Evaluation of rapid staining techniques for cytologic diagnosis of intracranial lesions

Sam N. Long, BVSc; T. James Anderson, BVM&S, PhD; Fenella H. A. Long, BVM&S, MVSc, MACVSc; Pamela E. J. Johnston, BVM&S, PhD

Objective—To evaluate 4 rapid supravital stains and 3 preparation techniques for use in the intraoperative diagnosis of intracranial lesions.

Animals—10 dogs and 1 cat euthanatized for intracranial lesions.

Procedure—Specimens were taken from lesions and slides prepared, using 3 techniques: touch impression, medium-pressure impression, or smear preparation. Preparations were then stained with 4 stains: modified Wright stain, May-Grünwald-Giemsa, toluidine blue, and zynostain and examined in a blinded randomized fashion. Cytologic diagnosis was compared with histopathologic diagnosis and classified on the basis of identification of the pathologic process and specific diagnosis into the following categories: complete correlation, partial correlation, or no correlation.

Results—An overall diagnostic accuracy of 81% (107/132) was achieved on the basis of a combination of partial and complete correlation. Of the stains examined, modified Wright stain appeared to be most accurate, with complete correlation in 17 of 33 (52%) specimens and partial correlation in 12 of 33 (36%) specimens. Of the preparation methods, touch preparation and smear preparation provided the most accurate results, with an overall diagnostic accuracy of 82% (36/44) for both methods. However, smear preparations appeared to be of greater diagnostic value, with fewer nondiagnostic specimens, compared with touch preparations.

Conclusions and Clinical Relevance—Cytologic preparations provide a useful diagnostic tool for the intraoperative diagnosis of intracranial lesions. All stains examined yielded promising results, the most accurate of which appeared to be the modified Wright stain. The smear preparation appeared to be the preparation method of greatest diagnostic value. (Am J Vet Res 2002;63:381–386)

Stereotactic techniques were first described in 1947 for the biopsy of intracranial lesions in humans. Over the past 2 decades the development of advanced imaging has led to refinement of these techniques, and image-guided stereotactic biopsy has now become a routine procedure in many neurosurgical institutions. Stereotactic needle biopsies for diagnostic purposes are minimally invasive and facilitate the diagnosis of previously inaccessible lesions with safety and reliability. The procedure is associated with low morbidity, with overall complication rates varying between 2 and 6% and with mortality rates of between 0 and 2.3%.

Consequently, the growing prevalence of stereotactic needle biopsies has resulted in neuropathologists in human medicine being presented with smaller and fewer specimens for evaluation. Rapid diagnostic techniques described for intraoperative diagnosis of CNS lesions include frozen tissue preparation and cytologic preparation. The advantages of cytologic preparations are that they provide a more rapid turnaround time than frozen tissue and retain more tissue for standard paraffin embedding for definitive diagnosis. As a result, intraoperative cytologic evaluation of smear preparations of CNS lesions has become a common practice in many neuropathologic institutions in human medicine. The aims of intraoperative cytologic evaluations are 2-fold: to confirm the diagnostic value of the specimen to minimize the number of specimens taken and to provide a provisional histopathologic diagnosis that facilitates patient management decisions in the early postoperative period.

Cytologic preparations routinely consist of smear or touch preparations. Although some reports suggest that touch preparations are considered less informative, others have demonstrated comparable results using this method, compared with the smear technique. Stains described include modified Wright stains, H&E, toluidine blue, and the Papanicolaou stain. Important features described include the large Betz neurons of the motor cortex and the numerous small granule cells of the cerebellum that, to the inexperienced cytologist, may be misinterpreted as a tumor of lymphoid origin.

Recently, stereotactic biopsy systems have been developed for use in animals, and their use for the diagnosis of intracranial lesions has been described. There are few reports in the veterinary literature describing the cytologic appearance of intracranial lesions. A recent report describes the cytologic evaluation of smear prepar-
rations of intracranial neoplasms for intraoperative diagnosis following stereotactic biopsy and conventional open craniotomy. However, this report described only the use of a single stain and preparation technique. The cyto logical appearance of meningiomas and a glioma, using air dried smears obtained from impression or needle aspiration, have been described. A report comparing the accuracy of cytologic with histopathologic diagnoses in 100 tumors of various organs included 4 intracranial neoplasms and suggested an overall diagnostic accuracy of 83% on the basis of cytologic methods.

Given the advantages of stereotactic biopsy over conventional craniotomy for the diagnosis of intracranial lesions, it is likely that this procedure will become more common in the field of veterinary neurology. The aims of the study reported here were to evaluate the accuracy and diagnostic value of 4 rapid supravital stains in common use in veterinary institutions (modified Wright stain, May-Grünwald-Giemsa, toluidine blue, zynostain) and 3 preparation techniques (touch preparation, medium-pressure touch preparation, smear preparation) for the cytologic diagnosis of intracranial lesions.

