Prediction of Pathological and Radiological Nature of Glioma by Mass Spectrometry Combined With Machine Learning

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BACKGROUND: We have previously developed a medical diagnostic pipeline that employs mass spectrometry and machine learning. It does not annotate molecular markers that are specific to cancer but uses entire mass spectra for predicting the properties of glioma.

OBJECTIVE: To validate the power of our diagnostic method in predicting the pathological and radiological properties of glioma with a simple sample preparation procedure.

METHODS: A total of 10 patients with glioma and 4 nonglioma patients who went through surgical resection were enrolled in our hospital. A total of 1020 mass spectra were acquired from 88 specimens. In order to examine the prediction power of the diagnostic pipeline that we have developed, we performed 10-fold cross-validation for pathological and radiological findings and calculated agreement rates with the conventional methods such as pathological diagnosis (World Health Organization [WHO] grading, MIB-1 labeling index [LI], mutations in the isocitrate dehydrogenase [IDH]-1 gene, and positive 5-aminolevulinic acid [5-ALA] fluorescence) and radiological information (gadolinium [Gd]-enhanced area and high-intensity area on fluid-attenuated inversion recovery [FLAIR] imaging).

RESULTS: Prediction accuracy for WHO malignant grade was 91.37%. Those for MIB-1 LI ≥ 10% and IDH-1 mutation-positive were 82.84% and 87.75%, respectively. Our method achieved an accurate prediction of 95.00% for the 5-ALA-positive lesion. The present method displayed an accuracy of 82.36% in predicting the area of FLAIR hyperintensity and 81.27% for the Gd-enhanced area.

CONCLUSION: Our methodology achieved a higher rate of prediction of glioma in terms of pathology and radiology. Research is ongoing to develop a validation cohort to verify the biological profiles of glioma specimens.

KEY WORDS: Glioma, Machine learning, Mass spectrometry

Due to the infiltrative nature of glioma, the determination of margins between the tumor and surrounding normal brain tissue is often difficult under the conventional microscopic strategy. Since residual tumor directly affects the prognosis, maximal resection is ideal for maximizing survival after the intervention.1-4 To facilitate this goal, electrophysiological monitoring, navigation systems, photodynamic diagnosis using 5-aminolevulinic acid (5-ALA), and intraoperative pathological examination of frozen sections have been used routinely in clinical settings. However, even with the aid of these modalities, optimal resection is often difficult, since each technique has its own specific drawbacks.

In recent years, mass spectrometry (MS) has attracted wide attention from biologists due to the emergence of ambient ionization methods, especially in the biochemical field.5-13 The present method, probe electrospray ionization (PESI)-MS,
employs ambient ionization, using a solid needle to simultaneously act as a probe for picking up samples and as an emitter for ionizing the molecules in a tandem sequence.\textsuperscript{14-16} PESI-MS is advantageous in terms of profiling metabolites in a real-time manner. Laborious sequences of multiple sample pretreatments are not required. By taking advantage of ambient ionization, intraoperative usage becomes feasible.

From another perspective, most studies have focused on the specific molecular changes taking place in tumor cells during malignant transformation and subsequent progression. Indeed, previous MS studies in glioma focused only on specific targets such as N-acetylasparate (NAA) and 2-hydorxyglutarate (2-HG) to detect glioma malignancy and dehydrogenase mutation status, which have been already indicated as a biological marker by magnetic resonance spectroscopy (MRS) imaging.\textsuperscript{5,9} To identify the complicated glioma nature, we introduced a machine-learning system to utilize all the available spectra in discriminating malignancy from multiple perspectives.

This study attempts to predict the results of the conventional diagnostic tools, including histopathology and molecular information (World Health Organization [WHO] grade, MIB-1 labeling index [LI], a mutation in the isocitrate dehydrogenase [IDH]-1 gene, and 5-ALA fluorescence) by combining MS data and a machine-learning system. We also tried to identify the precise spatial distribution of tumors based on simultaneous magnetic resonance imaging (MRI).

**METHODS**

**Patient Population**

We conducted this retrospective analysis of tumor samples in 10 consecutive patients who received a preoperative diagnosis of glioma and underwent tumor resection between 2015 and 2016. Histologically, 3 tumors were diffuse astrocytoma IDH-mutant, 2 tumors were anaplastic astrocytoma, 1 tumor was glioblastoma IDH-mutant, and 4 tumors were glioblastoma IDH-wildtype; based upon WHO 2016 criteria. Four nonmalignant control samples were registered as lesions that had been resected for treatment from patients with other nonglioma diseases. Clinically, 1 patient had subarachnoid hemorrhage, 1 patient had a metastatic brain tumor, and 2 patients had Chiari malformation. All procedures performed in this study were in accordance with the ethical standards of the Declaration of Helsinki (1964) and its later amendments or comparable ethical standards. Informed consent was obtained from all subjects. This study was approved by the ethics committee at our university.

