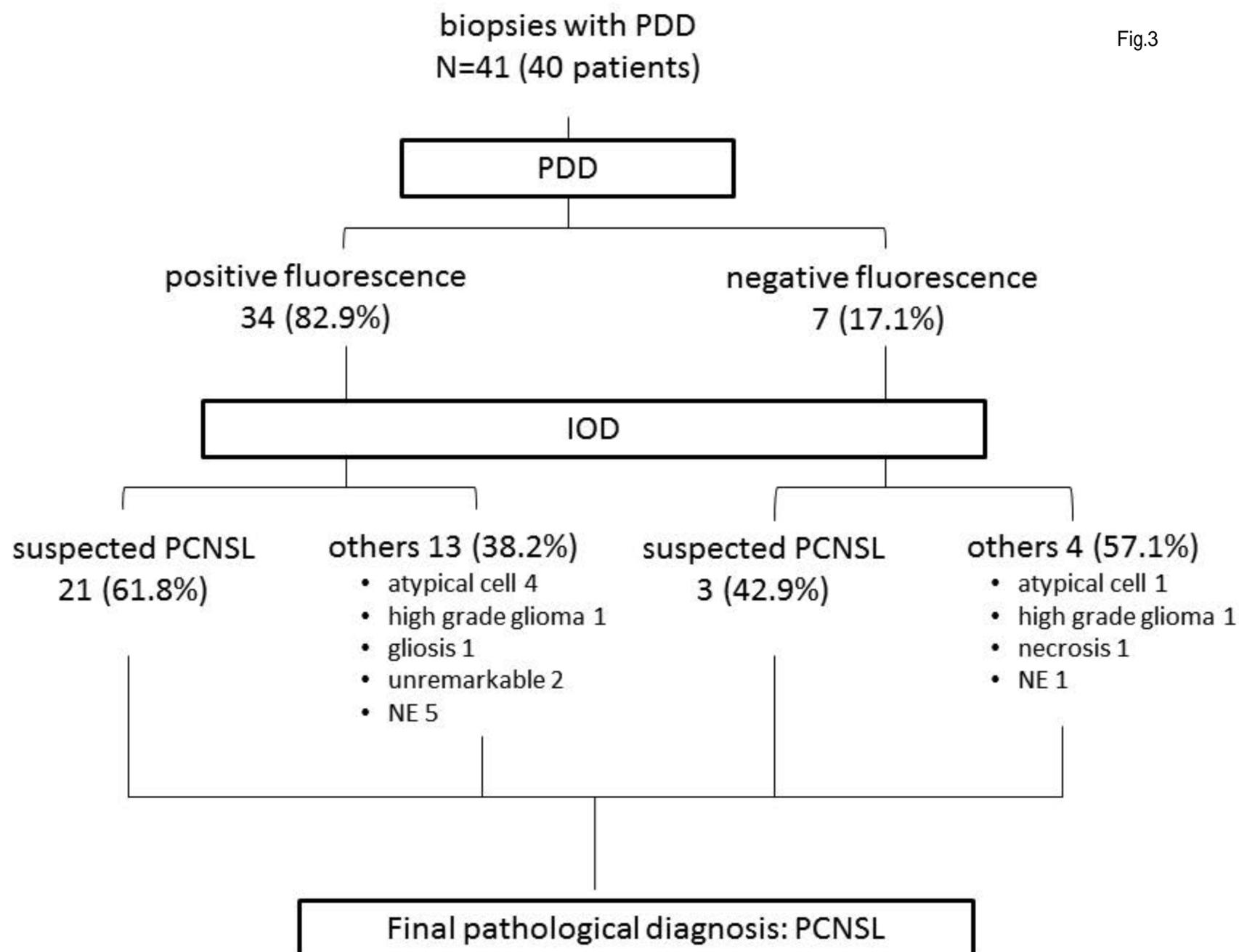
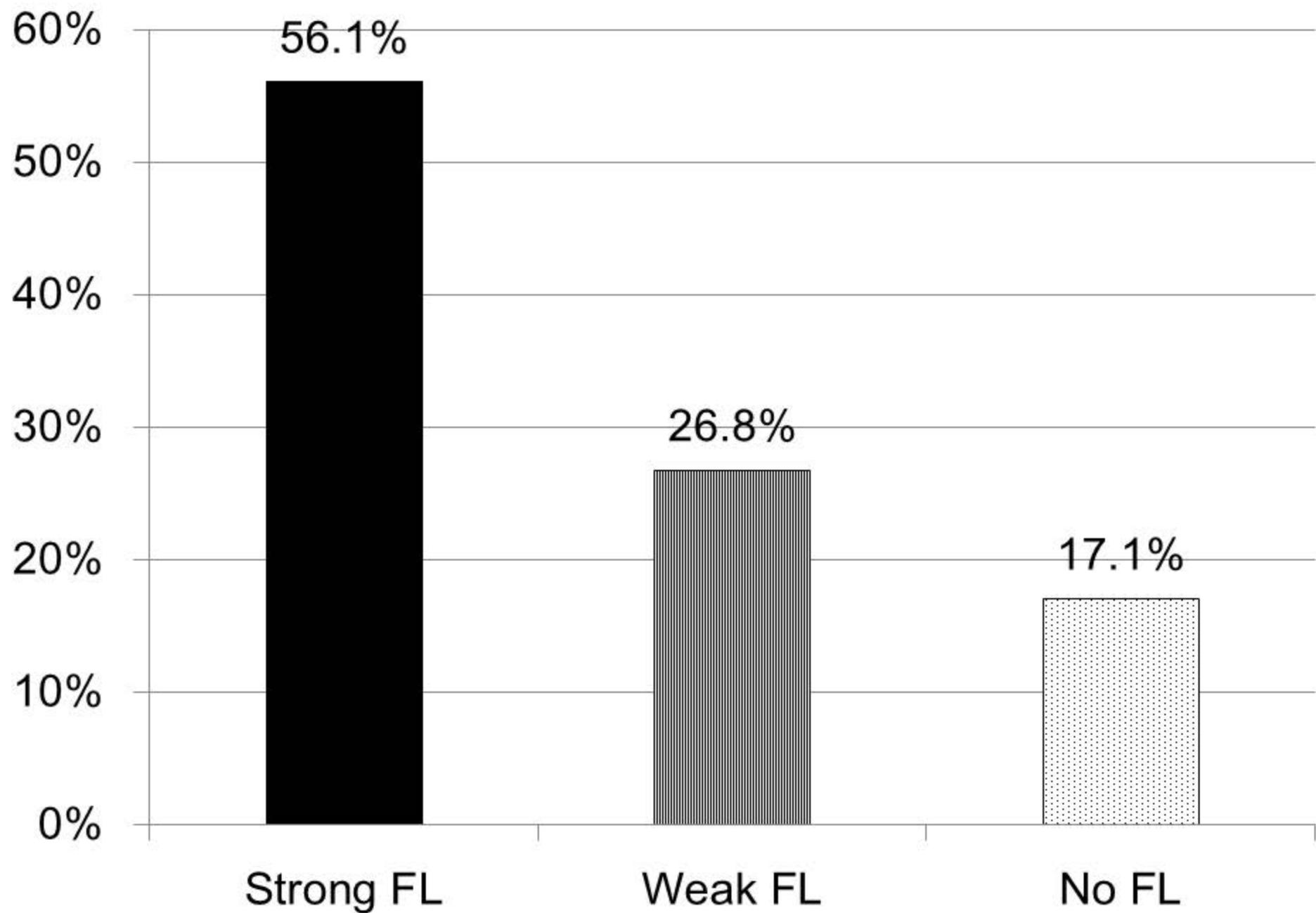


