ESSENTIALS OF HEAD AND NECK CYTOLOGY

Gia-Khanh Nguyen
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PREFACE

Tumors arising from the head and neck are numerous and have complicated and diversified histopathologic patterns. Cytodiagnosis of those neoplasms by fine needle aspiration is challenging and compounded with diagnostic pitfalls. However, with a representative cell sample and careful evaluation of different cellular and non-cellular components, a correct diagnosis may be safely made in the majority of cases.

This monograph is written for practicing pathologists in community hospitals, pathology residents and cytotechnologists who are interested in acquiring a basic knowledge in diagnostic cytology of head and neck tumors. It consists of four chapters describing the cytologic manifestations of important tumors of the thyroid, parathyroid, salivary glands, lymph nodes, soft tissues and brain. The text is concise and illustrations are abundant. For most tumors, cytologic and histologic images are presented side by side for cytohistologic correlation. Immunohistochemical features of commonly encountered neoplasms that are important for tumor typing and differential diagnosis are stressed.

For improvement of the future editions of the monograph, constructive comments and suggestions from the reader will be highly appreciated.

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Gia-Khanh Nguyen, M.D.

RELATED MATERIAL
(by the same author)

Essentials of Needle Aspiration Biopsy Cytology, 1991
Essentials of Exfoliative Cytology, 1992
Essentials of Cytology: An Atlas, 1993
Critical Issues in Cytopathology, 1996
Essentials of Fluid Cytology, 2009
Essentials of Gynecologic and Breast Cytology, 2010
To my family with love
Chapter 1

THYROID*

Fine needle aspiration (FNA) for cytologic evaluation of thyroid cancer was originally used by Martin and Ellis at the New York Memorial Hospital for Cancer and Allied Diseases in 1930 (1). However, this diagnostic procedure was subsequently found to have a limited value, and it was then discontinued at the above-mentioned institution (2,3). Thyroid FNA was not further developed and did not gain acceptance in the United States for nearly 50 years until the early 1980s when its diagnostic value was firmly demonstrated by Scandinavian investigators (4-8). The 1974 report by Crockford and Bain (9) and the 1979 paper of Miller and Hamburger (10) were apparently the first North American publications attesting to the value of thyroid FNA. This method of clinical investigation now is practiced worldwide and has become the cornerstone in the management of thyroid nodules (TN) (11-25).

INDICATION AND GOAL OF THYROID FNA

Thyroid nodular lesions are a common clinical problem. In the United States, 4 to 7% of adult population has a palpable TN (13). The incidence of thyroid cancer in a clinically solitary TN or in a multinodular goiter is equal and about 5% in non-endemic areas (26). TNs constitute the main indication for FNA, and the goal of this diagnostic procedure is to detect thyroid neoplasms for surgical resection and to identify non-neoplastic lesions that may be managed conservatively (23). This method of clinical investigation has reduced the number of diagnostic thyroid surgeries for TNs by 60-85%, and the difference in rates of thyroid surgery reflect the cytodiagnostic accuracy rates among different medical centers (24).

CONTRAINDICATIONS AND COMPLICATIONS OF THYROID FNA

The main contraindication to thyroid FNA is bleeding diathesis, as the formation of a large hematoma at the biopsy site may cause compression of the trachea and respiratory distress (13,23). Therefore, a bleeding time, PT and PTT should be ordered to screen this condition in all patients prior to thyroid FNA. This diagnostic procedure, if properly performed, is almost free of complications. Subcutaneous hematoma at the biopsy site, accidental puncture of the trachea and local infection are rare complications (13). Hematoma may be prevented by local pressure of the overlying skin at the biopsy site (13). Tracheal injury is manifested by minimal and transient hemoptysis. Seeding of thyroid cancer cells along the needle tract is also an exceedingly rare complication with FNA (13).

* This chapter is an update of the article "Fine-needle aspiration cytology of the thyroid: an overview" by Nguyen GK, Lee MW, Ginsberg J, Bilodeau D, Wragg T. Cytojournal, 2005; 2:12. It has received the Best Article Award – 2005 from the Cytojournal Award Committee.
PROCUREMENT AND PREPARATION OF CELL SAMPLES

Procurement of cell samples. Obtaining an adequate or satisfactory cell sample for cytologic evaluation is not simple, and interpreting thyroid cytology is challenging and requires expertise (13,23). To perform thyroid FNA, the TN is identified by palpation, and a 22- to 25-gauge and 4.5-cm-long needle is commonly used to procure cell samples from at least three different areas of any TN. Usually, only dermal anesthesia is required. Depending on personal preferences, FNA of a TN may be performed either with or without a syringe (13). However, for cystic thyroid lesions, the cyst contents should be evacuated first by FNA with a syringe. The gland is then carefully examined by palpation. If a residual nodule is found, it should be aspirated. If the TN is difficult to identify by palpation the patient should be referred to a radiologist for FNA under ultrasonographic guidance (13,22-24). Since the thyroid is rich in capillary blood vessels the needle aspirate usually contains a large amount of peripheral blood that may be reduced by limiting the biopsy procedure to about five seconds or by using the FNA technique without aspiration (13).

Preparation of cell samples. For cytological evaluation, smears should be appropriately prepared and stained. Depending on the amount and nature of the thyroid needle aspirate one of the following preparation techniques is used: (a). A small drop of thyroid aspirate is put near the frosted end of a glass slide and is quickly and gently smeared by a cover slip. (b). A small drop of thyroid aspirate is put on a glass slide and gently crushed with a second slide that is then separated vertically from the first one. (c). A small or medium-sized drop of thyroid aspirate is put near the frosted end of a slide that is placed on a table. A second slide is used to spread the aspirated material in the same manner used to prepare a peripheral blood smear. (d) Cytospin smears should be prepared from the liquid contents of all cystic thyroid lesions. (e). Excess of aspirated material should be used for preparation of a cell block that may show diagnostic tissue fragments on sectioning. It is important that a small drop of aspirated material is used for smear preparation, as if a large drop of aspirate material is used, an unevenly thick smear may be obtained, and at the end of the slide a thick and bloody cell film may be formed. This will obscure the cellular details of underlying thyroid cells and tissue fragments, making their evaluation extremely difficult, if not impossible.

Routine staining methods. Depending on personal preference, either air-dried and Romanowsky-stained smears or ethanol-fixed and Papanicolaou-stained smears are prepared. For Papanicolaou staining, the smears must be fixed quickly before drying with 95% ethanol or with a commercial spray fixative. A delay in fixation will result in air-dried artefactual changes with loss of cellular details. Air-dried smears for staining with one of the Romanowsky modified methods (Wright stain, May-Grunwald-Giemsa or Diff-Quik method) now are widely used, as air-drying artefactual changes can be avoided. However, nuclear details in Romanowsky-stained smears are not as well-
visualized as in wet-fixed and Papanicolaou-stained smears. A parallel use of air-dried and wet-fixed smears is usually recommended, as these two staining methods are complementary (13,22,23). Fixation of aspiration smears in Carnoy solution for 3-5 minutes may be used to lyse red blood cells prior to staining with the Papanicolaou method.

**SPECIMEN ADEQUACY**

Obtaining an adequate cell sample is a prerequisite to the success of thyroid cytology. Therefore, immediate microscopic assessment of the needle aspirate by a pathologist or a cytotechnologist is desirable. If the first sample is judged inadequate for cytological evaluation, the TN can be re-aspirated immediately. If a rapid evaluation is not available, multiple FNAs of different areas of the TN should be performed.

The rate of inadequate or unsatisfactory specimens reported in the literature range from 2-21% (means 17%)(15)*. Currently, criteria for specimen adequacy vary from institution to institution. Some investigators require that an adequate sample should contain five to six groups of well-preserved and well-visualized follicular cells with each group containing 10 or more cells (12). One group requires multiple punctures of the TN to be evaluated, with at least six properly prepared smears and a minimum of 8-10 tissue fragments of well-preserved follicular epithelium on each of two slides (25). Another group requires 10 clusters of follicular cells with at least 20 cells in each cluster (13). The Papanicolaou Society of Cytopathology Task Forces on Standard of Practice does not specify any numbers and groups of thyroid follicular epithelial cells for specimen adequacy (23). Two practical exceptions to these adequacy criteria are applied: (a) a benign colloid nodule may be suggested if a large amount of thick colloid material is present, regardless of the number of follicular epithelial cell clusters (23); or, (b) if a cell sample contains one or two small clusters of malignant or highly atypical cells, it should be reported as malignant or suspicious for malignancy and not as unsatisfactory or inadequate for cytodagnosis (23). Thyroid FNA under ultrasonographic guidance achieved higher rates of adequate cell samples, in the range of 79-99.3% (mean 91%) (21, 27-36). Ultrasound-guided thyroid FNA proved to be useful in sampling TNs smaller than 2 cm in greatest dimension, complex or solid-cystic TNs (27-36) and abnormal thyroid beds (35,36).

* An unsatisfactory rate of less than 2% has been obtained by FNA of TNs without suction with a syringe and an excessive suction was believed to be the leading cause of the problem. Oertel YC. Assessment of the cost of thyroid FNA cytology. Am J Clin Pathol. 2009; 131:146.
CYTODIAGNOSIS AND ITS LIMITATIONS

The cytodiagnosis of TNs by FNA is complex for the following reasons (26):

a. overlap of cytological patterns between neoplastic and non-neoplastic lesions.
b. overlap of cytological features between various neoplasms.
c. coexistence of non-neoplastic and neoplastic processes and multiple malignancies.

For a practical diagnostic approach, the cytological findings of thyroid lesions may be divided into seven main groups, as recommended by the Papanicolaou Task Force on Standard of Practice (23)(Table 1). These groups are heterogeneous and consist of both neoplastic and non-neoplastic lesions that may show either similar or specific cytological manifestations (23). A non-diagnostic group is added as some TNs yield inadequate or non-specific cytological findings.

