CT-assisted stereotactic brain biopsy: value of intraoperative frozen section diagnosis

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SUMMARY In 100 recent CT-guided brain biopsies, the value of intraoperative histologic examination using frozen section technique was evaluated. In 87 of these cases, the biopsy was performed stereotactically. In the remaining 13 cases, a CT-guided free hand technique was used. Of the 100 biopsies performed, adequate tissue for histopathologic diagnosis was obtained in 97, and in three the biopsy was nondiagnostic. In 61 procedures the initial biopsy specimen was adequate for diagnosis. Two specimens were required in 25 and in the remaining cases it was necessary to obtain three to four biopsy specimens before a definitive diagnosis could be made. Ultimately, the histologic diagnosis was made on frozen section examination in 93 of the cases. The lesions identified were neoplastic disease in 83 cases, vascular disease in seven, infectious disease in five, demyelinating disease in one, and radiation necrosis in one. Comparison between the frozen section diagnosis and the final diagnosis based on the permanent sections revealed that they matched in 89 cases (92%). Of the 83 cases of neoplasms the exact grade of malignancy was determined by frozen section examination in 71 (85%). Comparison between the size of tissue samples obtained and the ability to make a final diagnosis revealed that even if the specimen volume was less than 2 mm³, the biopsy was generally successful. The disadvantages of the small sample size obtained through needle biopsy are best overcome by careful targeting and assessment of sample quality by intraoperative frozen section examinations, which will give the definitive diagnosis in most of the cases without paraffin-embedded sections.

Despite the high resolution of computed tomography (CT), and magnetic resonance imaging (MRI), the specificity of these modalities remains limited.¹⁻⁶ Therefore, therapeutic decisions must be based upon an unequivocal histological diagnosis.

In 1947 Spiegel *et al* first introduced the use of stereotactic techniques in obtaining biopsy material from human subcortical structures.⁷ Today, these basic principles remain the same; however, the combination of stereotactic techniques and computed tomography has made biopsy of even minute lesions

in highly functional areas both safe and reliable.⁸⁻¹⁵

While stereotactic biopsy increases the accuracy of obtaining appropriate tissue for precise diagnosis, specimens obtained by the initial needle passage are sometimes insufficient for a definitive histologic interpretation. Microscopical examination of such specimens provides the ability to determine the necessity of additional needle passages. Thus, rapid, intraoperative histological examination of each biopsy sample by a well-trained neuropathologist will provide complete and reliable information on a minimum amount of tissue and avoid the risk of further biopsy. We review our experience on 100 brain biopsies, in which a CT-guided needle biopsy and intraoperative frozen section examination was performed for diagnosis of a variety of pathological entities. The value of intraoperative frozen section diagnosis in CT-guided brain biopsy is evaluated.

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Table 1 Location of biopsied lesions (N = 97)

<i>Supratentorial</i>	86
Cerebral hemisphere	
Left	39
Right	15
Corpus callosum	5
Basal ganglia	4
Thalamus, hypothalamus	16
Pineal region	1
Ventricle	
Lateral	2
Third	4
<i>Infratentorial</i>	11
Midbrain	3
Pons/medulla	6
Cerebellum	2
Total	97

Table 2 Final diagnosis of biopsied lesions (N = 97)

<i>Neoplastic lesion</i>	83
Astrocytoma	
Fibrillary	18
Protoplasmic	9
Gemistocytic	2
Malignant	23
Glioblastoma multiforme	7
Other gliomas*	4
Neuroblastoma	2
Malignant lymphoma	2
Other primary brain tumour†	5
Metastatic tumour	11
<i>Vascular lesion</i>	7
Haematoma	2
Infarction	5
<i>Infectious lesion</i>	5
Others‡	2
Total	97

*Ependymoma (1); choroid plexus papilloma (1);

oligodendroglioma (1); subependymoma (1).

†Hamartoma (1); germinoma (1); craniopharyngioma (1);

neuroepithelial cyst (1); pineoblastoma (1).

‡Multiple sclerosis (1); radiation necrosis (1).

