Intra-Operative Diagnosis in Surgical Neuropathology: A Study of 120 Cases with Reference to Squash Cytology

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Objective : *To present results of intra-operative consultation in surgical neuropathology and discuss the diagnostic guideline for squash cytology.*
Material and Method : *The intra-operative pathological diagnosis of 120 neurosurgical specimens was compared with the final histologic diagnosis. Squash preparation was used solely in 83 cases, frozen sections alone in 3 cases, and both techniques in the remaining. An algorithm for cytologic diagnosis was described.*
Results : *The intra-operative pathological diagnoses in neurosurgery were completely (83%) and partially (13%) correlated with the final results.*
Conclusions : *Intra-operative diagnosis in surgical neuropathology is reliable. Squash cytology is highly recommended as an alternative approach.*

Keywords : *Squash cytology, Intra-operative pathological consultation, Surgical neuropathology, Neurosurgery*

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Neurosurgical service is enhanced by the use of intra-operative diagnosis, particularly concerning specimens suspected of neoplasms. Techniques applied for the rapid diagnosis encompass the traditional cryostat sections and smear preparation. Even though it has been shown repeatedly that the squash technique for cytology is extremely valuable [1-7], surprisingly, on a recent survey by Firlik *et al*, approximately one-fourth of the neuropathologists did not prefer the method [8]. The lack of training and experience may have made them uncertain with the accuracy of smear [8]. Furthermore, although the cytologic features of the nervous system lesions have occasionally been displayed [9-11], no systematic cytomorphological approach is available. Herein, the authors present results of intra-operative diagnosis of 120 neurosurgical specimens and discuss the cytologically-based diagnostic guideline for squash cytology.

Material and Method

One hundred and twenty specimens from different patients operated in the Division of Neurosurgery, Department of Surgery, Chulalongkorn Hospital, from August 2001 to October 2003, were examined by smear technique and/or frozen sections for rapid intra-operative diagnosis. Thirty cases of this series have previously been reported [12]. Two to four representative areas, 1-2 mm in dimension, were selected from the fresh specimen. Each was placed on a labeled glass slide, and then gently crushed by the second slide held at a right angle. The smeared slide was immediately immerged in 95% ethanol. Hematoxylyn and eosin (H&E) was the primary staining method; Toluidine blue was occasionally used for detection of mast cells. Frozen sections were performed sporadically. The remaining tissue was fixed in 10% formalin and embedded in paraffin wax for permanent sections. A diagnostic algorithm (Fig. 1) was used for cytologic diagnosis. Neoplasms were classified according to the latest classification [13]. All intra-operative and final diagnoses were made by the first author, with occasional use of immunohistochemical study. When the final diagnosis was in doubt, panel diagnosis was obtained by all collaborators. The intra-operative and final diagnoses were compared using the categories below:

Category I Completely correct intra-operative diagnosis: The intra-operative and final diagnoses

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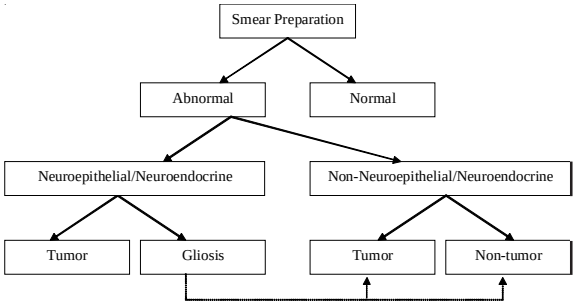

Fig. 1 Schematic approach for cytologic diagnosis of the nervous system lesions

were exactly the same.

Category II Incompletely correct intra-operative diagnosis: The intra-operative diagnosis was partially correlated with the final. For example, it provided the correct histologic type of tumor, but failed to designate degree of malignancy or to diagnose all histologic types of mixed lesions.

Category III Incorrect intra-operative diagnosis: The intra-operative diagnosis was incorrect in the histologic type of lesion, or offered only the presence or absence of abnormal tissue.

Category IV Deferred diagnosis: The intra-operative diagnosis was deferred to permanent section.

Results

Of the 120 cases, 58 and 62 patients were male and female, respectively. The age range was between 3 months to 89 years. Most of the lesions (88%) were neoplasms. In 98 cases (82%), the intra-operative diagnosis fell into category I (see material and method for definitions) (Table 1). Categories II, III, and IV were observed in 16 (13%), 3 (2.5%), and 3 (2.5%) cases, respectively. The majority of diagnoses (83/120) were made solely on squash preparation. Diagnosis of three cases (neurofibroma, cavernous hemangioma, and giant cell tumor of the spine) relied on frozen sections alone as the tissues produced unsatisfactory smears. Both techniques were applied in the remaining (34 cases). Cytologic features of selected neuroepithelial/ neuroendocrine (Fig. 2) and non-neuroepithelial/ neuroendocrine (Fig. 3) lesions were presented.