**Table 1. Cytodiagnostic Groups with Commonly Encountered Thyroid Nodular Lesions**

1. Benign colloid nodule
   - Solitary colloid nodule
   - Prominent nodule in MNG
   - Macrophillic adenoma
2. Cellular microfollicular lesion
   - Microfollicular adenoma
   - Low-grade follicular carcinoma
   - Hyperplastic microfollicular lesions in HT or MNG
3. Hurthle cell lesion
   - Hurthle cell adenoma
   - Hurthle cell carcinoma
   - Hyperplastic Hurthle cell nodule in HT or MNG
4. Primary malignant tumor
   - Papillary carcinoma
   - High-grade microfollicular carcinoma
   - Insular carcinoma
   - Medullary carcinoma
   - Anaplastic carcinoma
   - Lymphoma
5. Cystic lesions
   - Benign colloid nodule
   - Papillary carcinoma
   - Other thyroid neoplasms
6. Thyroiditis
   - Acute thyroiditis
   - Hashimoto's thyroiditis
   - Subacute thyroiditis
7. Other lesions
   - Graves' disease
   - Metastatic cancer
8. Non-diagnostic category

* HT, Hashimoto's thyroiditis; MNG, multinodular colloid goiter
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In October 2007, the National Cancer Institute hosted “The NCI Thyroid Fine Needle Aspiration State of the Science Conference”*. A recommended diagnostic terminology/classification of thyroid FNA interpretation was as follows: Benign, follicular lesion of undetermined significance, follicular neoplasm, suspicious for malignancy, malignant and non-diagnostic. Each diagnostic category has specific follow-up recommendations. Those terminology/classification and recommendations need to be evaluated further for their practical diagnostic value in patient care.

1. Benign Colloid Nodule. This group includes solitary benign colloid nodules and prominent benign colloid nodules in a multinodular colloid goiter. These two lesions are the most common ones in population at large and they are characterized histologically by large thyroid follicles distended with thick colloid material (Figure 1.1). A benign colloid nodule yields in FNA abundant, thick colloid material with cracking or bubble pattern and sheets of benign follicular epithelial cells in “honeycomb” arrangement (Fig. 1.2). Clusters of slightly hyperplastic Hurthle cells may be present (12,22,23,25). The cytological differential diagnosis between a benign colloid nodule and a macrofollicular adenoma of the thyroid is extremely difficult if not impossible, as the two lesions usually show abundant, thick colloid and similar follicular cells (22,23).

Figure 1.1. Histology of a multinodular colloid goiter showing large thyroid follicles distended with thick colloid (HE, x 100).

Figures 1.2. FNA Cytology of benign colloid nodule:
A: thick, deep blue colloid material with cracking pattern.
B. Thick, deep blue colloid material with bubble pattern.
C. A monolayered sheet of benign follicular epithelial cells with “honeycomb” pattern.
(Diff-Quik, A and B x 250; C x 400).

2. Cellular Microfollicular Lesion. This group includes hyperplastic microfollicular nodules in a multinodular colloid goiter or Hashimoto’s thyroiditis, a
microfollicular adenoma, and a well-differentiated follicular carcinoma. These lesions are characterized histologically by small thyroid follicles with or without a small amount of colloid material. They are the most challenging ones to diagnose cytologically (22,23-25). They are commonly reported as a microfollicular lesion or tumor with a recommendation for surgical excision (13,22-24). FNA from a cellular microfollicular lesion usually reveals abundant follicular cells in clusters, acini and small monolayered sheets (Figs.1.3). The individual cells show scanty, ill-defined cytoplasm and oval nuclei with regular nuclear contours and inconspicuous or prominent nucleoli.

Cellular microfollicular lesions of the thyroid fall into the diagnostic category of indeterminate or suspicious lesions (14,15,22), and in one large series 14% of microfollicular lesions were malignant (12).

Figures 1.3. Cellular microfollicular lesions showing in FNA cells with round nuclei arranged in acini and small monolayered sheets (Pap, A x 160, B x 400).

3. Hurthle Cell Lesion. Diagnosis of Hurthle cell lesions is a challenge in thyroid cytology. A hyperplastic Hurthle cell nodule in a Hashimoto’s thyroiditis or in a multinodular colloid goiter and a Hurthle cell neoplasm display similar cytologic findings (22-25,37,38). The presence of numerous lymphocytes or a large amount of
thick colloid material in the needle aspirate may indicate a hyperplastic Hurthle cell nodule in Hashimoto’s disease or a multinodular colloid goiter, respectively (38). Hurthle cell adenoma and carcinoma usually show similar cytologic findings that are characterized by sheets and clusters of polygonal epithelial cells with abundant, granular, eosinophilic or basophilic cytoplasm, oval nuclei with regular nuclear contours and conspicuous or inconspicuous nucleoli (22-25) (Figures 1.4). The presence of syncytial clusters of Hurthle cells with or without prominent nuclei (25) and abundant naked tumor cell nuclei has been reported to be a feature of Hurthle cell carcinoma (38).

When a Hurthle cell lesion is detected by FNA, surgical excision is usually indicated for further histologic study (38). Thyroid Hurthle cell lesions fall into the cytodiagnostic category of indeterminate lesions or suspected malignant lesions (14,15,22), and 13% of Hurthle cell lesions were malignant in one large series (12).

Figures 1.4. Histology and FNA cytology of a Hurthle cell neoplasm.
A and B. FNA of a Hurthle cell lesion showing Hurthle cells singly and in loose monolayered sheets (A: Pap, x 400; B: Diff-Quik, x 400).
4. Primary Malignant Tumors. This group includes papillary, high-grade follicular, insular, medullary and anaplastic carcinomas, and lymphoma. These lesions commonly show distinctive cytologic features that permit a correct identification in the majority of cases (13, 24, 25). An insular carcinoma or poorly differentiated carcinoma yields small cells similar to those of a high-grade microfollicular carcinoma (39).

4a. Papillary carcinomas (PC) is the most common thyroid malignant tumor and account for about 70% of all thyroid solid cancers. PCs may be divided into conventional PC with well-formed papillae with fibrovascular cores and PC variants that are composed of micro and macrofollicular, oncocytic, trabecular, tall-cell, columnar-cell and diffuse sclerosing variants. The tumor cells nuclei display nuclear crowding and overlapping and nuclear grooves and intranuclear cytoplasmic inclusions.

Conventional PC is characterized in FNA by the presence of thick or thin papillary tissue fragments with fibrovascular cores (Figures 1.5). Sheets of tumor cells showing focal nuclear crowding and overlapping, irregular nuclear contours, nuclear grooves (NG) and intranuclear cytoplasmic inclusions (INCI) are present. Psammoma bodies and benign metaplastic squamous cells may also be present (13, 22, 24, 25) (Figures 1.6 and 1.7). These nuclear changes are recognized with less difficulty in Papanicolaou-stained cell samples, but they may be difficult to identify in cell samples stained with the Romanowsky staining method (13, 22, 23). However, a presence of minute true papillary tissue fragments with fibrous vascular cores even without the identifiable above-mentioned nuclear changes is indicative of a PC. These papillary tissue fragments should be differentiated from thick and large follicular epithelial cell clusters with vascular transgression that may be found in FNA from different types of non-papillary epithelial neoplasm of the gland (40).
Figures 1.5. Papillary tissue fragments with thin (A) and thick (B) fibrovascular cores (Pap, A, x 100, B, x 40).
Figures 1.6. FNA cytology of conventional thyroid PC:
A. A sheet of tumor cell showing focal nuclear crowding with several cells displaying nuclear grooves (Pap, x 400).
B. A loose sheet of tumor cells showing focal nuclear crowding and 2 cells with INCIs (Diff-Quik, x 400).
C. Two psammoma bodies and a small amount of colloid (Pap, x 250).

Figure 1.7. A loose cluster of metaplastic squamous cells seen in FNA of a thyroid papillary carcinoma (Pap, x 400).

*Micro- and macrofollicular PCs* constitute a diagnostic challenge. A microfollicular PC may show in FNA follicular cells forming acini similar to those seen in the aforementioned cellular microfollicular lesions, and a macrofollicular PC may be easily mistaken for a macrofollicular adenoma or a benign colloid nodule cytologically, as nuclear grooves characteristic for a thyroid PC may not well be visualized (41-43). Ethanol-fixed and Papanicolaou stained cell samples reveal better PC nuclear features than air-dried and Romanowsky-stained tumor cells (Figures 1.8).
Figures 1.8 A and B. Thyroid PC, follicular variant:
A. Histology of a thyroid PC, microfollicular variant showing tumor cells with nuclear molding and grooves in follicular pattern (HE, x 250).
B. FNA cytology of a thyroid PC, microfollicular variant, showing tumor cells in acinar arrangement with some cells showing INCIs (Pap, x 400).
Figures 1-8 C and D: Thyroid PC, follicular variant showing in FNA:
C. Clusters and sheets of tumor cells and globules of thick colloid material.
D. Tumor cells in acinar arrangement showing nuclear crowding and overlapping and occasional INCIs (Diff-Quik, C, x 100, D, x 400).

**Other PC variants.** *Hyalinizing trabecular adenoma* is indistinguishable from a PC cytologically, as these two lesions yield cells with similar nuclear features (44). Recent molecular studies have suggested that this tumor is actually an *encapsulated trabecular variant of thyroid PC* (45). *Tall-cell PC* is characterized by the presence of tall tumor cells with well-defined, granular cytoplasm and nuclei with NGs and single or multiple INCIs, making at least 30% of the aspirated cells (46-51) (Figure 1.9). *Columnar-cell PC* shows no classic nuclear features of thyroid PC, but presence of clusters of columnar cells with palisading nuclei and the absence of classic nuclear changes of thyroid PC are cytologic features of this neoplasm (52) (Figure 1.10).