Materials and Methods

Material was examined from 10 dogs and 1 cat that had been euthanatized following diagnosis of intracranial lesions. The animals were admitted to the University of Glasgow Veterinary School from 2000 to 2001 for clinical signs referable to intracranial disease. All affected animals had mass lesions demonstrable either on computed tomography scans or at necropsy. Specimens were taken from each lesion within 30 minutes of euthanasia, using a standard core biopsy needle, and preparations were made in 1 of 3 ways: light-pressure touch impression, medium-pressure impression, and smear preparation. Touch impressions were generated by lightly applying a standard dry microscope slide to the tissue specimen. Medium-pressure impressions were generated in the same way as touch impressions but by applying a moderate amount of pressure. Smear preparations were generated as follows: the specimen was placed on the end of a standard glass microscope slide, the end of a second glass slide was then applied to the specimen, and using an appropriate amount of pressure, the 2 slides were drawn quickly and firmly apart.

Following slide preparation, slides to be stained with toluidine blue were immediately fixed in 95% ethanol for 60 seconds while all other slides were rapidly air-dried. Slides were then stained with 1 of 4 rapid supravital stains according to standard protocols: a commercially available modified Wright stain, May-Grünwald-Giemsa stain, 1% toluidine blue, and zynostain. All slides were cleared and mounted with coverslips following preparation.

Following specimen collection, the brain was fixed in neutral-buffered 10% formalin for 24 hours before being sectioned transversely at the level of the optic chiasm to allow adequate internal fixation. After fixation for a further 7 days it was then routinely processed for histologic examination following paraffin embedding, sectioning at 5 µm thickness, and staining with H&E.

The number of nondiagnostic specimens was recorded, and for final cytologic evaluation, 12 slides in total were examined from each affected animal, 1 of each combination of the 4 stain types and 3 preparation methods. These were selected at random from those specimens that were diagnostic. Slides of

Figure 1—Photomicrographs of smear preparations from an intracranial meningioma of a dog. A—Modified Wright stain; bar = 140 µm. B—May-Grünwald-Giemsa stain; bar = 70 µm. C—Toluidine blue; bar = 70 µm. D—Zynostain; bar = 70 µm.
cytologic preparations were examined by 1 pathologist (FHAL) in a blinded randomized fashion. Diagnosis was made on the basis of pathologic process (ie, normal, inflammation, neoplasia, undetermined) and specific diagnosis (ie, inflammation, mesenchymal tumor, epithelial tumor, round cell tumor, undetermined). For purposes of classification, glial tumors (including astrocytomas and oligodendrogliomas) were classified as mesenchymal tumors, whereas meningeal tumors were classified as epithelial tumors.

Paraffin-embedded histologic sections were evaluated independently by a second pathologist (PEJJ) to give the definitive diagnosis. In comparing cytologic diagnosis with histopathologic diagnosis, results were classified for each specimen into the following categories: complete correlation, partial correlation, and no correlation. Complete correlation indicated correct identification of the pathologic process together with a correct specific diagnosis. Partial correlation indicated correct identification of the pathologic process but an incorrect specific diagnosis. No correlation indicated incorrect identification of the pathologic process and an incorrect specific diagnosis.

Results

The nature of the 11 specimens examined was as follows: 2 meningiomas, 1 oligodendroglioma, 1 choroid plexus papilloma, 1 pituitary macroadenoma, 2 lymphomas (1 primary and 1 metastatic), 2 adenocarcinomas (1 with local extension through the cribiform plate from a nasal tumor and 1 metastatic carcinoma from primary bronchogenic carcinoma), 1 instance of granulomatous meningoencephalitis, and 1 instance of gliosis surrounding intracranial hemorrhage of unknown origin.

The quality of cytoplasmic and nuclear staining with all 4 stains was generally excellent (Fig 1–3). When staining results were examined independently of preparation method, modified Wright stain with complete correlation in 17 of 33 (52%) specimens and partial correlation in 12 of 33 (36%) specimens appeared to be the most accurate. May-Grünwald-Giemsa stain and zynostain had similar results, with complete correlation in 17 of 33 specimens (52%) for both stains and partial correlation in 9 of 33 (27%) specimens for May-Grünwald-Giemsa stain and in 10 of 33 (30%) for zynostain. Toluidine blue had the least accurate results, with complete correlation in 15 of 33 (45%) specimens and partial correlation in 10 of 33 (30%). Overall, the May-Grünwald-Giemsa stain had more reliable results than other stains, with only 22% (16/73) nondiagnostic specimens. This compared with 27% (19/70) nondiagnostic specimens with zynostain, whereas modified Wright and toluidine blue stains had comparable results, with 31% (22/71) nondiagnostic specimens with each stain.