Patients and methods

During the period April 1983 to June 1986, 100 CT-guided needle biopsies of brain lesions were performed on 98 patients at Emory University Hospital. In 87 cases, biopsies were performed with CT guidance (General Electric 8800 scanner), using the multiplanar stereotactic head frame (Howland Industries, Stanton, California) and software to produce simultaneous axial, coronal, and sagittal reconstructions, allowing a three-dimensional assessment of the target tissue.⁹ In the remaining 13 cases the biopsy was performed by a free hand technique. The biopsy procedure was generally carried out under local anaesthesia, except in children younger than 12 years. The patients included 53 males and 44 females, aged from 1 to 89 years (mean 37.5). The anatomical origin of the specimens is given in table 1.

Specimens were obtained with an 18-gauge guillotine needle, usually from the periphery or contrast enhancing edge (if present), and the centre of the lesion shown on CT scan. Specimens were placed on a flat piece of rubber glove and then wrapped in a towel to keep them moist. The specimens were examined immediately by the pathologists and the approximate volume of the sample was evaluated in many cases. Frequently, a small sample was taken for electron microscopy, and then the remainder of the specimen was frozen and sectioned in a cryostat (American Optical Cryostat, Buffalo, NY), which was kept at -20°C . A small mount comprising of embedding medium for frozen sections was prepared on a chuck inside the cryostat and then frozen with the aid of a commercially available freon spray. After freezing, sections were cut at 3 or 4 μm thickness. The slides were then stained with haematoxylin and eosin, and toluidine blue (0.7% solution). The frozen specimens were subsequently fixed in 10% formalin solution and were processed through a routine paraffin embedding technique. The histopathological findings of the frozen section, and paraffin-embedded sections were interpreted by the senior author (YT) in each case. Depending on the frozen section diagnosis and CT findings, one or more tissue samples were usually then taken, until frozen section diagnosis was established. In some cases it was decided to wait for paraffin-embedded sections rather than subject the patient to the additional risk of further biopsy.

The final pathological diagnosis was the result of rapid intraoperative frozen section examination, histological examination of paraffin-embedded materials, and sometimes electron microscopic or immunocytochemical examinations. This diagnosis was compared with the clinical impression and frozen section diagnosis in each case. Special attention was paid to any discrepancies and these were reviewed in conjunction with an assessment of sample volume and type of lesions.

Results

After combined CT-guided biopsy and intraoperative frozen section examinations, an adequate sample for histological confirmation of lesion type was obtained in 97 of the 100 biopsies. In the three nondiagnostic

Table 3 Correlation of clinical impression with final histopathological diagnosis (N = 97)

Clinical diagnosis	Agreement with final histopathological diagnosis?	
	Yes	No
Primary brain tumour	60	9*
Primary vs metastatic tumour	7	0
Primary tumour vs radiation necrosis	3	0
Tumour vs infectious lesion	7	0
Tumour vs infarction	4	0
Infarction	2	0
Others†	4	1‡

*Included is a case in which the clinical diagnosis of "brain tumour of glial origin" was identified histologically as "organising haematoma". This was subsequently confirmed by the patients' clinical course.

†Haematoma (1); abscess vs infarction (1); multiple sclerosis (2); encephalitis (1).

‡In this case a clinical diagnosis of "multiple sclerosis" was identified histologically as "gemistocytic astrocytoma arising in a demyelinating focus".

Fig 1 (A) MRI scan of a cystic lesion of the midbrain in a 12 year old boy. TE 30 ms, TR 540 ms. Based upon the clinical impression and radiographic findings, a working diagnosis of a brainstem glioma was made. (B) CT scan obtained at the time of stereotactic biopsy. The axial image as well as reconstructed coronal and sagittal images are seen. The simulated trajectory between entrance and target points is also depicted. (C) Biopsy specimen examined by frozen section demonstrating a neuroepithelial cyst. This tissue was lost in the paraffin-embedded materials. Hematoxylin and Eosin, $\times 200$.

biopsies, the procedure was terminated before an adequate tissue sample for confirmation of lesion type was obtained. However, these three biopsies were performed early in our series and it was thought additional biopsy would expose the patient to an unacceptable risk. Subsequently, a more aggressive approach in obtaining tissue for a definitive diagnosis has been adopted without increased morbidity. Analyses mainly on the 97 biopsies, in which a definitive histological diagnosis was obtained, are described below.