Figure 1.19. FNA cytology of thyroid PC, tall cell variant showing pleomorphic tumor cells with some cells having an elongated configuration and cytoplasmic tails. A tumor cell with an intranuclear cytoplasmic inclusion is present (Diff-Quik, x 400).
Figure 1.10. Thyroid PC, columnar cell variant, showing tall, columnar tumor cells without characteristic nuclear features of a conventional PC (Pap, x 400).

**Diffuse sclerosing PC** can be confidently suggested when abundant benign squamous cells admixed with lymphocytes, follicular epithelial cells with nuclear features of papillary carcinoma and a few psammoma bodies are noted (53,54) (Figures 1.11).

Figs.1.11. FNA cytology of thyroid PC, diffuse sclerosing variant showing isolated and a sheet of benign metaplastic squamous cells admixed with lymphocytes and a psammoma body. (A: HE, x 160; B x 400).

**Oncocytic variant PC** is a rare neoplasm. Histologically, it is characterized by fibrovascular cores covered with follicular cells with extensive oncocytic change with nuclear crowding and overlapping. INCl's may be present. In FNA thick papillary tumor tissue fragments with fibrovascular cores are present as well as single and clustered oncocytes (40).
Solid/trabecular variant PC is an uncommon tumor. It yields in FNA thick sheets or anastomotic cords of tumor cells showing nuclear crowding, and INCIs and nuclear grooves are present* (Figures 1.12).

Figures 1.12. Thyroid PC, solid/trabecular variant.
A. Histology of trabecular variant thyroid PC (HE, x 160).
B. Thick anastomotic cords of tumor cells in FNA (Pap, x 250).

4b. High-grade follicular carcinoma and insular carcinoma are characterized by sheets and acinar clusters of pleomorphic epithelial cells with prominent nucleoli (22,24,25,39) (Figures 1.13 and 1.14).

Figures 1.13. High-grade follicular carcinoma of the thyroid.
A. Histology of the tumor (HE, x 250).
B. Tumor FNA showing malignant glandular cells in cohesive clusters (Pap, x 400).
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Figures 1.14. Thyroid insular carcinoma:
A. Histology of the tumor (HE, x 250).
B and C. FNA of the tumor showing tumor cells in a large, cohesive three-dimensional clusters or nests. (B and C. Pap, x 400).

4c. **Medullary carcinoma** shows in FNA a mixture of single and clustered polygonal cells and spindle tumor cells that may display INCIs (22,24,25). The tumor cell cytoplasm may show intracytoplasmic pink azurophil granules that are well-visualized by MGG or Diff-Quik stain and stains positively with calcitonin antibody. Irregular fragments of amyloid material that stains positively with Congo red may be seen (Figures 1.15 and 1.16).
Figure 1.15. Histology of thyroid medullary carcinoma showing solid sheets of polygonal cells and focal amyloid deposit (HE, x 250).
Figures 1.16. Thyroid medullary carcinoma cytology: 
A. Tumor FNA showing dyshesive plasmacytoid tumor cells and intracytoplasmic azurophil granules,
B. Loosely clustered spindle tumor cells with scant, ill-defined cytoplasm and
C. An irregular fragment of amyloid in smear background.
(A: Diff-Quik, x 400; B: Pap, x 400 and C: Pap, x 100).

4d. Anaplastic carcinoma consists of two main histologic subtypes: Giant-cell and spindle-cell subtypes. Depending on the histologic subtype, an anaplastic thyroid carcinoma displays in FNA pleomorphic large, bizarre cancer cells with prominent nucleoli or spindle cancer cells admixed with a variable amount of necrotic debris (Figures 1.17).
Figures 1.17. Cytology of thyroid anaplastic carcinomas:
A. Giant-cell type tumor showing single, large and bizarre malignant cells.
B. Spindle-cell type tumor showing spindle malignant cells with scant, ill-defined cytoplasm (A and B, Pap, x 400).

4e. *Non-Hodgkin lymphoma* is usually of large cell type and yields in FNA cells similar to those of a lymph node involved by the same neoplastic process. A *thyroid Hodgkin’s disease* is characterized by Reed-Steinberg cells admixed with benign lymphoid cells and eosinophils (13,24,25).

5. **Cystic Lesions.** Benign cysts account for the majority of thyroid cystic lesions. They are formed as the result of hemorrhagic degeneration of a benign colloid nodule. FNA from a benign colloid cyst may show colloid material admixed with benign follicular epithelial cells and hemosiderin laden macrophages. However, any thyroid neoplasm may undergo hemorrhagic necrosis and become a cystic lesion (13,22-25). Of the thyroid neoplasms, PC tends to undergo marked hemorrhagic degenerative change. FNA from the tumor commonly shows a large amount of blood and the cystic lesion tends to recur rapidly (23). Cytological examination of the aspiration smears usually reveals a large amount of blood and rarely tumor cells. However, sections from the cell block prepared from the needle aspirate may show diagnostic papillary tissue fragments with fibrovascular cores and nuclear features of a PC (23) while that of a benign colloid nodule will show no true papillary tissue fragments with fibrovascular cores or nuclear features of a thyroid PC (Figures 1.18 and 1.19).
6. Thyroiditis. **Acute thyroiditis** is clinically evident and it is not a target for FNA. It shows in FNA necrotic debris and abundant polymorphonuclear leukocytes. **Hashimoto’s thyroiditis** and **subacute thyroiditis** commonly have fairly distinctive clinical findings. Rarely, these lesions may present as a nodular lesion mimicking a thyroid neoplasm. **Hashimoto’s thyroiditis** is characterized by the presence of numerous benign lymphoid cells admixed with benign follicular cells and Hurthle cells that may have a bizarre morphology (Figure 1.20).
Figures 1.20. Hashimoto’s thyroiditis:
A. Histology of Hashimoto’s thyroiditis (HE, x 250).
B. Hashimoto’s thyroiditis showing in FNA abundant benign lymphoid cells and a Hurthle cell cluster (Pap, x 400).

Subacute thyroiditis may yield clustered epithelioid cells, scattered lymphocytes and a few multinucleated giant cells containing up to one hundred nuclei (13,22-25,37) (Figures 1.21). It should be born in mind that Hashimoto’s thyroiditis may harbor hyperplastic follicular and Hurthle cell nodules, and these two nodules are cytologically indistinguishable from a cellular follicular neoplasm and a Hurthle cell neoplasm, respectively (37,38). Surgical excision of these lesions is required for histologic study.
Figures 1.21. Subacute thyroiditis:
A. Histology of subacute thyroiditis (HE, x 160).
B, C. A large multinucleated giant cell and a syncytial cluster of epithelioid cells with carrot-shaped nuclei seen in FNA of a subacute thyroiditis (Diff-Quik, x 400).
7. Other Nodular Lesions. *Graves’ disease* may rarely present as a nodular thyroid lesion (55). It yields clusters of follicular cells with cytoplasmic vacuoles that may contain pink material or “flare cells” (Figure 1.22). However, this finding is non-specific for Graves’ disease (13).

![Figure 1.22. A group of “flare cells” showing intra cytoplasmic pink globular material in FNA of a Graves’ disease (Diff-Quik, x 400).](image)

*Metastatic cancers* to the thyroid are common in patients with advanced cancers arising from other body sites (25). However, solitary metastatic cancer to the thyroid gland presenting as a palpable TN is uncommon*. For unknown reasons, renal cell carcinoma is the most common metastatic neoplasm to the thyroid, and cases of clinically occult renal cell carcinoma presenting initially as a large thyroid mass have been documented (25). Cytodiagnosis of metastatic cancer to the thyroid is relatively straightforward as metastatic cancer usually displays a cytologic pattern distinctive from those of a primary thyroid carcinoma (25). However, a cytological differential diagnosis between a metastatic renal cell carcinoma of clear cell type and a primary thyroid carcinoma with clear cell change may be difficult, and immunocytochemical staining of aspirated tumor cells with thyroglobulin antibody will be helpful to identify the aforementioned primary thyroid cancer (Figure 1.23). Metastatic cancer to the thyroid is associated with a very poor prognosis with death occurring within 6 months (25).

*Lee WM, Batoroev YK, Odashiro NA, Nguyen GK. Solitary metastatic cancer to the thyroid: a report of 5 cases with fine-needle aspiration. Cytojournal. 2007; 4:5.*
8. **Non-Diagnostic Category.** The lesions in this category are highly diversified and may be any lesions listed in the above seven categories. In this category the FNA yields non-diagnostic or inadequate cellular materials. In one study, cystic thyroid lesions yielded non-diagnostic cell samples at initial FNA in about 50% of cases (12). In the Mayo Clinic experience, repeating the FNA in the cases with initial non-diagnostic needle aspirates revealed diagnostic material in 30 to 80% of cases (12,15). Other investigators found that thyroid re-FNA was of limited value (59). If the re-aspiration is still non-diagnostic, ultrasound-guided FNA should be performed. Ultrasound-guided FNAs yield adequate cytologic materials in about 91% of cases (27-36). Patients with no specific risk factors for thyroid malignancy and a non-diagnostic FNA who refuse a re-biopsy may be managed conservatively. While patients in the high-risk group should have their TNs removed for histologic study, and an increase in nodule volume alone is not a reliable predictor of malignancy, as most solid and benign TNs grow in size (57).

**DIAGNOSTIC ACCURACY AND ERRORS**

In a review of seven large series totaling 18,183 thyroid FNAs, Gharib and Goellner found that the biopsy technique had a sensitivity rate varying from 65 to 98% (mean 83%), and that its specificity rate varied from 72 to 100% (mean 92%) (15). The false-negative rate varied from 1 to 11.5% (mean 5.2%), and the false-positive rate varied from 0 to 7.7% (mean 2.9%) (15). The overall cytodiagnostic accuracy rate of thyroid FNA approached 95% according to some reported series (13).

**ADJUNCTIVE DIAGNOSTIC VALUE OF ANCELLARY TECHNIQUES**

Ultrafast Papanicolaou stain selectively swells the nuclei of papillary thyroid carcinoma, making their nuclear grooves disappear and making the swollen nuclei look like "watery grapes", while this staining method has no effect on nuclei of a follicular adenoma (21). This artifactual change is due to the disorganization of nuclear lamins and permits a
confident distinction between a follicular adenoma and a follicular variant papillary carcinoma (21).