Fig.4



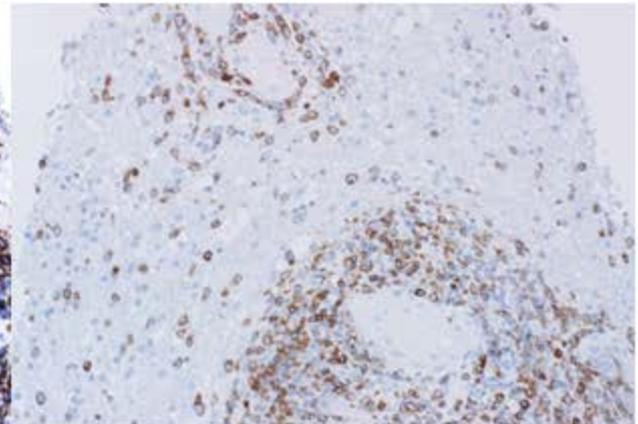
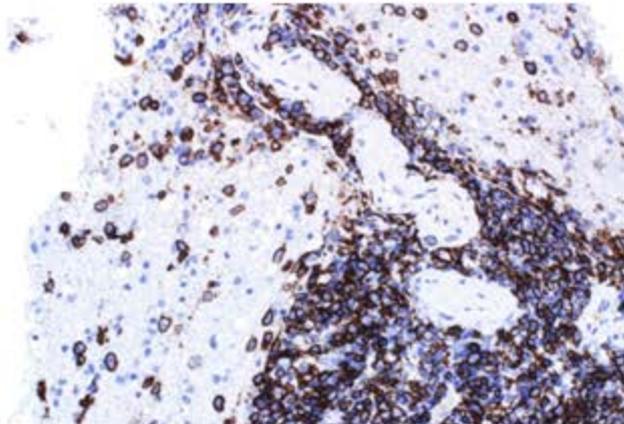
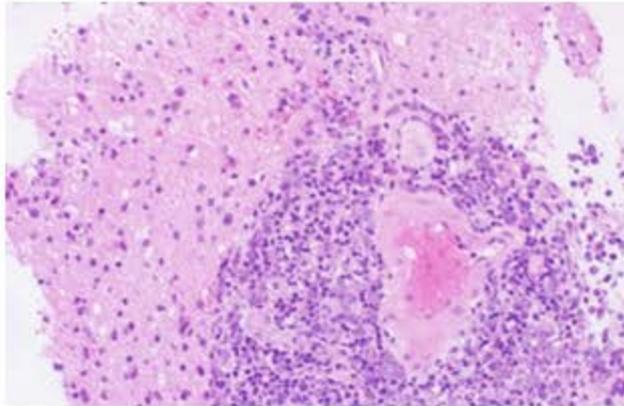
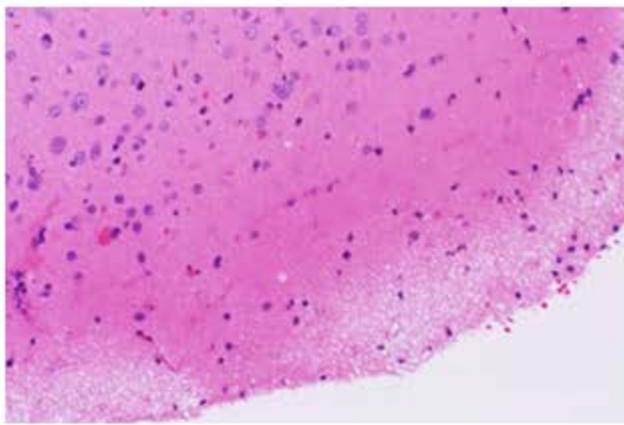


Fig.5