1 General aspects

The diagnoses based upon the histological findings are presented in table 2. The majority of the lesions in this series were tumours of the glioma group ($N = 63$), with the malignant astrocytoma being the most frequent ($N = 23$). An ependymoma, oligodendroglioma, subependymoma and choroid plexus papilloma were each encountered once. Metastatic tumours were less frequent and were identified in 11 cases. Two neuroblastomas, corroborated by ultrastructural examination, and two malignant lymphomas, corroborated by immunocytochemical studies were also seen. Rarely encountered were craniopharyngioma, germinoma,

neuroepithelial cyst of the midbrain, and pineoblastoma.

2 Correlation of clinical impression to final histopathological diagnosis (table 3)

A clinical impression of a primary brain tumour was confirmed histologically in 60 cases. In eight cases histological examination altered the clinical diagnosis as to the tumour type. This included four metastatic tumours, which were diagnosed clinically as malignant astrocytoma in three cases and glioblastoma multiforme in one case. Two neuroblastomas and one lymphoma were diagnosed clinically as malignant astrocytomas; and one neuroepithelial cyst was initially diagnosed as a low grade astrocytoma (fig 1). In one other case the diagnosis had to be changed to "organising haematoma" which, clinically, was suspected to be a brain tumour of glial origin. Organising haematoma as a definitive diagnosis without evidence of neoplastic growth was confirmed by serial CT scans and the subsequent clinical course of this patient. A clinical impression of "primary vs secondary tumour" was found to be a primary brain tumour in all seven cases. A clinical impression of "tumour vs radiation necrosis" was found to be tumour in two and radiation necrosis in one. Seven cases with a suspected diagnosis of "tumour vs infectious lesion" were found to be tumour in four and infection in the remaining three cases.

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3 Number of tissue samples

There were 93 cases, in which the numbers of biopsy samples were evaluable (table 4). Among 80 cases in which a stereotactic biopsy was performed, the initial specimen, which was usually obtained from the periphery of the lesion delineated on CT was sufficient for exact histological diagnosis in 51 cases (64%). A negative report on the initial sample necessitated an additional specimen in 21 cases. In six cases, three specimens were necessary, and two patients had four or more specimens taken before obtaining an appropriate biopsy sample for histological diagnosis. Among the 13 cases, in which free hand biopsy with CT guidance was performed, the initial specimens were sufficient for histological diagnosis in six cases (46%). In two cases, two specimens were necessary, three patients had three specimens, and two patients had four specimens taken before a histological diagnosis could be made.

4 Volume of sample

The volume of sample, from which a definitive histological diagnosis was obtained, was evaluable in 71 biopsies. In 48 biopsies (67%) the volume of sample was less than 2 mm³. The sample tended to be bigger in cases of metastatic tumours, malignant astrocytomas and glioblastomas than in those of low grade astrocytomas. However, there was no definite correlation between the volume of samples and type of lesion (table 5). The total sample volumes of the three cases in which the biopsy was nondiagnostic, ranged from 4 to 17 mm³.

5 Accuracy of intraoperative frozen section diagnosis

A correlation of the diagnosis obtained from frozen section and that obtained from paraffin-embedded sections was carried out in 100 cases (table 6). Among the 97 biopsies in which a definitive histologic diagnosis was obtained, the diagnosis based on the frozen sections was confirmed by the examination of the postoperative paraffin-embedded sections in 89 biopsies as to tumour type or type of lesion.

In the 83 cases of neoplasm, the exact grade of malignancy identified by frozen section examination was confirmed by paraffin section examination in 71

Table 5 Sample volume and type of lesion (N = 49)

Type of lesion	Mean sample volume (mm ³) (range)
Benign astrocytoma (N = 19)	2.3 (0.5-12)
Malignant astrocytoma/glioblastoma multiforme (N = 23)	3.7 (0.5-20)
Metastatic tumour (N = 7)	3.5 (0.5-10)

(85%) (fig 2). In seven cases, the frozen section diagnosis had to be changed in tumour type or grading after examination of the paraffin-embedded sections. This included four malignant astrocytomas, which were diagnosed as "fibrillary or protoplasmic astrocytoma" on frozen section; one glioblastoma diagnosed as a malignant astrocytoma (fig 3); one malignant astrocytoma, which was diagnosed as "malignant neuroglial tumour" on frozen section; and one "malignant tumour" (no details concerning the tumour type could be determined on frozen section), which had to be changed to metastatic carcinoma.