Immunostaining with thyroid peroxidase antibody has been reported to be of value in distinguishing these two lesions, as malignant and benign follicular cells commonly stain negatively and positively with this antibody, respectively (58). All malignant tumors, regardless of histologic types, arising from the thyroid express Thyroid Transcription Factor-1 (TTF-1)*. Tumor cells from follicular epithelial neoplasms react positively with thyroglobulin antibody, except anaplastic carcinoma and medullary carcinoma*. Cells from follicular epithelial carcinomas are negative for CEA and neuroendocrine markers including calcitonin while those of a medullary carcinoma react positively with CAE and neuroendocrine antibodies (58a). Thyroid carcinomas express vimentin and cytokeratins, and HBME-1 and galactin-3 are useful although not completely specific markers for differentiated thyroid carcinomas (58a). Ploidy determination has no value in distinguishing a follicular adenoma from a follicular carcinoma (59-62) and immunostaining for p53, Ki-67 and Bcl-2 has no value in separating benign from malignant Hurthle cell tumors (63).

Genetics-molecular studies have been extensively carried out on tissue samples of different types of thyroid neoplasm since the past decade (64). However, only a few genetics-molecular studies on thyroid cells obtained by FNA have been recently published. Human telomerase reverse transcriptase (hTERT) gene expression, using reverse transcriptase-polymerase chain reaction, has been identified as a promising diagnostic marker in distinguishing benign from malignant tumors in materials obtained by FNA. It was found that 90 and 92.8% of thyroid carcinomas were positive for hTERT while 35 and 61.5% of benign thyroid nodules were positive for hTERT, respectively (65, 66). Among the thyroid tumors with positive hTERT, there were eight of eight papillary, two of two Hurthle cell and three of four follicular carcinomas (65). BRAF point mutation and RET/thyroid PC rearrangements were found in 38% of thyroid PCs and refined the diagnosis of thyroid PC in five of fifteen cell samples that were considered either indeterminate or insufficient by cytology (67,68). No mutation was found in FNAs of follicular adenomas and non-toxic nodular goiters (67). These molecular markers were of adjunctive diagnostic value when the FNA diagnosis of TN was equivocal (65-68). Powerful molecular techniques including microarray analysis and molecular profiling may have a significant role in the future evaluation of TNs, while providing impetus for further insight into the molecular pathogenesis of both benign and malignant TNs (69-72). Moreover, such techniques may allow deeper insight into both loss and gain of function of unidentified genes by examining panels of genes rather than one or a limited number of potential gene candidates. By analysis of cancer gene profiles for a cohort of 62 thyroid samples, Finley et al (69) were able to distinguish between benign and malignant thyroid tumors.

They reported a sensitivity of 91.7% and specificity of 96.2% for the detection of thyroid carcinomas of various types, including thyroid PC and its follicular variant and follicular carcinoma (69). Distinction of benign and malignant thyroid tumors and molecular classification of follicular thyroid tumors by gene profiling suggests that these powerful techniques may have significant diagnostic potential when used with FNA cytology (70, 71). Molecular profiling may also permit the distinction between primary and metastatic malignancies when dealing with multiple suspicious nodules at various sites. Using material retrieved by FNA, Schoedel et al (72), compared loss of heterozygosity (LOH) patterns and demonstrated genetic kinship of multifocal carcinomas in the thyroid and a separate nodule in the lung, supporting a diagnosis of metastatic thyroid carcinoma to the lung rather than an independent lung neoplasm.

At present, techniques such as microarray analysis are limited by the amount of RNA that can be retrieved from a sample, thereby often limiting analysis to surgically resected samples. However, refinement of these techniques may make them applicable to FNA, with extraction of RNA from a cell block from which molecular analysis of FNA material may have significant diagnostic benefit.

**SUMMARY**

FNA is basically used as a screening technique to detect TNs that require a surgical excision and TNs that can be managed conservatively. The key for the success of thyroid FNA consists of an adequate or representative cell sample and expertise in thyroid cytology. The FNA cytologic manifestations of TNs may be classified into 8 working cytodiagnostic groups consisting of a few heterogenous lesions each to facilitate the differential diagnosis: benign colloid nodule, cellular microfollicular lesion, Hurthle cell lesion, primary malignant tumor, cystic lesions, thyroiditis, other lesions and non-diagnostic category. Immunocytochemistry is helpful in tumor typing and recent application of diagnostic molecular techniques to aspirated thyroid cells proved to be useful in separating benign from malignant TNs in several cases of indeterminate lesions.
REFERENCES


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Chapter 2

SALIVARY GLANDS AND OTHER NECK MASSES

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A. SALIVARY GLANDS

The history of salivary gland FNA cytology can be traced back to the 1950s. Zajdel of France and Zajicek of Sweden have initially developed this diagnostic procedure in the early years of the 1950s, and its value in patient care was widely recognized in Europe some 20 years later. In North America, this method of investigation was adopted for patient care in the 1980s, after a long period of reluctance. FNA of salivary gland mass lesions now is practiced worldwide. However, it is still underutilized despite of numerous published papers on this topic. The main reasons are the complexity of the histopathology of salivary gland tumors and their relative rarity, resulting in poor cytohistologic correlations and lack of pathologist's experience in interpreting salivary gland tumor cytology.

INDICATION AND GOAL OF FNA

Enlarged salivary glands and salivary gland mass lesions are targets of FNA for cytologic evaluation. The only relative contraindication for this diagnostic procedure is the presence of a bleeding disorder. The goal of FNA of a salivary gland lesion is to triage the lesion for appropriate treatment into:

1. Normal versus abnormal tissue.
2. Neoplastic versus inflammatory.
3. If neoplastic, epithelial versus non-epithelial with the most specific diagnosis possible.
4. If inflammatory assessment of subtype with the most specific diagnosis possible.

TECHNICAL CONSIDERATIONS

The techniques of FNA of salivary gland mass lesions are similar to those of a thyroid nodule. Usually, a 25-gauge needle is used. Depending on personal preference, FNA can be performed with or without a syringe. Usually anesthesia is not required but topical benzocaine or injected lidocaine is recommended for intraoral lesions. For submandibular lesions the patient should be cautioned that some blood may appear in the mouth and reassured that this will be transient and relieved by rinsing.

For a cystic lesion, the cyst contents must be evacuated first. Note the viscosity and appearance of the fluid. Any residual palpable nodule, if present, should be sampled as
it may contain diagnostic cells. Large volumes of fluid may allow cell block preparation. Small volumes can be smeared or processed as a monolayered preparation (cytospin, Thin Prep, etc.). Both the ethanol-fixed Papanicolaou and air-dried Romanowsky (Diff-Quik, MGG) staining methods are commonly used to stain the obtained cell sample. The use of both stains is complementary and provides more information for cytologic evaluation of salivary gland lesions. Occasionally immunocytochemical staining with selected antibodies may be useful for tumor typing. Mucin stains (mucicarmine, periodic-acid Schiff with prior diastase digestion (PASD)) may at times be useful. Stains for acid-fast bacilli or fungal elements and cultures should be done if a tuberculous or fungal infection is suspected.

ANATOMY, HISTOLOGY AND CYTOLOGY OF NORMAL SALIVARY GLANDS

There are six major salivary glands: two parotid glands, two submandibular glands and two sublingual glands. The parotid gland is a serous gland with Stensen’s excretory duct that is lined by a single layer of columnar epithelium. The parotid gland has a superficial and a deep lobe with facial nerve between the two lobes and a few intraglandular lymph nodes. The submandibular gland is seromucinous with mucous cells predominant and has Wharton’s excretory duct that is also lined by a single layer of columnar epithelial cells. Unlike the parotid glands, the submandibular glands do not contain lymph nodes. The sublingual gland is also seromucinous and opens directly into the oral cavity. There are 500 to 1000 minor salivary glands that are located beneath the oral epithelium and that open directly into the oral cavity.

FNA of normal salivary gland usually reveals a mix of acinar and ductal cells with acinar cells predominant. Acinar cells are cuboidal or pyramidal in shape with vesicular, bland, basally located nuclei and granular or vacuolated cytoplasm. They appear in uniform cell clusters in continuity with intercalated ducts, forming a ductal-acinar complex. Intercalated duct cells are cuboidal in shape and show scant cytoplasm and round, bland nuclei (Figures 2.1 and 2.2). In older people fat is often present and focal oncocytic change may be apparent.

Figure 2.1. Histology of a normal serous salivary gland showing acinar cells in acini separated by a variable amount of benign fat. A few small excretory ducts are also present (HE, x 100)
Figure 2.2. FNA cytology of a normal serous salivary gland showing acinar cells in acinar arrangement and a small excretory duct (Pap, x 250).

**BENIGN MASS LESIONS:**

*Chronic sialadenitis* is most commonly present as a mass lesion of the submandibular gland and may clinically mimic a neoplasm of the salivary gland. FNA in the early phase is variably cellular with mainly small cohesive fragments of ductal cells and a variable amount of acinar epithelium admixed with lymphocytes and plasma cells (Figure 2.3). FNA of longstanding lesions may be hypocellular, consistent with a gland fibrosis and yields only scant fragments of ductal epithelium, some of them may show squamous metaplasia. Inflammatory cells, other than a small amount of crushed lymphoid tissue may not be apparent. Chronic sialadenitis in patients with previous radiation for oral cancer may yield in FNA fragments of ductal epithelium with post radiation change that can mimic a recurrent carcinoma.

Figure 2.3. Chronic sialadenitis showing in FNA a benign large sheet of ductal epithelium and abundant lymphoid cells (Pap, x 250).
Granulomatous inflammations such as sarcoidosis, tuberculosis, and cat scratch disease may cause a salivary gland enlargement, simulating a neoplasm. Sarcoidosis is characterized by clustered epithelioid cells with elongated or “carrot-shaped” nuclei and ill-defined cytoplasm admixed with lymphocytes (Figures 2.4).