When preparation method was examined independently of stain type, the preparation method with the greatest diagnostic accuracy appeared to be the touch preparation, with 24 of 44 (55%) specimens having complete correlation and 12 of 44 (27%) specimens hav-
The smear preparation had similar results, with 21 of 44 (48%) specimens having complete correlation and 15 of 44 (34%) specimens having partial correlation (Fig 4). The medium-pressure preparation yielded 21 of 44 (48%) specimens with complete correlation and 14 of 44 (32%) specimens with partial correlation. However, when these results were correlated with the percentage of diagnostic specimens, the smear preparation appeared to be most useful, because only 19% (20/103) were nondiagnostic specimens. This compared favorably with both other methods, with 30% (27/90) nondiagnostic specimens created using the medium-pressure touch preparation and 35% (32/92) nondiagnostic specimens created using the touch preparation. Overall, 66 of 132 (50%) specimens had complete correlation with histopathologic diagnosis, 41 of 132 (31%) had partial correlation, and 28% (79/285) nondiagnostic specimens were found.

Overall the number of specimens in which the cor-

Table 1—Overall diagnostic accuracy on the basis of histopathologic diagnoses, using 4 rapid supravital stains and 3 preparation techniques on intracranial lesions from 10 dogs and 1 cat.

<table>
<thead>
<tr>
<th>Histopathologic diagnoses</th>
<th>No. of correct pathologic processes identified*</th>
<th>No. of correct specific diagnoses made*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningioma (syncytial)</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Lymphoma (metastatic)</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Lymphoma (primary)</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Adenocarcinoma (nasal)</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Choroid plexus papilloma</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Granulomatous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meningoencephalitis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma (metastatic)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Meningioma (transitional)</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Gliosis secondary to hemorrhage</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Number out of 12 specimens.
Pathologic processes identified = Normal, neoplastic, inflammatory, or undetermined. Specific diagnoses = Inflammation, mesenchymal tumor, epithelial tumor, round cell tumor, or undetermined.
rect pathologic process was identified was high (Table 1). However, although specimens from some types of lesions (ie, syncytial meningioma, primary lymphoma, and the metastatic carcinoma) had an accurate specific diagnosis, others (granulomatous meningoencephalomyelitis, glosis, pituitary adenoma, choroid plexus papilloma, transitional meningioma) did not.

**Discussion**

The results of our study suggest that when the combination of pathologic process and specific diagnosis is used, cytotologic examination provides an accurate diagnostic method that is useful for the intraoperative diagnosis of intracranial lesions. The overall diagnostic accuracy of 81% (107/132) is similar to other reports in the veterinary and human literature. However, it is difficult to compare diagnostic accuracy with that quoted in other reports. Most human studies evaluate diagnosis made in conjunction with imaging studies and consultation with a neurosurgeon, unlike our study. Furthermore, the assessment of diagnostic accuracy in other reports is not straightforward, as accuracy can be evaluated in various ways. Most studies in humans include only 2 categories of accuracy: correct or incorrect. If these terms are not further defined it may be unclear which criteria need to be fulfilled for a correct diagnosis. Most reports assess the accuracy of diagnosis by comparing cytologic examination with histopathologic diagnosis, although 1 report has evaluated cytologic diagnosis using survival analysis and found that cytologic diagnosis reliably predicted mean survival time. Few reports exist in the veterinary literature evaluating the accuracy of intraoperative diagnosis, and to the authors' knowledge no reports exist evaluating the intraoperative diagnosis of intracranial lesions. As stated previously, 1 report evaluating the accuracy of cytologic diagnosis in 100 animals with various lesions included a small number of brain tumors. Another report evaluating the accuracy of intraoperative diagnosis using frozen section examination in 194 specimens reported an overall accuracy rate of 93%, with a correct specific diagnosis achieved in 83% of specimens and correct identification of the pathologic process in an additional 10% of specimens. This report included 8 biopsy specimens from the nervous system, 4 of which were intracranial lesions.

Intraoperative diagnoses are often broad and are further narrowed by evaluating paraffin embedded sections. For this reason, we considered it more appropriate to have 3 categories of correlation between cytologic and histopathologic diagnosis. In this way partial credit could be given to the cytologist for making a tentative diagnosis that may be clinically useful despite a lack of final specific diagnosis. One of the most important factors for diagnosis appeared to be familiarity of the cytologist with the stain used. Toluidine blue, a stain that is rarely used in our institution, was less accurate than other stains. At the same time the 2 most accurate stains, modified Wright stain and zynostain, are in common use in our institution. However, the number of specimens examined in our study was small, and more work needs to be performed to further evaluate the effects of experience on diagnostic accuracy with respect to stain type.