Discussion

Similar to several previous studies [1-8], the authors demonstrated once again the high accuracy of intra-operative pathological diagnosis in neuro-surgery. Although several staining methods have been applied for squash preparation [7, 9], the authors

Table 1.

Categories of Intra-operative Diagnosis	n
Category I: Completely correct diagnosis	**98**
Pilocytic astrocytoma	2
Subependymal giant cell astrocytoma	2
Low-grade diffuse astrocytoma	4
Anaplastic astrocytoma	1
Glioblastoma	6
Oligodendroglioma	2
Ependymoma	2
Central neurocytoma	1
Medulloblastoma	4
Melanotic neuroectodermal tumor of infancy	1
Meningioma	16
Neurilemmoma	13
Neurofibroma	1
Germinoma	5
Lymphoma	2
Langerhans cell histiocytosis	2
Plasmacytoma	1
Craniopharyngioma	2
Chordoma	3
Pituitary adenoma	4
Giant cell tumor of spine	1
Lipoma	1
Hemangioblastoma	1
Cavernous hemangioma	1
Metastatic adenocarcinoma	10
Infarct	1
Organizing hematoma	1
Gliosis	2
Granuloma	4
Aspergillosis	2
Category II: Incompletely correct diagnosis	**16**
Mixed oligoastrocytoma	1
Anaplastic oligodendroglioma	1
Anaplastic mixed oligoastrocytoma	1
Anaplastic ependymoma	1
Pineal parenchymal tumor, intermediate	1
Dysembryoplastic neuroepithelial tumor	1
Atypical/malignant meningioma	4
Craniopharyngioma	1
Mixed germinoma/endodermal sinus tumor	1
Squamous cell carcinoma	1
Metastatic small cell carcinoma	1
Cyst with xanthogranulomatous reaction	1
Granuloma	1
Category III: Incorrect diagnosis	**3**
Pineocytoma	1
Peripheral primitive neuroectodermal tumor	1
Rosai-Dorfman disease	1
Category IV: Deferred	**3**
Pilocytic astrocytoma	1
Hemangioblastoma	1
Gliosis	1
Total	**120**

