Bone-invading meningiomas can arise from both the skull base and the convexity. Bone involvement, a major concern in meningioma surgery, is proved to be an independent predictor of tumor recurrence and is associated with an increased rate of disease progression and decreased survival. Histopathological studies have documented bone invasion in 20%–68% of patients affected by meningioma, mainly in the context of hyperostosis. However, bone infiltration has been described in 10%–40% of cases with no hyperostosis, thus making imaging insufficient to predict cranial invasion.

Predictive value of intraoperative 5-aminolevulinic acid–induced fluorescence for detecting bone invasion in meningioma surgery

Clinical article

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Object. Bone invasion is a major concern in meningioma surgery, since it is predictive of the recurrence of cranial involvement, morbidity, and mortality. Bone invasion has been reported in 20%–68% of studies with histopathologically confirmed data. Unfortunately, radical resection of bone invasion remains challenging. The aim of this study was to assess the role of 5-aminolevulinic acid (5-ALA) fluorescence in guiding the resection of bone-invading meningiomas. To this purpose, the sensitivity, specificity, and positive and negative predictive values of 5-ALA in detecting meningioma bone invasion were evaluated.

Methods. Data from 12 patients affected by bone-invading meningiomas (7 with skull base and 5 with convexity meningiomas) who had undergone surgery with the assistance of 5-ALA fluorescence and neuronavigation between July 2012 and March 2013 at the Department of Neurosurgery of Padua were retrospectively analyzed. To evaluate the sensitivity and specificity of 5-ALA fluorescence in detecting meningioma tissue, a pathologist analyzed 98 surgical bone samples under blue light, according to different fluorescence patterns. Magnetic resonance images and CT scans were obtained pre- and postoperatively to determine the extent of bone invasion resection.

Results. The rate of 5-ALA–induced fluorescence of both tumor and bone invasion was 100%. Based on the pathological examination of bone specimens, 5-ALA presented a sensitivity of 89.06% (95% CI 81.41%–96.71%) and a specificity of 100% in detecting meningioma bone invasion, while the positive and negative predictive values were 100% and 82.93% (95% CI 71.41%–94.45%), respectively. At the postoperative stage, MRI did not detect cases of meningioma bone invasion, whereas CT scans revealed residual hyperostosis in 2 cases.

Conclusions. In summary, 5-ALA fluorescence represents a suitable and reliable technique for identifying and removing bone infiltration by meningiomas. However, further studies are needed to prove the clinical consequences of this promising technique in a larger population.

Key Words • 5-aminolevulinic acid fluorescence • 5-ALA • oncology • intracranial meningioma • microsurgery • meningioma bone invasion • hyperostosis
thermore, while the removal of invaded bone is crucial for a good clinical outcome, it is challenging to completely achieve. Intraoperative MRI and neuronavigation have been recently advocated to maximize resection, but techniques specifically focusing on the intraoperative identification of bone invasion are lacking. 5-Aminolevulinic acid (5-ALA) fluorescence has been proved to be a useful tool in malignant glioma surgery, improving both the extent of resection and the survival of patients. Preliminary data on 5-ALA fluorescence of meningiomas have been recently reported, but the technique’s effectiveness in both detecting and removing bone infiltration remains unclear.

Methods

Aims and End Point of the Study

The purpose of this study was to assess the role of 5-ALA fluorescence in guiding the resection of bone-invasive meningiomas. To evaluate the sensitivity, specificity, accuracy, and positive and negative predictive values of the technique in detecting meningioma bone invasion, the bone samples collected during surgery were analyzed according to different fluorescence patterns. Since the Simpson resection grading system does not evaluate bony invasion, we radiologically assessed the amount of tumor resection, bone invasion, and hyperostosis by comparing preoperative and postoperative MR images and CT scans.