**Surgical Procedures**

Glioma surgery was performed to achieve maximal resection while preserving neurological function. Approximately 3 h before starting surgery, a single oral bolus of 5-ALA at a dose of 20 mg/kg body weight was administered to the patient. Conversion of 5-ALA into protoporphyrin IV predominantly takes place in the tumor region, where fluorescence was visualized under a microscope (Pentero; Carl Zeiss, Oberkochen, Germany). In all cases, preoperative MRI (pMRI) and intraoperative MRI (iMRI) were performed in an iMRI suite (IMRIS VISIUS Surgical Theatre; IMRIS, Winnipeg, Canada) with a ceiling-mounted 3.0-Tesla (T) moveable magnet. Neuronavigation was used in all cases with Stealth Station S7 planning software (Medtronic, Dublin, Ireland). Preoperative fluid-attenuated inversion recovery (FLAIR) and contrast-enhanced T1-weighted imaging (T1-WI) were overlaid for navigation. The resection margin of the tumor was principally defined as signal hyperintensity on FLAIR for grade II and contrast-enhanced area on T1-WI for grades III and IV, on navigation-based pMRI and iMRI images.

**Sample Collection**

We resected tumors in accordance with the navigation. Obtained tissues were mainly processed for routine pathology, while small amounts (≥2 mm\(^3\)) of tissues were used for MS. Histological and imaging data from each obtained specimen were registered by the following criteria: for histological diagnosis, WHO grade, MIB-1 LI, and IDH-1 mutation were used; for radiological diagnosis, tumor location, intensity on FLAIR images, enhancement on contrast-enhanced T1-WI, and positive 5-ALA fluorescence were used. Control samples were tissues resected for the purposes of treatment from patients with other nonglioma diseases.

Specimens for MS were divided into 5 to 20 pieces, depending on the tissue volume obtained. Those specimens met the following criteria: no preoperative chemotherapy or irradiation; and volume of the specimen ≥ 2 mm\(^3\). All specimens were immediately stored at −80°C until MS analysis.

**Pathology**

**Histological Staining**

Brain specimens were routinely processed for paraffin sectioning, involving cutting into 3-μm sections and staining with hematoxylin and eosin. Histopathological diagnosis was performed based on the 2016 WHO classification by expert neuropathologists.

**MIB-1LI**

Immunostaining with MIB-1 antibody (1:5 dilution; Leica Biosystems, Nussloch, Germany) was performed using a standard avidin-biotinylated immunoperoxidase methodology with microwave retrieval. The area of tumor showing the most staining was used to determine the LI. Only nuclear staining was interpreted as positive. MIB-1 LI was determined for each tumor by a neuropathologist.

**IDH-1 Mutation**

IDH-1 mutation was verified with immunohistochemical staining with monoclonal anti-R132H-IDH1 protein (1:4000; Dianova, Hamburg, Germany). The expression of IDH mutations was
determined semiquantitatively by assessing the proportions of positively stained tumor cells. Cases in which >10% of cells were stained were defined as positive.

Method for MS

PESI is a derivative of electrospray ionization (ESI) and uses a solid needle to act as a probe for picking up samples and as an emitter for ionizing molecules.13 PESI-MS is constructed on a precision actuator system, with the stroke of the needle probe set to <10 μm from the sample surface. This enables the reduction of the sample volume to the order of picoliters.

First, 110 μl of 50% ethanol was added to the specimen thawed in a 0.5-ml tube and homogenized with a disposable pestle (Argos Technologies, Elgin, IL). The homogenate was centrifuged at 500 g for 1 min, and 95 μl of supernatant was collected and diluted in an equal volume of 50% ethanol. A total of 85 μl of extractant was analyzed by PESI-MS, which was performed as described previously.11 All MS analyses were performed on each specimen in positive-ion mode, and groups of mass spectral data were handled separately in further sequences. The whole procedure from sample preparation to judgment by learning machine is summarized in Figure 1.