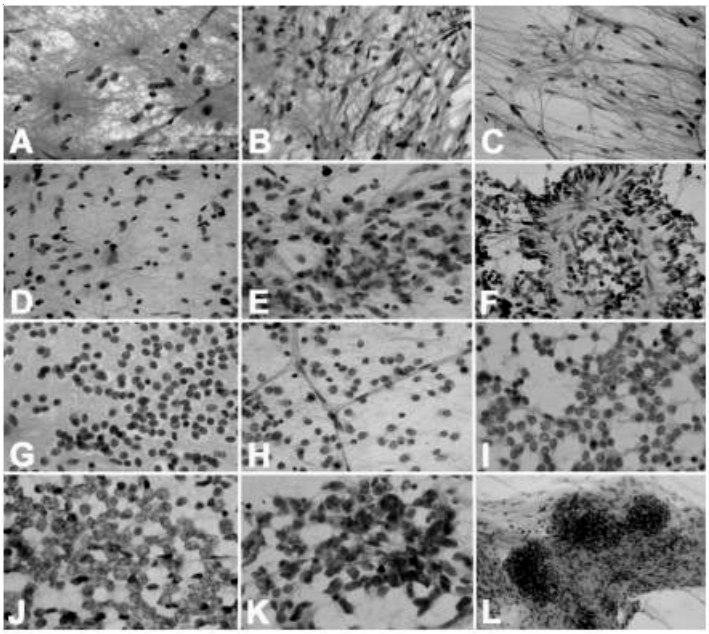

Fig. 2. Cytologic Features of Neuroepithelial/Neuroendocrine Lesions. Glial processes are distinctive on squash preparation. In reactive gliosis (A), astrocytes have abundant omni-directional glial processes. Piloid gliosis (B) is characterized by the presence numerous Rosenthal fibers, thus mimicking pilocytic astrocytoma. Careful examination of this smear revealed a few epithelial sheets of adamantinomatous craniopharyngioma (not shown). Bipolar astrocytic cells with long hair-like processes feature pilocytic astrocytoma (C). Fibrillay astrocytoma (D) contains naked atypical nuclei, embedded in fine fibrillary meshwork. Hypercellular smear of glioblastoma (E) is depicted; note several tumor cells with prominent astrocytic processes. Well-organized perivascular pseudorosettes of ependymoma (F) are demonstrated. Monotonous round cells with finely stippled chromatin pattern and indistinct nucleoli feature central neurocytoma (G), oligodendroglioma (H), and pituitary adenoma (I). Clinical correlation can generally resolve these diagnostic confusions. Although sea of plump blue round cells with coarsely-stippled chromatin and indistinct nucleoli is characteristic of medulloblastoma (J), these nuclear features resemble those of metastatic small cell carcinoma (K). Presence of cohesive cellular clusters of the latter and different clinical settings allows the distinction. Glomeruloid endothelial proliferation (L) in glioblastoma is shown. (A-L, H&E-stained squash preparation)

found that the routine H&E stain provided excellent cytologic details, particularly the cytoplasmic processes of glial lesions. An algorithm for cytologic evaluation was discussed (Fig. 2) to provide the diagnostic framework for pathologists who are not familiar with the squash preparation.

According to the authors' scheme, the smear is first determined whether it is normal or abnormal [9, 10]. The normal cortex and white matter yield good smears easily. They are low in cellularity and contain normal-appearing neurons and neuroglia. Glial processes are not distinct in normal circumstances. The uniform small round hyperchromatic granular neurons of the cerebellum must not be mistaken for embryonal tumors, especially medulloblastoma. Small pieces of ependyma, choroid plexus, and arachnoid membrane should not be confused with the neoplastic process. Smears of adenohypophysis are much less in cellularity compared to a pituitary adenoma, and the normal pituitary gland comprises polymorphous population of endocrine cells with different cytoplasmic tinctures. Arterioles and venules, which seem thickened on smears, should not be regarded as endothelial proliferation.

Once considered abnormal, the smear is then designed whether it is a neuroepithelial/neuroendocrine (NN) or a non-NN lesion. The authors group neuroendocrine and neuroepithelial tumors together as they share several similar cytologic features, particularly the stippled chromatin pattern. Common neuroendocrine tumors affecting the brain include pituitary adenoma and metastatic neuroendocrine carcinoma. Cytologic features suggesting NN lesions consist of the following: 1) Soft texture of the specimen (which typically produces good smears), 2) Rigid cellular glial processes (astrocytic, ependymal and sometimes oligodendroglial tumors), 3) Perivascular arrangement with processes approaching the vascular lumen (astrocytic and ependymal tumors), 4) Fine fibrillary background (oligo-

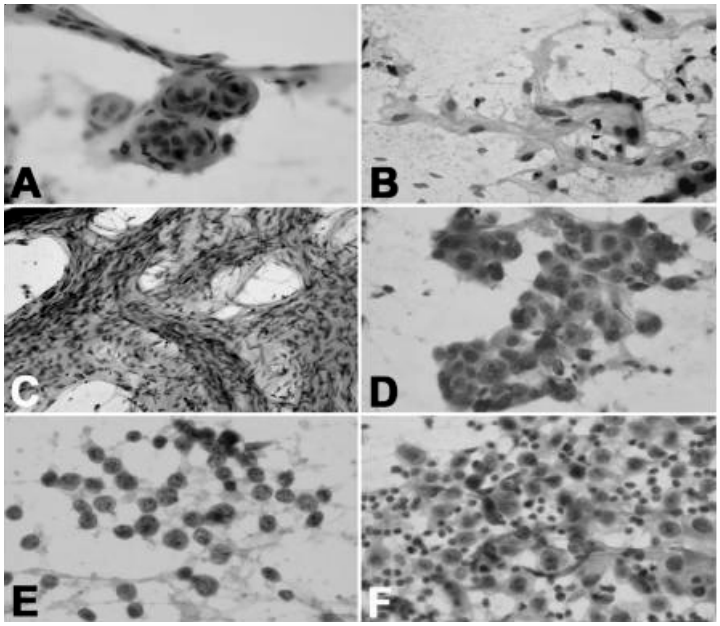

Fig.3 Cytologic Features of Non-Neuroepithelial/Neuroendocrine Lesions. Whorls (A) of meningioma are depieted. In cases where whorls are scant or absent, scattered tumor cells in the background of smear with wispy cytoplasm (B) are highly diagnostic. Schwannoma (C) does not smear well and tends to form compact fascicles with "twisted robe" appearance. In contrast to a meningioma, scant tumor cells spill out of the clusters. Metastatic adenocarcinoma (D) forms cohesive clusters; tumor nuclei are obviously malignant. Lymphoma (E) contains large discohesive cells, with visible nucleoli and scant cytoplasm. In germinoma (F), large round tumor cells with prominent nucleoli are intermixed with small lymphocytes. (A-F, H&E-stained squash preparation)

dendroglial tumors, neurocytoma and embryonal tumors), 5) Rosenthal fibers (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and piloid gliosis), 6) Round nuclei with finely stippled chromatin (neurocytoma, oligodendroglioma, pineocytoma, and pituitary adenoma), 7) Carrot-shaped nuclei with coarsely stippled chromatin (embryonal tumors, metastatic neuroendocrine carcinoma), and 8) Endothelial proliferation (gliomas, embryonal tumors, and neuroendocrine tumors).