A diagnostic error in frozen sections concerning the presence of tumour tissue (false-negative diagnosis) occurred in three cases. This included one malignant astrocytoma, in which the specimen was estimated as 5.5 mm³ in total sample volume and showed only gliosis and oedema on frozen section; one glioblastoma multiforme, whose frozen section specimen revealed only necrotic tissue and was nondiagnostic. The total sample volume of this latter biopsy was estimated as 6.5 mm³. Lastly was a case of a gemistocytic astrocytoma arising in a demyelinating focus, in which the frozen section showed only the evidence of demyelination (fig 4). Multiple sclerosis was the clinical impression of this patient.

In two biopsies, the intraoperative frozen section diagnosis could not be confirmed in the permanent sections since the lesions identified by frozen section were lost in the paraffin-embedded materials (fig 1). In these cases, the frozen section diagnosis had been relied upon for practical purposes, and this was confirmed by the patient's clinical course.

Among the biopsies of non-neoplastic lesions, the frozen section diagnosis had to be changed from astrocytic gliosis to multiple sclerosis in one case. Frozen section of this case showed astrocytic gliosis without evidence of demyelination, but the paraffin-embedded section contained a demyelinating focus. Other non-neoplastic lesions were correctly diagnosed by frozen section.

In the three nondiagnostic biopsies, frozen and paraffin-embedded sections contained only astrocytic proliferation, suggesting the border zone of a pathological process.

Table 4 Number of biopsy specimens necessary for diagnosis

	1	2	3	4
Stereotactic biopsy (N = 80)	51	21	6	2
Free hand technique (N = 13)	6	2	3	2
Total (N = 93)	57	23	9	4

Fig 2 Biopsy specimen of a malignant astrocytoma, in which frozen and paraffin-embedded sections were in agreement. Numerous pleomorphic and multinucleate forms can be seen. (a) Frozen section. (b) Permanent section. Hematoxylin and Eosin $\times 900$.

Discussion

The indications for CT assisted stereotactic biopsy are well-established. Nevertheless, while this procedure has been shown to be safe and particularly effective for deep-seated lesions, there are several factors which influence its reliability. The first and probably the most important factor is the selection of the target for biopsy.^{8 10-18} Although biopsy from a contrast-enhancing rim will most often yield a diagnosis, several investigators have noted that viable tumour tissue may be obtained from areas of enhancement and areas of low density.^{10-12 16} In addition, a diagnosis of malignant astrocytoma based upon a biopsy of the contrast-enhancing rim may be confidently diagnosed as a glioblastoma if necrosis is found within the low density centre. In our series, the initial tissue sample was usually obtained from the periphery or contrast-enhancing rim (if present) of the lesion delineated on CT. This was checked histologically by frozen section preparation and was found to contain the lesion in 61% of cases. Thus, in 39% it was necessary to take two or more subsequent samples before the definitive histopathological diagnosis was made. The second factor which influences the reliability of CT-guided biopsy is the size of the specimen sent for histological examination. This small sample size has been considered to be not only the essential limitation in making a diagnosis but also the most common cause of misdiagnosis.¹⁹ As demonstrated in our series, however, the accuracy of diagnosis does not always increase proportionately with the size of the specimen. A sample volume of 2 mm³ proved to be sufficient for diagnostic evaluation in 67% of cases. Clearly, these factors are not mutually exclusive. The pathologist is dependent upon the neurosurgeon to obtain an optimal specimen for histological study and the neurosurgeon must rely on the expertise of the pathologist to inform him of a diagnostic biopsy. Thus, the combination of CT-assisted

stereotactic technique and precise intraoperative frozen section diagnosis has been most useful in eliminating the undesirable (that is, nondiagnostic) outcome of the biopsy procedure.