In tuberculous sialadenitis, necrotic debris containing acid-fast bacilli and inflammatory cells is commonly found. Langhans giant cells and epithelioid cells may also be observed. In cat scratch disease epithelioid cells, lymphocytes and polymorphonuclear leukocytes can be seen.

Pleomorphic adenoma or mixed tumor accounts for over 75% of all salivary gland tumors (SGT) with 75% arising from the parotid gland, 5-10% from the submandibular gland and 10% from minor salivary glands. The tumor occurs more commonly in adult female patients over 30 years of age, and 75% of them arise from the superficial lobe of the parotid gland. Histologically, pleomorphic adenomas (PA) are circumscribed masses composed of a chaotic mix of cuboidal ductal cells in sheets or small tubules surrounded by spindle or plasmacytoid myoepithelial cells drifting off into a chondromyxoid stroma. Mature cartilage
may occasionally be found (Figure 2.5). Sebaceous or metaplastic squamous or mucinous epithelium may occasionally be focally present.

Figure 2.5. Histology of a pleomorphic adenoma of the parotid (HE, x 200).

In most cases, the FNA cytodiagnosis of PA is straightforward. Aspirates are usually cellular with a mix of thick cohesive clusters of benign glandular epithelial cells, dyshesive ragged groups of plasmacytoid or spindle-shaped cells and oval, plasmacytoid myoepithelial cells with bland nuclei dispersed in mucoid or fibrillar chondromyxoid material. These findings are usually readily identifiable by Papanicolaou stain but Romanovsky stains (Diff-Quik or MGG) make it easier to see the metachromatically stained stroma and facilitates the diagnosis (Figures 2.6 and 2.7).
Figures 2.6. Cytology of a pleomorphic adenoma of the parotid:
A. A thick cluster of benign epithelial cells.
B. Chondromyxoid stroma containing stellate and elongated cells with fibrillary cytoplasmic extensions (A and B: Pap, x 400).
Pitfalls may be encountered in cases of PA. In some cases, the epithelial or fibromyxomatous component predominates the smear pattern. As a rule, a minute amount of mucoid or chondroid material should raise the index of suspicion for a PA, and a repeat FNA should be performed for further evaluation. However, some cases of low-grade mucoepidermoid carcinoma (MEC) or acinic cell carcinoma may have a myxoid matrix that mimics a PA on FNA (Figure 2.8). An FNA with numerous epithelial cell clusters with glandular spaces containing round amorphous and metachromatic material may mimic an adenoid cystic carcinoma (Figure 2.9). An FNA with abundant mucoid material may be mistaken for a low-grade MEC. Of course, the opposite is also true, that malignant tumors may be mistaken for PA. Minor degrees of epithelial atypia, usually rare, may be encountered in a PA and can usually be ignored. However, groups of cells with marked epithelial atypia should be noted as malignant transformation may develop in longstanding tumors (carcinoma ex-pleomorphic adenoma).
Figure 2.9. Adenoid globules in a PA similar growth pattern of an adenoid cystic carcinoma (Pap, x 200).

**Monomorphic adenoma** has several histologic variants such as adenolymphoma or Warthin’s tumor, oxyphilic adenoma or oncocytoma, basal cell adenoma, myoepithelioma and sebaceous adenoma.

**Warthin’s tumor** is the most common monomorphic adenoma of the salivary gland and accounts for 5-10% of all benign SGTs. The tumor predominantly arises from the lower pole of the parotid gland in patients over 60 years of age with a smoking history. It often has a characteristic soft, boggy texture to palpation. It usually undergoes cystic degenerative changes with turbid, rust-colored fluid contents and it is bilateral in 10-15% of cases. Histologically, it consists of oncocytic epithelial cells in solid and glandular pattern with stroma containing abundant lymphocytes often with germinal center formation (Figure 2.10). FNA reveals numerous benign lymphocytes, a variable number of “sheet-like” fragments of oncocyes that have abundant, granular cytoplasm and granular necrotic debris. Degenerated oncocyes or ghost cells may be present in variable number. Rare atypical oncocyes or atypical metaplastic squamous cells may be present and these cells may be mistaken for malignant squamous cells (Figures 2.11).

Figure 2.10. Histology of a parotid Warthin tumor (HE, x 250).
Oncocytoma is a rare salivary gland tumor that presents as a slow-growing, painless, firm well-circumscribed nodule. It is characterised in FNA by abundant oncocytic cells that are predominantly arranged in cohesive monolayered sheets (Figures 2.12). Cystic or inflammatory changes are usually not apparent or minimal.
ESSENTIALS OF HEAD AND NECK CYTOLOGY

Figures 2.12. Histology and cytology of a salivary gland oncocytoma:
A. Tumor tissue section showing benign oncocytes in solid pattern.
B. A cohesive monolayered sheet of oncocytes showing round, monomorphic nuclei.
(A: HE, x 250; B: Diff-Quik, x 400).

Myoepithelioma is a rare tumor consisting exclusively of myoepithelial cells. It is characterised histologically by solid sheets of spindle tumor cells and amorphous granular stroma. In FNA the tumor is characterized by single and clustered spindle cells with scant cytoplasm and elongated nuclei arranged in a nonspecific pattern. There may be an amorphous background material that stains metachromatically with the Diff-Quik technique (Figures 2.13 and 2.14). The tumor cells show nuclear staining for p63 and express S-100 protein.
Basal cell adenoma is a rare neoplasm accounting for about 2% of all salivary gland tumors. The tumor occurs more commonly in adult patients in 6th decade of life and 75% of them arise from the parotid gland. There are two histologic variants: classic and membranous
basal cell adenomas. Both variants have distinctive cytologic manifestations. The tumor cells are cuboidal in shape with round, bland nuclei and scant cytoplasm. They occur in large cohesive masses or sheets. In **classic basal cell adenoma**, a small amount of basement membrane material is present (Figures 2.15).

Figures 2.15. Classic basal cell adenoma a salivary gland. A. Histology of a classic basal cell adenoma (HE, x 200). B and C. The adenoma yields in FNA large and small cohesive groups of small benign tumor cells with round, bland nuclei (Diff-Quik, A x100; B x 400).
The membranous variant is characterized by abundant basement membrane material. It shows in FNA small round tumor cells surrounding round, granular, eosinophilic bodies, mimicking an adenoid cystic carcinoma (Figures 2.16).

![Figures 2.16. Membranous basal cell adenoma: 
A. Histology of membranous basal cell adenoma showing sheets of tumor cells admixed with a large amount of pink basement membrane material (HE, x 200).
B. FNA of the tumor showing cells arranged in acini containing a large amount of basement membrane material, mimicking an adenoid cystic carcinoma (Diff-Quik, x 400).](image)

A sebaceous adenoma yields in FNA cells similar to those of a basal cell adenoma, but many cells with sebaceous differentiation are seen.

Schwannoma is a rare neoplasm arising from facial nerve. FNA of the tumor will cause radiated pain. Histologically, a schwannoma has a biphasic pattern with cellular Antoni A areas consisting of interwoven fascicles of spindle cells with elongated nuclei in palisade forming Verocay bodies and relatively hypocellular Antoni B areas containing spindle cells in a loosely cellular background (Figures 2.17). The two above mentioned components can be identified in tumor needle aspirates (Figures 2.18).
Figures 2.17. Histology of a schwannoma showing an area of Antoni A in figure A and areas of Antoni B and Antoni B in figure B (HE, x 250).
**Pilomatrixoma** is a benign skin adnexal tumor that occurs in the head and neck area (occasionally mimicking a parotid tumor) or the upper extremity usually before the age of 20 years. The growth especially in young children can be rapid raising concern for malignancy. FNA material is usually cellular with a mix of cohesive clusters of undifferentiated or epidermoid basaloid cells, anucleated cells in clusters “ghost cells”, multinucleated giant cells and necrotic debris. Mitosis may be present in undifferentiated basal cells (Figure 2.19).
Figures 2.19. FNA of a pilomatrixoma showing:
A. Undifferentiated small basaloid cells (Pap, x 400).
B. Clusters of small basaloid cells and anucleated cells (ghost cells) (MGG, x 200).
C. Foreign body type of multinucleated giant cell (MGG, x 400).
D. Fragments of anucleated squamous cells (Pap, x 100).
MALIGNANT EPITHELIAL TUMORS

*Mucoepidermoid carcinoma* occurs more commonly in adult males. It is the most common cancer of the salivary glands with 5-10% arising from major glands and 10% from minor glands. Large tumor may undergo cystic degenerative change with mucous contents admixed with inflammatory cells. A mucoepidermoid carcinoma (MEC) may be classified as low or high-grade, depending on the degree of nuclear atypia of the epithelial cells, the extent of mucinous differentiation and the growth pattern (Figures 2.20 and 2.21). Mucus-secreting cells that have abundant, vacuolated cytoplasm and bland nuclei are abundant in low-grade tumors but rare in high-grade tumors. High-grade tumors may resemble non-keratinizing squamous cell carcinoma and mucus cells are rare and difficult to visualize without staining with mucicarmine or PASD. Low-grade tumors have a 5-year survival rate greater than 90% while high-grade tumors have a 5-year survival rate ranging from 20-40%.
Figures 2.20. Low-grade mucoepidermoid carcinoma.
A. Histology of a low-grade MEC with cystic change (HE, x 250).
B. & C. FNA of the tumor showing a sheet of benign appearing squamoid cells in B and two clusters of mucus secreting epithelial cells in C (B & C, Pap, x 400).
D. Thick granular mucus from a low-grade MEC (MGG, x 200).
E. Epidermoid cells and mucous cells (Pap, x 500, oil immersion).
Adenoid cystic carcinoma accounts for 10% of all SGTs and represents a greater percentage of malignant tumors arising in minor salivary glands. The tumor is commonly seen in patients between 40-60 years of age. Histologically, it is composed of a monomorphous population of small tumor cells with scant cytoplasm, so called basalloid cells. Small nucleoli may be present. The tumor cells are arranged in solid sheets, trabeculae or lobules with cystic spaces or cribriform arrangements that contain either eosinophilic basement membrane-like material or mucus (Figure 2.22). FNA from the tumor reveals three-dimensional spherical clusters of tumor cells wrapping around globules of basement membrane-like material with surrounding dispersed naked tumor cell nuclei. Cylindrical cell groups with central matrix cores, solid ball-like cell groups without matrix and irregular sheets of tumor cells with round, empty spaces may be present. Careful examination of sharply marginated cell groups may reveal a thin rim of surrounding basement membrane material (Figures 2.23). Matrix material and globular bodies stain pink with H&E, blue with Papanicolaou
stain and purplish (metachromatic) with the Diff-Quik or MGG method. Importantly, unlike PA, the background does not contain dispersed myoepithelial cells.