Gliosis, a reactive process resulting from various stimuli, has to be distinguished from gliomas particularly low-grade lesions. Both could demonstrate a similar cellularity. Numerous radiating and tapering glial processes favor the reactive process, and the presence of foamy histiocytes generally argues against the diagnosis of low-grade gliomas [13]. Once gliosis is considered, care should be taken to find out the possible etiology.

Cytologic features of non-NN lesions vary considerably, and many times this broad group of diseases is considered when the above NN features are absent. Nevertheless, the presence of cohesive cellular clusters or epithelial sheets, tightly-packed spindle cells, whorls, large round cells with prominent nucleoli and discernable cytoplasm, points towards non-NN tumors. Meningioma, schwannoma, lymphoma, germ cell tumor, and metastatic cancers are the common non-NN tumors affecting the brain. Features favoring non-NN non-neoplasms include the presence of numerous inflammatory cells, epithelioid histiocytes, and red ischemic neurons.

Although the current series is relatively small, with this diagnostic guideline, it was able to produce a high accuracy rate of intra-operative diagnosis comparable to that of the previous studies [1-8]. Erroneous intra-operative diagnosis was due mainly to unrepresentative sample, resulting in undergraded tumors or failure to diagnose mixed lesions. It should be emphasized, however, that in practice intra-operative diagnosis should be made in correlation with the clinical data. A cytologic approach, with reference to the topographic distributions and radiographic appearances of the nervous system lesions, has recently been reviewed [11]. Combination of these approaches should fully enhance the accuracy of squash cytology.

To summarize, in addition to the present demon-stration of the high accuracy of intra-operative pathological diagnosis in neurosurgery, a diagnostic algorithm for squash cytology was provided. Squash

cytology is highly recommended for practicing pathologists dealing with neurosurgical specimens.

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การวินิจฉัยระหว่างการผ่าตัด ในศัลยประสาทพยาธิวิทยา: การศึกษาผู้ป่วย 120 ราย โดยเน้นเทคนิคทางเซลล์วิทยาโดยวิธีบีบ

ชนพ ช่วงโชติ, สมฤทัย ช่วงโชติ, สำรวย ช่วงโชติ

วัตถุประสงค์ *: เพื่อนำเสนอผลการวินิจฉัยทางพยาธิวิทยาระหว่างการผ่าตัด ในประสาทศัลยกรรมและนำเสนอแนวทางในการวินิจฉัยทางเซลล์วิทยาด้วยการบีบ*

วิธีการศึกษา *: ผู้วิจัยได้ศึกษาเปรียบเทียบผลการวินิจฉัยทางพยาธิวิทยาระหว่างการผ่าตัดกับผลการวินิจฉัยขั้นสุดท้ายของชิ้นเนื้อทางประสาทศัลยกรรม ในผู้ป่วยจำนวน 120 ราย ใน 83 รายใช้เทคนิคเซลล์วิทยาโดยวิธีบีบอย่างเดียวในการวินิจฉัย ใน 3 รายใช้การตัดชิ้นเนื้อแช่แข็ง และในรายที่เหลือใช้ทั้งสองเทคนิคร่วมกัน ผู้วิจัยได้นำเสนอแนวทางการวินิจฉัยทางเซลล์วิทยา*

ผลการศึกษา *: เมื่อเปรียบเทียบกับผลการวินิจฉัยขั้นสุดท้ายพบว่า ผลการวินิจฉัยทางพยาธิวิทยาระหว่างการผ่าตัดถูกต้องทุกประการคิดเป็นร้อยละ 83 และมีความถูกต้องบางส่วนร้อยละ 13*

สรุป *: การวินิจฉัยทางพยาธิวิทยาระหว่างการผ่าตัดของผู้ป่วยศัลยกรรมระบบประสาท มีความแม่นยำสูงแนะนำให้ใช้เทคนิคเซลล์วิทยาโดยวิธีบีบ เพื่อให้การวินิจฉัย*