Machine Learning of Mass Spectra

Up to 14 representative mass spectra from each specimen were generated using LabSolutions version 5.82 SP1 software (Shimadzu, Kyoto, Japan), and processed for database construction using Moyashi version 0.1.0 software (https://github.com/akchan/moyashi). The obtained 1020 spectra (m/z range 10.0-1199.9, m/z bin width: 0.1) were dimensionally reduced by restricting the m/z range (10.0-1199.9) and by averaging every 10 intensities to increase the bin width to 1. Then every spectrum (m/z 10-1199) was normalized by dividing the absolute intensity at each m/z value by the median intensity of the spectrum. Classifiability of the spectra (eg, nontumor and WHO grade II vs WHO grades III-IV, MIB1 LI ≥ 10% vs MIB1 LI < 10%, and IDH-1 mutation + vs IDH mutation −) was tested using partial least squares (PLS) regression and logistic regression (LR) as described in the previous study.17 The classification accuracy of the LR was evaluated by standard 10-fold cross-validation, where spectra from a same patient may appear both in training and validation sets.

RESULTS

Patient Characteristics

All clinical and demographic data of patients are summarized in Table. The 14 patients included 6 men and 8 women, with a mean age 43.6 yr (range, 15–81 yr). Samples were obtained from 4 tumor-free brains, 3 cases of grade II glioma, 2 cases of grade III, and 5 cases of grade IV. In total, 88 specimens from these patients were analyzed by PESI-MS. A total of 1020 mass spectra were acquired by positive-ion mode. Figure 2 shows representative mass spectra for specimens.

Predictive Accuracy of our Method for Pathological Diagnosis

The predictive outcome of our method showed 91.37% for nontumor and grade II vs grades III and IV (Figure 3). While PLS analysis showed some overlap of plots between nontumor/grade II/grades III and IV, each appeared to form a concrete group in positive-ion modes (Figure 4). When it comes to the genetic mutation linked with pathology, mean MIB-1 LI was only 18.2% (range, 0%-60%), where the clinical cut-off was set to 10%. In this case, the predictive power of our method to the pathology was 82.84% (Figure 3). While immunohistochemistry with anti-IDH1R132H antibody identified only 4 positives among the 14 cases, our method achieved 87.75% in IDH1 mutation. Similar values were obtained for predicting 5-ALA-positive and -negative specimens (Figure 3).

Predictive Accuracy of our Method for the Radiological Diagnosis

Radiological findings were defined during surgery as signal hyperintensity on FLAIR for grade II and contrast-enhanced area on T1-WI for grade III or IV based on the navigation information. Our method was able to predict the high-intensity area on FLAIR from the iso-intensity region with an agreement rate of 82.36% to that of radiological diagnosis. In the case of gadolinium (Gd)-enhanced and nonenhanced areas on MRI, the prediction rate was 81.27% (Figure 5).

DISCUSSION

The present study tested the diagnostic prediction power of our new method16 for glioma, in which the entire spectrum from a certain m/z window was fed into the learning machine. Our method is based on the machine learning system to discriminate the entire spectrum pattern of glioma tissues between the interest groups. This is a new perspective completely divergent from the authentic tumor marker-based diagnosis or some prevailing applications of AI to imaging diagnosis. Here we used 1020 datasets of mass spectra derived from both glioma and nontumor brain tissue to construct a database. Furthermore, the performance of this method was confirmed by cross-validation. All sampling points were confirmed on neuronavigation based on pMRI and iMRI, and precisely linked to the pathological diagnosis and radiological information. This system achieved high prediction rates for each of the various clinical parameters, including tumor grading (>90%), MIB-1 LI ≥ 10% (>82.5%), IDH-1 mutation (87%), high-intensity area on FLAIR (82%), Gd-enhancement (81%), and 5-ALA positivity (95%). Therefore, the present findings suggest that our method can be implemented based on parameters other than the pathological diagnosis.

A previous attempt to use MS in the intraoperative diagnosis of glioma was based on desorption electrospray ionization (DESI)-MS.2 That was a unique trial in which glioma margins were determined by the mass spectra corresponding to NAA. The higher diagnostic accuracy in that study2 appears to explicitly confirm the utility of their method in glioma surgery. However, these previous MS studies in glioma focused only on a specific m/z window corresponding to the known mass spectrums on MRS.
which have already been detected as a biological marker. To identify whole spectra without any prejudice or emphasis on a specific mass spectrum, we established a mass spectral database to cope with the pathological and radiological diagnoses, which focus on changes in cellular profiles, tissue integrity, and water and metal contents.

From this view, the present study is the first to link MS data to the specific data of critical clinical parameters in neurosurgery. Notably, the excised lesions determined by neuronavigation proved extremely reliable, since navigation accuracy was secured by reregistration of neuronavigation to images taken by 3-T iMRI. This ensured that all samples taken from the tissues corresponded precisely to the region of interest to be analyzed, and each specimen was accurately linked to pathological and radiological information.