Figure 2.22. Histology of an adenoid cystic carcinoma of the parotid (HE, x 200).
Acinic cell carcinoma is a rare tumor and accounts for about 2% of all SGTs. It occurs predominantly in the parotid gland in women in their middle years. It is also occasionally seen in children. The tumor often has a solid growth pattern although papillary, tubular and cystic patterns are not unusual. The tumor cells resemble serous acinar cells, pyramidal in shape with finely vacuolated cytoplasm (Figure 2.24) or shows coarse cytoplasmic granules. FNA from the tumor reveals abundant polygonal cells with variably abundant granular or foamy delicate cytoplasm and round nuclei with fine chromatin, distinct micro or occasionally macro nucleoli. The tumor cells are seen arranged singly or in monolayered sheets often with fine capillaries. There is an absence of ductal epithelial fragments or cells (Figures 2.25 and 2.26). A background of stripped nuclei resembling lymphocytes is commonly noted. These nuclei should be distinguished from true lymphocytes that can also be present. Giemsa stain may reveal coarse intracytoplasmic metachromatic granules that are helpful in making a correct cytodagnosis (Figure 2.27). These granules are PASD positive but mucicarmine negative, a helpful finding for separating an acinic cell carcinoma from a low-grade MEC which is sometimes a problem.
Figures 2.24. Histology of an acinic cell carcinoma of the salivary gland (HE, x 200).

Figures 2.25. An acinic cell carcinoma yields in FNA monolayered sheets of tumor cells with vacuolated cytoplasm in “honeycomb” pattern (Pap, A x 100; B x 400).
Figure 2.26. Dispersed polygonal tumor cells with eccentrically located round nuclei and foamy cytoplasm in FNA of an acinic cell carcinoma (Pap, x 400).

Figure 2.27. Loose aggregate of polygonal tumor cells with eccentrically located round nuclei, foamy microvacuolated cytoplasm and metachromatic intracytoplasmic granules in acinic cell carcinoma (MGG, x 400).

Other carcinomas arising from the salivary glands are very rare and consist of adenocarcinoma, squamous cell carcinoma and undifferentiated carcinomas of large and small cell types. The cytologic manifestations of these cancers are similar to those of the same histologic types arising from other anatomic sites.

OTHER MALIGNANT TUMORS

Malignant mixed tumor has three variants: (1) carcinoma expleomorphic adenoma that is characterised by identifiable benign cellular elements of a pleomorphic adenoma and malignant epithelial cells that are commonly of glandular type, (2) tumor composed of both malignant epithelial and stromal cells, and (3) tumor with characteristics of pleomorphic adenoma but with distant metastases. The FNA of a malignant mixed tumor reflects its characteristic cellular components.
**ESSENTIALS OF HEAD AND NECK CYTOLOGY**

*Malignant nerve sheath tumor* is a rare tumor of the salivary gland. It is characterized histologically by malignant spindle or epithelioid cells arranged in solid pattern (Figure 2.28). It yields in FNA single and loosely clustered malignant cells with well-defined cytoplasm. Tumor cells with elongated “tails” may be seen (Figures 2.29).

Unlike benign neural tumors, S-100 protein is often only weakly positive.

Figure 2.28. Histology of a malignant nerve sheath tumor (HE, x 200).

Figures 2.29. A malignant nerve sheath tumor of the parotid yields in FNA:

A. A loose cluster of spindle cells with well-defined, thick cytoplasm.
B. A few isolated oval tumor cells with some showing cytoplasmic extension or tails.
Embryonal rhabdomyosarcoma is an exceedingly rare tumor arising from salivary glands or oral mucosa. An example of embryonal rhabdomyosarcoma arising from the hard palate showing round tumor cells in syncytial sheets with empty round spaces mimicking an adenoid cystic carcinoma is illustrated below (Figures 2.30). The excised tumor proved to be a rhabdomyosarcoma by immunohistochemistry and electron microscopy.

Figures 2.30. Embryonal rhabdomyosarcoma of hard palate:
A. Histology of the tumor (HE, x 250).
B. Tumor FNA reveals cohesive sheet of malignant round cells (Pap, x 400).

Primary lymphomas of salivary glands are commonly of non-Hodgkin's type. Extranodal lymphoma may arise from a background of salivary gland with autoimmune chronic sialadenitis, usually in middle-aged women with Sjögren syndrome. Lymphoma can rarely arise in the parotid but it is usually part of a more systemic disease.

Metastatic cancers to the salivary glands account for about 8% of all SGTs and...
commonly occur in 4-6 decades of life. Squamous cell carcinoma and melanoma are the most frequent metastatic tumors usually occurring in patients with a prior history of cancer in the head and neck area. Occasionally, metastases are from a lung or kidney cancer. Metastatic cancers should be considered the most likely possibility when the salivary gland tumor FNA reveals a squamous cell carcinoma, melanoma, high-grade adenocarcinoma or small cell carcinoma, although primary tumors of those types do rarely occur.

**TUMOR-LIKE LESIONS**

1. **Sialadenosis** is a rare non-inflammatory lesion that is secondary to secretory dysfunction or metabolic disorders such as diabetes mellitus, thyroid insufficiency, alcoholism, sex hormone change. FNA in this case reveals only normal salivary gland tissue.

2. **Salivary gland duct cyst** usually yields in FNA only benign cyst contents (watery clear fluid, foamy histiocytes, cholesterol crystals and perhaps a few mixed inflammatory cells) (Figure 2.31). Caution should be exercised when the cystic fluid is mucoid, even if hypocellular, as a MEC may be predominantly cystic and mucous cells may resemble histiocytes.

3. **Kuttner’s tumor** or **chronic sclerosing sialadenitis** occurs most commonly in submandibular glands and is seen in patients in 40-50 years of age as a hard usually painless well-circumscribed mass. The lesion may be the end stage of a chronic inflammatory process with fibrosis caused by lithiasis, radiation and disorder of salivary gland secretion.

![Figure 2.31. Non-specific cyst contents showing a few foamy histiocytes and cholesterol clefts (MGG, x 200).](image)
4. **Benign lymphoepithelial lesion** is characterized by a bilateral or unilateral autoimmune chronic inflammation of salivary and lacrimal glands (Figure 2.32). When the lesions are limited to these sites it may be classified as Mikulicz syndrome; if associated with a systemic collagen vascular disease it is classified as Sjögren syndrome. The FNA cytology usually displays features of a follicular lymphadenitis with a polymorphous population of lymphoid cells. Normal salivary gland cells are usually not seen and only remnants of degenerated ductal epithelium are identified.

Patients with Sjögren syndrome are at increased risk for non-Hodgkin lymphoma, and this needs to be considered when examining the FNA material. The term “Mikulicz syndrome” is no longer used to describe enlargement of salivary and lacrimal glands secondary to leukemic, lymphomatous or amyloid infiltration, tuberculous infection or sarcoidosis.

Figures 2.32. Histology (A) and FNA cytology (B) of a lymphoepithelial lesion of the parotid showing in both specimen an admixture of lymphoid cells and epithelial cells (A: HE, x 200; B: Diff-Quik, x 400).
ESSENTIALS OF HEAD AND NECK CYTOLOGY

CYTODIAGNOSTIC ACCURACY OF SALIVARY GLAND TUMORS

Klijanienko et al reviewed 18 large series of salivary gland tumors including their own and found that FNA of SGTs is a safe diagnostic procedure with no risk of tumor implantation along the needle tract, in contrast to open surgical biopsy that may cause extensive tumor spreading. It has a sensitivity varying from 62 to 98%, a specificity varying between 85 and 100% and an accuracy rate within 81 to 97% range. For benign tumors a diagnostic accuracy of 92.5% has been reported. For malignant SGTs a diagnostic accuracy rate of 65.7%, a false-positive rate of 3.3% and a false-negative rate of 11.9% have been reported.

B. CYSTIC LESIONS

Six important cystic lesions of the neck are considered: thyroglossal duct cyst, branchial cleft cyst, thymic cyst, dermoid cyst, metastatic squamous cell carcinoma with cystic degenerative change and metastatic cystic papillary thyroid carcinoma.

1. Thyroglossal duct cyst is a congenital lesion arising from the thyroglossal duct that fails to disappear during the 6th or 7th weeks of fetal life. Most thyroglossal duct cysts are located on the midline of the anterior neck and is connected to the hyoid bone. A few are located laterally and rarely it is located within the thyroid. Most lesions are encountered during childhood or adolescence. It is lined by either a squamous or columnar epithelium and thyroid follicles may be present in the cyst wall (Figure 2.33). Rarely a cancer may develop from a thyroglossal duct cyst, and most of these malignant tumors are thyroid papillary carcinomas. The cyst yields in FNA benign cyst contents containing foamy histiocytes, anucleate squames and squamous cells, similar to the contents of a branchial cleft cyst (Figure 2.34). Thyroid follicular epithelial cells are not seen, in most cases.
2. **Branchial Cleft Cyst** is located lateral to hyoid bone. It is a congenital malformation developed from the branchial cleft (most commonly the second branchial cleft). Most of them are lined by a squamous epithelium and the subepithelium is heavily infiltrated with benign lymphoid cells with or without germinal centers. It shows in FNA anucleated, “ghost” squames and benign squamous cells. Benign lymphocytes may be present. If the cyst is infected abundant polymorphonuclear leukocytes are noted and squamous cells with nuclear atypia may be encountered (Figures 2.35).
3. **Thymic Cyst** arises from remnants of the thymopharyngeal duct that fails to involute. It commonly occurs in the mediastinum and rarely in the neck. When it occurs in the neck, it is most frequently located in the anterior cervical triangle and is readily mistaken for a branchial cleft cyst clinically. It is lined by squamous epithelium and shows remnant of thymic tissue within its wall. The lesion yields in FNA abundant benign squamous cells and anucleated squames. Hassall corpuscles are rarely seen.