As a means of making a rapid intraoperative diagnosis, smear preparations have long been used, particularly among pathologists of the European school.^{15 19-26} Eisenhardt and Cushing²² and subsequently Badt²⁰ were among its early advocates; however, its use became widespread only after the introduction of the wet film technique by Russell *et al.*²⁶ The principal advantages of the smear technique are its technical simplicity, the ease with which several tiny fragments of tissue can be screened, and the clarity of detail. Smear preparations, however, often destroy tissue architecture, and are variable in thickness so that the degree of cellularity may be difficult to evaluate. McMenemey emphasised the difficulties and dangers of misinterpretations particularly when attempting to grade astrocytomas; as only 66% of diagnoses based on smear preparations were confirmed by paraffin-embedded sections in his series.²³ More recent reports have shown a much higher degree of accuracy which in part is related to improved stereotactic techniques. Marshall *et al.*²⁴ reported a correct diagnosis in 94% of cases and Ostertag *et al.*¹⁵ has had success with the smear technique in 95% of his cases. Unfortunately, the number and volume of biopsy samples required for diagnosis were not specified in these articles. Interestingly, in the report by Ostertag *et al.*, a diagnosis based upon paraffin-embedded sections was not possible in 31% of the cases.¹⁵

Despite the reported accuracy of the smear technique, a definitive diagnosis can be obtained intraoperatively utilising the frozen section technique.^{11 13 14 27} In the series by Bullard *et al.*, diagnosis was established on frozen section examination of the first biopsy specimen in 65% of cases.¹¹ Additional specimens were necessary in the remaining cases before a diagnosis could be made. However, in approximately 50% of cases in which the first biopsy sample was non-diagnostic on frozen section, the paraffin-embedded section examinations proved to be diagnostic. Ultimately, a diagnostic biopsy was obtained in 49 of 50 cases. In our series, among 83 neoplasms, histological diagnoses based upon frozen sections matched with the final diagnosis in tumour type in 78 cases (94%), and the exact grade of malignancy in 71 (85%). It should be noted, however, that in neurosurgical pathology, one must be cautious in grading the malignancy of a tumour based upon the histological appearance of limited samples alone. Clinical data, such as the age of the patient, the tempo and duration of symptoms, the radiographic findings and the precise anatomical location of the lesion are

Table 6 Correlation of frozen section diagnosis with paraffin embedded sections (N = 97)

Type of lesion	Positive correlation*	Negative correlation
Neoplastic (N = 83)		
Presence of tumour	78	5†
Type of tumour	76	7
Grade of malignancy	71	12
Non-neoplastic (N = 14)		
Type of lesion	13	1

*Frozen section diagnosis was confirmed by paraffin embedded sections.

†Included are two cases, in which tumour was present only in the frozen section, and three cases in which tumour was not present in the frozen section but was identified in the paraffin embedded sections.

Fig 3 Biopsy specimen in which there was disagreement between frozen and paraffin-embedded sections as to tumour grade. (a) Frozen section depicting features of a malignant astrocytoma. Hematoxylin and Eosin $\times 900$. (b) Permanent section demonstrating a zone of necrosis and surrounding pseudopallisading, making the diagnosis of a glioblastoma multiforme more appropriate. Hematoxylin and Eosin $\times 60$.

Fig 4 Biopsy specimen in which a false negative diagnosis was made on frozen section. (a) Frozen section depicting a focus of demyelination. Hematoxylin and Eosin $\times 900$. (b) Paraffin embedded section from the same tissue block which revealed a small focus of a gemistocytic astrocytoma arising within a demyelinating plaque. Luxol Fast Blue $\times 450$.

very important in considering the grade of malignancy. In this respect, the pathologist must be familiar with the clinical spectrum of neurological disease and must be aware of the fact that a knowledge of CT-histology correlations by the neurosurgeon may enhance the value of the biopsy procedure.

Although frozen section preparations have been accused of frequently obscuring the fine cellular detail,²¹ an accurate judgement of the type of lesion and often the grade of malignancy can generally be made on frozen section examination alone. It is clear, however, that the combination of CT-guided stereotactic techniques and the meticulous application of the frozen section technique is responsible for this high degree of accuracy.

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