The modified Wright stain and May-Grünwald-Giemsa stain are Romanowsky-type stains that use 2 dyes to differentiate cellular elements. Along with zynostain, these stains were chosen for evaluation because of their availability, speed of preparation, ease of use, and their common use in veterinary institutions. Toluidine blue, although commonly in use in human neuropathologic institutions, has yet to become popular in veterinary cytopathologic evaluations. The advantages of this stain in cytotologic examination of the nervous system include speed of use and the ability to stain astrocytic processes. The quality of cytoplasmic and nuclear staining with all 4 stains was generally excellent (Fig 1–3). May-Grünwald-Giemsa and modified Wright stains produced good cytoplasmic and stromal detail, whereas zynostain and toluidine blue allowed better visualization of nuclear detail, as has been described elsewhere. Whereas the Romanowsky-type stains and zynostain are based on air-drying techniques, toluidine blue relies on wet fixation. A number of differences exist between wet-fixed and air-dried specimens: the smearing technique is less important for well-fixed specimens but important for air-dried specimens; air drying increases cell size, whereas wet fixation decreases cell size; and cell loss is high with wet fixation, compared with air-drying techniques. This last point is less relevant in smear preparations, where a large amount of material is transferred to the slide, but important in touch preparations, where fewer cells adhere to the slide surface. It is even more important for fibrous or firm specimens (eg, meningioma, malignant nerve sheath tumors), which imprint or smear poorly.

Of the 3 preparation techniques, touch preparation and smear preparation provided results of similar diagnostic accuracy, with touch preparation providing slightly greater accuracy for specific diagnosis. However, if the percentage of nondiagnostic specimens was taken into consideration, smear preparation was felt to be more useful, as fewer nondiagnostic specimens were found. This is especially relevant to stereotactic biopsy specimens, where it is desirable to take as few specimens as possible to achieve a diagnosis. Although a greater amount of tissue may be obtained with multiple aspirations, this increases the risk of hemorrhage, and consequently for brainstem lesions or other high risk locations only a single suction aspiration biopsy may be performed for safety reasons. Most human neuropathologists use the smear technique for cytologic examination of CNS lesions. In smear preparations of small specimens not only the cytologic detail but also some tissue architecture is preserved, which may not be achieved with paraffin-embedded sections, and this proved helpful in the diagnosis of some lesions (Fig 4). Few reports have compared the diagnostic accuracy of touch and smear preparations in humans, but 1 report found that touch preparation was associated with lower diagnostic accuracy (76%), compared with the smear technique. This may be the result of artificial alterations in the tissue resulting from manipulation, disruption, and crushing of the biopsy specimen. However, although smear preparations are most commonly used, it has been reported that less forceful touch preparations can be useful for metastatic carcino-
ma and lymphoma but usually are not adequate for gliomas. Technical problems with the touch preparation may also complicate interpretation of specimens. For example, excessively thick cytologic preparations lead to clumping and distortion and may suggest a more anaplastic lesion. Firm tumors with abundant fibrous connective tissue stroma may not shed cells on the glass slide, which may lead to an underestimation of the degree of malignancy. In general, soft and friable tissues lend themselves to the smear technique, whereas firm or fibrous material may require a frozen section for adequate examination. Although our study found that the touch and smear preparations were of similar diagnostic accuracy, the greater difficulty in performing the touch preparation was reflected in the greater proportion of nondiagnostic specimens. Given the total number of nondiagnostic specimens, it would appear that several slides should be prepared from each specimen to maximize the possibility of a diagnostic specimen.

Perhaps 1 of the most interesting features of our study was the considerable variation in diagnostic accuracy among specimens from different lesion types (Table 1). With some of these specimens, such as those of granulomatous meningoencephalomyelitis and gliosis, this is unsurprising as gliosis and inflammation provide diagnostic challenges. The distinction between reactive gliosis and the infiltration zone of glial tumors constitutes a difficulty for diagnosis, as no reliable morphologic criteria can be defined to distinguish reactive astrocytes from neoplastic cells. Most specimens from these lesions had a misdiagnosis of mesenchymal neoplasms of glial origin.

The misdiagnosis of the transitional meningioma is likely to have occurred because of difficulties with the classification system used in this study. Cytologic features suggestive of meningioma include cells with epithelial and mesenchymal characteristics and a tendency toward cell clustering. The dual-cell morphologic characteristics within a single tumor are expected in light of the mixed fibroblastic and epithelioid components often seen histologically. One report of the cytologic characteristics of canine meningiomas has suggested that on the basis of cytologic features alone, a diagnosis of neoplasia may be possible, but a specific diagnosis of meningioma can only be made on the basis of cytologic features together with tumor location and imaging characteristics. With the choroid plexus papilloma and pituitary adenoma, misdiagnosis occurred because of interpretation error. However, most specimens of all 3 tumors were correctly identified as neoplastic, although the type of neoplasia was incorrectly identified as mesenchymal. This may be the result of the lack of familiarity of the cytologist with cytologic preparations of these tumors.

References