4. **Cervical Dermoid and Epidermoid Cysts** are rare lesions that usually occur on the neck midline. The two lesions are lined by squamous epithelium and differentiate from each other by the presence of skin appendages within the wall of the former. It shows in FNA abundant benign squamous cells and anucleated squames, similar to those of the 3 above mentioned cysts.
5. **Metastatic Squamous Cell Carcinoma with cystic degeneration.** The needle aspirate shows acute inflammatory cells, necrotic debris and atypical to frankly malignant squamous cells that are present singly, in clusters and sheets (Figure 2.36). However, about 5% of FNA samples have only benign appearing squamous cells, mimicking the aspirate of an infected branchial cleft cyst; and in patients over 40 years of age there should be a high level of suspicion for metastatic carcinoma. The primary tumor is not infrequently a small, undiagnosed occult cancer arising in the ipsilateral tonsil or base of the tongue that may require specialized diagnostic imaging techniques, blind biopsy or ipsitosilectomy to detect.
6. Metastatic Papillary Thyroid Carcinoma with marked cystic change accounts for about 1% of cases. FNA may reveal only a clear cystic fluid that shows only foamy histiocytes on cytospin preparations. It should be born in mind that epithelial cells in fluid usually round up and develop cytoplasmic vacuoles mimicking foamy histiocytes. Therefore, cell blocks should be prepared from all fluid samples to detect minute tissue fragments that may provide more information for a correct diagnosis. A high index of suspicion is needed with a recommendation for clinical follow-up and repeat sampling if the lesion recurs. For illustrations, the reader is referred to Chapter 1 on Thyroid. It should be kept in mind that salivary gland tumors such as PA, Warthin's tumor, low-grade MEC, acinic cell carcinoma and papillary cystadenoma may show extensive cystic change and mimic a cyst clinically.

C. MENINGIOMA

Primary extracranial meningioma is a rare tumor arising from the arachnoid cap cells located extracranially within the sheaths of nerves or vessels. It may originate from the sinonasal tract and it should be distinguished from an intracranial meningioma with extracranial/extraspinal extension. Clinically, epistaxis, nasal obstruction and facial deformity may be present. Rarely, it appears as a lateral cervical mass lesion. The tumor may range up to 8 cm in greatest dimension with a mean of 3 cm. Histologically it consists of a variety of histologic patterns, most commonly meningotheliomatous, that is characterized by lobules of cells with whorl formation, indistinct cell borders and bland, oval nuclei with delicate chromatin. Intranuclear cytoplasmic inclusions and psammoma bodies are common. The tumor yields in FNA cohesive clusters of meningothelial cells with thin, defined cytoplasm
and oval nuclei with finely granular chromatin and small nucleoli. Psammoma bodies are rarely observed (Figures 2.37).

Figures 2.37. Cervical meningotheliomatous tumor:
A. Histology showing nests of tumor cells with a psammoma body (HE, x 200).
B. Tumor FNA showing a large sheet and a cluster of tumor cells with bland nuclei and thin, ill-defined cytoplasm (Diff-Quik, x 400).

D. CAROTID BODY TUMOR

This neuroendocrine neoplasm is an uncommon and slow-growing lesion and it is the most common paraganglioma of the head and neck. The lesion occurs primarily in adults averaging 40-50 years of age. It presents usually as a lateral neck mass located deeply to the anterior border of the sternocleidomastoid muscle below and mandibular angle, measuring about 4 cm on average. It is rarely bilateral or associated with paraganglioma in other sites. Exceptional tumors are functional causing hypertension that is secondary to catecholamine secretion. Histologically, the neoplasm is highly vascular and consists of 2 types of cells, “chief” and “sustentacular”, arranged in a
characteristic alveolar or Zellballen pattern. The “chief” cells are epithelioid in shape and contain catecholamine-bound neurosecretory granules. The supporting “sustentacular” cells are located at the periphery of the Zellballen and devoid of neurosecretory granules. The tumor yields in FNA pleomorphic cells with ill-defined granular cytoplasm and pleomorphic nuclei similar to cells aspirated from an adrenal pheochromocytoma (Figures 2.38). Nuclei have a finely speckled chromatin and generally lack visible nucleoli. On rare occasions, tumor cells arranged on ball-like cluster or Zellballen are observed. The tumor cell cytoplasm stains positively with neuroendocrine markers such as neuron-specific enolase and chromogranin antibodies. As excision is rarely complete tumor recurrence is common. Tumor recurrence is not an evidence of malignancy that is characterized only by metastasis. Malignant carotid tumors are very rare and indistinguishable from their benign counterparts histologically and cytologically.
Figure 2.38. Carotid body tumor:
A. Histology of the tumor showing nests of tumor cells separated by thin fibrovascular septae (HE, x 200).
B and C. Tumor FNA is cellular and shows ragged syncytial groups of spindle and epithelioid cells traversed by multiple delicate capillaries. The cells have hyperchromatic nuclei and a moderate amount of eosinophilic cytoplasm. Bizarre giant cells with degenerated nuclear change are present (Pap, B x 200 and C x 400).

E. SOFT TISSUE TUMORS

Soft tissue tumors of different types may occur in the head and neck. Benign tumors are more common than the malignant ones. Among the benign tumors lipoma is the commonest. FNA of a lipoma reveals clustered benign fat cells. A Merkel's tumor of the skin consists of round tumor cells with neuroendocrine differentiation. It yields in FNA small, round tumor cells with hyperchromatic nuclei showing nuclear molding and overlapping (Figure 2.39).
ESSENTIALS OF HEAD AND NECK CYTOLOGY

Figure 2.39. Merkel’s tumor yields in FNA clustered round tumor cells with nuclear molding and overlapping (MGG, A x 200 and B x 1000).

F. NASOPHARYNGEAL CARCINOMA

This is a poorly differentiated squamous cell carcinoma that is commonly associated with a marked lymphoid infiltration. It is more common in Asian countries and is related to Epstein-Barr virus infection. The primary tumor is often clinically occult and presents as a metastatic carcinoma to cervical lymph nodes of unknown primary. It shows in FNA undifferentiated malignant cells singly and in clusters. The tumor cells have a variable amount of pale, fragile cytoplasm with large vesicular, elongated or spindle nuclei with conspicuous nucleoli (Figures 3.40). Rare keratinizing tumor cells may be seen. The tumor cells are positive for cytokeratin and Epstein-Barr virus associated nuclear antigens and negative for lymphocyte makers.
Figure 3.40. A cluster of pleomorphic undifferentiated malignant epithelial cells with conspicuous nucleoli in FNA of a cervical lymph node with a metastatic nasopharyngeal carcinoma (Pap, x 400).

B. Tumor cells showing a positive nuclear staining for Ebstein-Barr virus RNA by in-situ hybridization (x 400).

G. PARATHYROID TUMORS

Parathyroid adenoma may present as a lateral neck mass and rarely as an intrathyroid nodule. It may undergo cystic degenerative change with clear, acellular liquid contents with a high level of parathyroid hormone. A parathyroid adenoma is almost always solitary and associated with hyperparathyroidism (nephrolithiasis, osteitis fibrosa cystica and diffuse osteopenia). Histologically, a parathyroid adenoma is usually composed of cells with clear or granular cytoplasm and monomorphic round nuclei (Figure 2.41).

Figure 2.41. Histology of a parathyroid adenoma showing polygonal epithelial cells with round nuclei and granular cytoplasm (HE, x 200).
Parathyroid adenoma usually yields in FNA abundant epithelial cells with scant, ill-defined cytoplasm and oval, bland nuclei arranged in acini and in syncytial sheets with nuclear crowding and overlapping (Figures 2.42). Abundant bare tumor cell nuclei are commonly seen. Intranuclear cytoplasmic inclusions may be present in some tumor cells. The needle aspiration cytology of parathyroid adenoma displays some overlapping features with those of a cellular microfollicular lesion or papillary carcinoma of the thyroid. Therefore, immunocytochemical staining of the tumor cells with parathyroid hormone and thyroglobulin antibodies is essential to differentiate a thyroid follicular lesion from a parathyroid neoplasm.

A parathyroid adenoma may consist of large pleomorphic oncocytic tumor cells with bizarre nuclei (Figures 2.43). Immunocytochemical staining of the aspirated tumor cells with parathyroid hormone and thyroglobulin antibodies will be helpful for tumor typing.
Parathyroid carcinoma is a rare tumor and most patients with parathyroid carcinoma have hyperparathyroidism. Asymptomatic non-functioning parathyroid carcinoma is uncommon. Histologically, a parathyroid carcinoma is usually composed of cells with clear or granular cytoplasm and little variation in nuclear size and shape. Bizarre tumor cells or tumor cells with oncocytic change may be present and mimic cells of an anaplastic carcinoma or oncocytic tumor, respectively. FNA of a parathyroid carcinoma reveals dysesive polygonal cells with enlarged, hyperchromatic nuclei and conspicuous nucleoli. Some tumor cells have a plasmacytoid configuration (Figures 2.44). A positive cytoplasmic staining of the tumor cell cytoplasm with parathyroid hormone antibody will confirm its parathyroid origin. Cases of parathyroid carcinoma showing cells mimicking a parathyroid adenoma or a low-grade thyroid follicular neoplasm has been reported.
Figures 2.44. FNA of a parathyroid carcinoma showing:
A. Dyshesive and loosely clustered polygonal tumor cells with defined, granular cytoplasm and enlarged, hyperchromatic nuclei.
B. A cluster of tumor cells showing strong cytoplasmic reaction to parathyroid hormone antibody. (A: Pap, x 400; B: ABC, x 400).