Patient Population

Between July 2012 and March 2013, 87 patients were surgically treated for intracranial meningiomas at the Department of Neurosurgery, Padua University Hospital, Padua, Italy. Among these patients were 12 (8 females and 4 males, median age 59 years [range 30–80 years]) who had presented with radiological evidence of bone invasion and underwent surgery with the assistance of 5-ALA fluorescence. Seven harbored skull base meningiomas and 5 had convexity meningiomas. One patient had repeat surgery for recurrent disease. Patient data, including surgical records, discharge letters, histological records, and imaging studies (CT and MRI), were collected. All patients signed a specific informed consent because of the off-label use of 5-ALA fluorescence in meningioma surgery.

Imaging Studies

All patients underwent MRI and CT head scanning before surgery. Computed tomography scans were acquired with both parenchymal and bone sequences. In 8 patients, CT scanning with 3D reconstruction was performed. Computed tomography scanning and MRI with gadolinium were performed in all cases within 3 months after surgery. Also postoperatively, an expert neuroradiologist not informed of the intraoperative data evaluated the meningioma, bone invasion, and extent of hyperostosis.

Surgical Technique and Bone Sample Collection

While the patient was under general anesthesia, surgery was performed with the aid of an operating microscope and microsurgical instrumentation. Two to 4 hours before surgery, patients orally received 20 mg/kg of 5-ALA, as previously described. All operations were performed using a Zeiss Pentero surgical microscope equipped with a fluorescent 400-nm ultraviolet light and filters. All patients underwent surgery in an MRI neuronavigation setting. Different approaches and techniques were adopted for skull base and convexity meningiomas. For skull base meningiomas (Fig. 1), after a standard frontopterional craniotomy, pathological bone in the cranial base was resected using a high-speed drill, while repeatedly shifting from white to blue light. After removing infiltrated bone, the dura mater was opened and meningioma was removed, again while shifting from white to blue light. Finally, the bone flap was placed on the surgical table (Fig. 2), examined under blue light, and fluorescent bone was drilled out. For convexity meningiomas (Fig. 3), location of the craniotomy site was guided by neuronavigation, the meningioma was removed along with its dural attachment, the bone flap was examined under blue light, and fluorescent bone was drilled away (Fig. 4). Several photographs obtained under white and blue light, showing cranial base meningioma invasion (C and D), intradural meningioma exposition (E and F), and final bone operculum inspection (G and H).
bone samples were collected during surgery, checked under blue and white light, and sent to the pathologist (Fig. 5). The bone specimens were divided in 2 groups: fluorescent and nonfluorescent. The nonfluorescent samples were recorded as coming from hyperostotic or nonhyperostotic bone.

Pathological Analysis

The fresh surgical specimens were cut into smaller fragments and fixed in formalin 5% for 12–24 hours to maintain the cellular integrity. After fixation in formalin, the bone fragments were placed in a decalcifying agent (Kristensen solution: sodium formate, formic acid, and distilled water) for several days, reduced to a size that could be easily processed, embedded in paraffin, and sectioned (Fig. 5).

Analysis of Data

True positive (TP), true negative (TN), false-positive (FP), and false-negative (FN) results were established according to an analysis of the pathological specimens. Sensitivity (SE) was defined as (TP)/(TP+FN), specificity (SP) as (TN)/(TN+FP), positive predictive value (PPV) as (TP)/(TP+FP), negative predictive value (NPV) as (TN)/(TN+FN), and accuracy (AC) as (TN+TP) over all patients. The 95% CIs were calculated as ± 1.96 \sqrt{\frac{SE*(1-SE)}{n}}, ± 1.96 \sqrt{\frac{SP*(1-SP)}{n}}, ± 1.96 \sqrt{\frac{PPV*(1-PPV)}{n}}, and ± 1.96 \sqrt{\frac{NPV*(1-NPV)}{n}}, where n is, respectively, (TP+FN), (FP+TN), (TP+FP), and (FN+TN).