From another standpoint, using an “authentic” tumor marker, we compared our method to results for mutations in IDH-1 and achieved a higher rate of prediction. Kanamori et al. also showed a higher level of IDH-1 mutation-positive diagnosis using MS. They analyzed the individual peak of a characterized metabolite,
FIGURE 2. Representative mass spectra of each category. Each category of mass spectra patterns is indicated in positive-ion mode. In all series, major peaks around m/z 800 include those classified as phospholipids, the major components of cell membranes and organelles. Note that even Grade IV glioma shows a spectral pattern very similar to that of Grade II.
2-HG, a product of mutant IDH that inhibits the activity of dioxygenases. Genetic markers of glioma such as 1p19q co-deletion are well-recognized parameters for glioma, but are not an ideal target for MS, because they give rise to very complex and broad changes in downstream products and metabolic networks. To address this issue, we employed machine learning to exploit whole spectra for discriminating cancer in multiple dimensions.

In terms of using machine learning with MS for glioma samples, Eberlin et al. reported a similar attempt that combined DESI-MS with machine learning. This achieved 79% agreement with conventional histological diagnosis. We used PESI-MS in lieu of DESI-MS and demonstrated the applicability of the present system to the diagnosis of hepatocellular and renal cell carcinoma (RCC). In some cases such as RCC, patterns of spectra changed drastically at the boundary between nontumor
and tumor lesions, whereas some other tumor cases did not show conspicuous differences in mass spectral fingerprints, suggesting the sensitivity of the present method. These results could indicate the pathological features that glioma cells have highly infiltrative surrounding tissues with a less clear margin between tumor and nontumor area.

Since PESI uses a metal needle with a very fine tip as the source of high voltage, the suppression effect is less pronounced when compared with conventional ESI. Moreover, laborious sample pretreatment and vacuum conditions are not required. The measurement finishes within about 1 min. However, DESI offers superior surface analysis to PESI, as that method enables almost intact analysis. In the future, with the development of smaller probes, MS systems will be able to be introduced to the operating room. Since our method provides rapid and accurate prediction within 10 min, the required time for surgical operations will be shortened.

**Limitations**

A major limitation of this study is that the relatively low number of patients and heterogeneity of the samples, as samples were collected from various points in tissues gained from the same glioma patients. Considering the region of interest specified by this method is very limited and small, technical variance in sampling greatly affects the outcome. For example, if the sample straddles both the tumor and nontumor area, the judgment may be varied depending on the ratio of each region. Therefore, a different validation cohort study is required. However, we verified diagnostic concordance rates with the results acquired from the conventional pathological and radiological methods for more than 1000 spectra. Then, all positional data for each sample were confirmed under the navigation and precisely linked to clinical information such as pathological and radiological data.

Another limitation is that we did not perform deoxyribonucleic acid sequencing to detect IDH mutation status. In this study, the IDH1 R132H mutation status was verified by immunohistochemical staining. We focused on IDH1 mutation status, not IDH2, by analyzing the concordance with the whole spectrum pattern in MS results. Further analysis is needed to take all possibilities of other IDH mutations into account.

Finally, as further steps toward achieving implementation of this technique to surgery, we are currently developing equipment with a much more compact probe to facilitate use during surgical operations. This system will allow real-time intraoperative histological diagnosis and contribute to the determination of tumor resection margins.

**CONCLUSION**

Implementation of our method with MS and machine learning achieved high prediction power to the diagnoses from either pathology or radiology. The present system dares to avoid the identification of tumor markers and may contribute to decision-making during surgery for glioma and improvement in the extent of resection.

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**Disclosures**

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

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COMMENT

The authors present an interesting pilot study using mass spectrometry (MS) analysis through probe electrospray ionization (PESI) combined with a machine learning approach to predict pathological and radiological characteristics of gliomas. MS analysis and machine learning algorithms are two emerging approaches increasingly used in neurosurgery. MS seems to be effective to provide, in a very few time, a large amount of data from tissue specimens about their biochemical composition that could be analysed to anticipate histological and biomolecular findings. Nevertheless, yet it is still not clear which could be the best methodology for an effective analysis of such an amount of data in order to provide clinical useful information. Modern machine learning algorithms that are able to elaborate thousands of MS spectra could provide an answer to this question. Accordingly, the present study deserves merit because combines two of the most up-to-date approaches to answer to an important clinical question that is the rapid characterization of gliomas tissue. I do strongly believe that MS will have an important role in gliomas tissue identification and resection in the next future: new surgical devices (MS scalpels) to perform MS analysis in vivo during gliomas resection have been already developed, and will be introduced in the clinical practice for a rapid in vivo identification of gliomas tissue through the analysis of MS spectra, probably using machine learning algorithms. That will definitively help surgeons in the OR to concretely achieve the gross total resection of neoplastic tissue, preserving the surrounding healthy brain.

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