Results

Pathological and Fluorescence Data

The pathological data showed (Table 1) evidence of bone invasion in all 12 patients. Ninety-eight bone samples were finally collected. Fifty-seven samples were fluorescent and 41 were not. Of the nonfluorescent samples, 23 were obtained from hyperostotic and 18 from non-
hyperostotic bone. Of the fluorescent samples, 36 were obtained from hyperostotic and 21 from nonhyperostotic bone. Bone invasion was confirmed in 65% (n = 64) of the samples collected, specifically, in 100% (57 of 57) of the fluorescent samples, in 30% (7 of 23) of the nonfluorescent hyperostotic samples, and in none of the nonfluorescent nonhyperostotic bone samples. In all patients, both meningioma and bone invasion were fluorescent. Meningioma was brightly fluorescent in 11 cases and weakly fluorescent in 1. Bone invasion was brightly fluorescent in 10 patients and weakly fluorescent in 2. Ten patients had WHO Grade I meningiomas, and 2 patients had WHO Grade II meningiomas. According to pathological examination of the 98 collected samples, 5-ALA presented a sensitivity of 89.1% (95% CI 81.41%–96.71%) and specificity of 100% in detecting meningioma bone invasion. Positive and negative predictive values of the technique were 100% and 82.9% (95% CI 71.41%–94.45%). Overall accuracy of the method was 92.9%.

### Extent of Resection and Radiological Findings

Macroscopic tumor removal was achieved in all patients (Simpson Grade I or II). In 8 patients the infiltrated dura was removed, and in 4 it was partially removed and partially coagulated. None of the patients required cranial reconstruction. In all 12 cases pathological examination confirmed the bone invasion that had been suggested by the radiological findings and intraoperatively detected under blue light. In 10 patients hyperostosis was radiologically reported and surgically confirmed. In all patients bone invasion detected intraoperatively was drilled away. Postoperative radiological evaluation (MRI and CT) confirmed complete removal of both intracranial meningioma and bone invasion in all cases. Two patients had a small residual non–contrast-enhancing hyperostosis.

### Discussion

5-Aminolevulinic acid fluorescence can detect bone invasion caused by meningiomas with a specificity of 100%, a sensitivity of 89%, a PPV of 100%, an NPV of 82.9%, and an accuracy of 92.9%. These results mean that fluorescent bone under blue light is certainly infiltrated by meningioma; on the other hand, nonfluorescent bone can contain areas of meningioma invasion in approximately 13% (7 of 41) of cases. In light of our data, we assert that the difficulty in identifying meningioma cells within the hyperostotic bone could be the reason for the lower sensitivity of 5-ALA fluorescence. Indeed, all cases of nonfluorescent bone samples with evidence of tumor infiltration on pathological analysis (false-positive, Type II error) were collected from hyperostotic bone. In addition, we can postulate that 5-ALA fluorescence allows complete resection of meningioma infiltration of nonhyperostotic bone. Among the nonhyperostotic bone, 100% of the fluorescent samples (21 of 21) and none (0 of 18) of the nonfluorescent samples presented tumor infiltration. At the postoperative

### TABLE 1: Clinical, pathological, and surgical data in 12 patients with bone invasion in meningioma surgery*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age (yrs)</th>
<th>WHO Tumor Grade</th>
<th>Site of Meningioma</th>
<th>Radiological Findings of Hyperostosis†</th>
<th>Pathological Data From Bone Samples</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fl+/T+</td>
<td>Fl−/T−</td>
</tr>
<tr>
<td>1</td>
<td>M/79</td>
<td>I</td>
<td>middle fossa</td>
<td>yes</td>
<td></td>
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<tr>
<td>2</td>
<td>F/38</td>
<td>I</td>
<td>sphenoorbital</td>
<td>yes</td>
<td>4</td>
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<tr>
<td>3</td>
<td>F/80</td>
<td>I</td>
<td>frontal conv</td>
<td>yes</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>M/79</td>
<td>II</td>
<td>sphenoorbital</td>
<td>no</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>F/57</td>
<td>I</td>
<td>parietal conv</td>
<td>yes</td>
<td>4</td>
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<tr>
<td>6</td>
<td>M/30</td>
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<td>parietal conv</td>
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<tr>
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<td>sphenoorbital</td>
<td>yes</td>
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<tr>
<td>8</td>
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<td>I</td>
<td>middle fossa</td>
<td>yes</td>
<td>5</td>
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<tr>
<td>9</td>
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<td>6</td>
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<tr>
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<td>F/54</td>
<td>II</td>
<td>frontal conv</td>
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<tr>
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<td>I</td>
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<tr>
<td>12</td>
<td>F/67</td>
<td>I</td>
<td>sphenoorbital</td>
<td>yes</td>
<td>6</td>
</tr>
</tbody>
</table>

* conv = convexity; Fl+ = fluorescent; Fl− = nonfluorescent; T+ = tumoral bone invasion; T− = no tumoral bone invasion.
† All patients had radiological evidence of bone invasion, and all patients had intraoperative fluorescence of tumor and bone.
stage, MRI did not reveal cases of meningioma bone invasion. Indeed, residual nonfluorescent hyperostosis was reported in 2 cases, but no evidence of bone enhancement was reported. However, we must emphasize that we do not know whether residual tumor was present in the 2 cases in which hyperostotic bone remained. Long-term follow-up will probably allow assessment of the recurrence rate in the potentially involved bone. Only additional studies with a different design can really assess the clinical implications of the present approach. Studies on 5-ALA fluorescence in meningioma surgery have recently appeared in the literature;[3][13] Coluccia et al.[4] performed a 33-case study with an intraoperative 5-ALA–induced tumor fluorescence rate of 94% and the absence of a correlation between fluorescence intensity and histological grading. Our study confirms both the very high rate of fluorescence of meningiomas and the lack of a correlation with the grade of malignancy. However, a previous report by our group documented a case of nonfluorescent hyperostotic meningioma in which neither the tumor nor the bone infiltration was fluorescent.[9] In the literature, the issue of 5-ALA fluorescence in bone-invading meningioma surgery has not been extensively addressed as yet. So far, 4 cases have been described: 3 single-case reports and 1 case from a series of 33 patients. In 3 of the 4 cases the meningioma was WHO Grade II. In all cases the tumor was fluorescent and surgery was radical.

Strengths and Weaknesses of the Study

Strengths of this study are as follows: homogeneity of the population, consistent surgical approach, histopathological confirmation of all surgical samples, and radiological assessment (both MRI and CT scanning) of the extent of resection. The main limits of the study are the relatively small number of patients and the “preselection” of patients since we do not have data on cases with uncertain preoperative imaging (doubtful bone invasion) that would generate a more accurate estimate of the real sensitivity and specificity of 5-ALA.

Significance of Results and Indications for Further Research

The design of our study does not promote changing the current resection strategy in patients affected by bone-infiltrating meningioma. However, our data do show that 5-ALA fluorescence can be effective in detecting contrast-enhanced infiltration of bone and consequently facilitating its removal. By providing for the “selective” removal of infiltrated bone, this technique may potentially reduce the number of large bone defects (due to excessive removal of infiltrated bone) and, consequently, cranial reconstructions. This last issue must certainly be proved by further study. The clinical impact of 5-ALA–guided resection of bone-invading meningiomas is yet to be defined. Additional studies are needed to determine if 5-ALA fluorescence can improve tumor resection rate and patient survival.

Conclusions

5-Aminolevulinic acid fluorescence has already been proved to be helpful in meningioma resection. We report on the reliability and utility of the technique in detecting and guiding the removal of bone infiltrated by tumor. Further studies are needed to assess the clinical consequences of this promising strategy.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Della Puppa, Lombardi, Rolma, Munari, Cecchin, Sciienza. Acquisition of data: Della Puppa, Rustemi, Troncon, Rolma, Sergi, Gardiman. Analysis and interpretation of data: Della Puppa, Gioffrè, Rolma, Cecchin, Gardiman. Drafting the article: Della Puppa, Rustemi, Troncon. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Della Puppa. Statistical analysis: Cecchin.

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