**SUMMARY**

Cytology of salivary gland tumors is complex. It is possible to predict the exact tumor type with a high accuracy when unequivocal cytologic criteria are present. It should be born in mind that sampling error may lead to misinterpretation as there is an overlapping of histologic and cytologic patterns in several salivary gland neoplasms. However, exact tumor typing is not necessary at preoperative stage as it rarely influences management. When tumor typing is equivocal, it would be helpful to the surgeon if the cytopathologist offers a differential diagnosis with a preference if possible. Parathyroid neoplasms show an overlapping cytologic pattern with thyroid follicular lesions in most cases.
REFERENCES

LYMPH NODES

Fine needle aspiration for cytologic evaluation of enlarged lymph nodes was initially performed in 1911 by Grieg and Gray who identified trypanosomes to support their clinical diagnosis of sleeping sickness. In 1921 Guthrie documented a case of Hodgkin disease correctly diagnosed by FNA and in 1930 Martin reported a large series of FNA of different anatomic sites including lymph nodes. Since then several papers documenting the utility of FNA in diagnosing enlarged lymph nodes have appeared in the literature. Lymph node FNA for cytologic evaluation now is practiced worldwide.

INDICATIONS AND GOALS OF FNA

Enlarged cervical lymph nodes are prime targets of FNA for cytologic evaluation. This diagnostic procedure is a safe, convenient, minimally invasive and economical method of clinical investigation. The goals of lymph node FNA include the identification of:
1. A reactive hyperplasia
2. An infection (bacterial or fungal)
3. A suspected metastatic cancer
4. A Hodgkin disease
5. A transformation of a low- to a higher-grade Non-Hodgkin lymphoma (NHL).

The value of FNA in the cytodiagnosis of NHL is controversial. Most investigators, including the writer, still think that an accurate diagnosis of NHL can only be made by histologic, cell marker and molecular studies of lymph node tissue, as cell marker studies of a small number of tumor cells may yield unreliable information leading to an incorrect tumor typing. However, several recently published studies have reported a high diagnostic accuracy rate of NHL by FNA coupled with cell marker and/or molecular studies. Since enlarged cervical lymph nodes are superficial, therefore they can be easily sampled by excisional or incisional biopsy under local anesthesia for tissue diagnosis. A primary cytodiagnosis of mediastinal, intraabdominal and retroperitoneal NHL by FNA and ancillary techniques is an acceptable only in patients in whom a thoracotomy, mediastinoscopy or laparotomy to obtain tissue for histologic diagnosis are contraindicated. For metastatic cancer, the great advantage is that a lymph node FNA can, not only make a correct diagnosis, but it can avoid an ugly seeding of tumor cells into avascular planes and scarring that may be caused by incisional biopsy.

CONTRAINDICATION AND COMPLICATIONS

Bleeding disorder constitutes the only contraindication for cervical lymph node FNA. Hematoma formation often occurs but it can be controlled by digital compression of
the biopsy site. Focal lymph node infarction is observed in 4% of excised biopsied lymph nodes with prior FNA but it rarely interferes with histologic evaluation of the subsequently excised lesions. A pneumothorax may occur in FNA of a low cervical or supravacuolar lymph node.

TECHNICAL CONSIDERATIONS

FNA of an enlarged lymph node is best performed by non-aspiration technique using a 23- to 25-gauge needle without a syringe. Dermal anesthesia of the overlying skin with 1 ml of xylocain is usually necessary as multiple samples will be taken to secure an adequate cell sample for routine cytologic evaluation and cell marker studies. A smear should be prepared and stained by the Diff-Quik technique to evaluate the FNA adequacy. If an infection is suspected, an additional cell sample should be obtained for bacterial or fungal culture. Usually cell samples are either fixed in 95% ethanol or air-dried for staining by the Papanicolaou method and the Diff-Quik technique, respectively. Additional materials should be obtained for cell block preparation for immunohistochemical studies, if indicated. If a lymphoma is suspected a tissue biopsy with fresh tissue samples saved in RPMI solution, according to usual lymphoma protocols for cell marker and cytogenetics studies.

LYMPH NODE GROUPS AND THEIR COMMON PATHOLOGY

Cervical lymph nodes are clinically divided into 9 groups, as outlined below. Lymphoma may involve any groups, but rarely groups 2 and 4. These nodal groups are more commonly associated with certain pathologic conditions, according to Chen and her associates:

1. Preauricular: metastases from the face skin, scalp and parotid cancers.
2. Postauricular: usually inflammatory conditions of face skin, scalp, external ear or viral infection.
3. Submandibular: metastases from cancers of mouth floor, cheek, submandibular salivary glands, face skin, maxillary sinus and anterior tongue.
4. Submental: metastases from lip, mouth floor and nose cancers.
5. Jugulodigastric: metastases from cancers arising from oropharynx, submandibular salivary glands or tonsils.
6. Mid jugular: metastases from thyroid, larynx or pharyngeal cancers.
7. Lower jugular: metastases from cancers thyroid, larynx or pharynx.
8. Supraclavicular: metastases from cancers arising below the clavicle (lung, breast, esophagus, stomach, ovary and occasionally thyroid).
9. Posterior cervical, lower and upper: inflammatory conditions or metastases from cancers of nasopharynx or face skin, neck or scalp.
NON-NEOPLASTIC LESIONS

Reactive hyperplasia is a common condition that is most often caused by viral infection in children and young adults, and it is less common in adult patients. Cytologically, it is characterized by a polymorphic cell population consisting of lymphoid cells at various stages of transformation. Tangible-body macrophages are usually noted (Figures 3.1). The hyperplastic lymphoid cells express both kappa and lambda light chains. Similar cytologic findings are seen in FNAs from a lymph node with Castleman's disease or toxoplasmosis lymphadenitis, HIV-associated lymphadenopathy and dermatopathic lymphadenitis. In the case of a dermatopathic lymphadenitis, in addition to a polymorphic lymphoid cell population, a few melanin-laden macrophages are seen. A hyperplastic lymph node may consist predominantly of small lymphoid cells. In this situation, cell marker studies, either by flow cytometry or immunocytochemistry should be done to rule out a small-cell NHL.

Figures 3.1. Reactive lymph node showing in FNA numerous lymphoid cells at different stages of maturation and a plasma cell (Diff-Quik, x 400).

Inflammatory/infectious conditions

Sarcoidosis is a systemic granulomatous disease of unknown etiology affecting young and middle aged adults. A variety of tissues are involved but lung and lymph nodes in the neck and mediastinum are commonly affected. It is characterized in FNA by the presence of granulomas containing C-3, V- and boomerang-shaped epithelioid cells, multinucleated giant cells and lymphocytes (Figures 3.2).
Acute bacterial lymphadenitis yields in FNA purulent material and bacterial culture is the best means to identify the causative agent.

Fungal lymphadenitis occurs more commonly in AIDS victims and usually yields in FNA variable findings. Some show only purulent material and others may yield only granulomas or abundant histiocytes admixed with other inflammatory cells. In other cases, fungal elements admixed with inflammatory cells are seen. *Histoplasmosis capsulatum* and *Coccidioides immitis* can be seen in Papanicolaou stained materials (Figures 3.3). Histiocytes may show intracytoplasmic vacuoles containing microorganisms that are referred as “negative images”. These “negative images” are only observed in air-dried and Romanowsky-stained cytologic preparations and they are not seen in ethanol-fixed and Papanicolaou-stained smears. Fungal elements are best demonstrated by Gomori silver (GMS) or periodic acid-Schiff stain.
Figures 3.3: Lymph node with histoplasmosis infection showing in FNA:
A. Numerous foamy histiocytes with vacuolated cytoplasm (negative images) that are only seen in air-dried smear stained with Romanowsky stain (Diff-Quik, 500).
B. Cell block section showing numerous fungi within histiocytes (GMS, x 500).

Cat scratch disease is the most common cause of chronic benign lymphadenopathy in North America and it is self-limited. Enlarged lymph nodes are tender and may be matted together. Early lesions begin with a monocytoid B-cell proliferation followed by necrosis and neutrophilic infiltration forming stellate microabscess. The causative agent bacillus \textit{Bartonella henselae} may be demonstrated by Steiner and Steiner staining method with variable results. Culture is the best way for confirmation of the disease.

\textit{Mycobacterial lymphadenitis}. The lesion is more commonly seen in people from third world countries. In North America mycobacterial lymphadenitis occurs mainly in immunocompromised individuals (eg. AIDS victims). It is characterized cytologically by extensive necrosis. Granulomas with epithelioid histiocytes, multinucleated giant cells of Langhans, neutrophils and intra and extracellular bacilli may be seen. Acid-fast bacilli may be demonstrated by staining the FNA or cell block sections with the Ziehl-Neelsen (ZN) technique (Figures 3.4 and 3.5).
Figures 3.4. Tuberculous lymphadenitis showing in FNA:
A. Large amount of necrotic debris admixed with clustered epithelioid cells and scattered polymorphonuclear leukocytes (Diff-Quik, x 100).
B. A large cluster of epithelioid cells showing elongated, oval or curved nuclei and ill-defined cytoplasm (Diff-Quik, x 400).
Figures 3.5. Tuberculous lymphadenitis:
A. A cohesive cluster of epithelioid cells forming a multinucleated giant cell of Langhans (Diff-Quik, x 400).
B. Clustered epithelioid cells and a multinucleated giant cell of Langhans (Pap, x 400).
C. Numerous acid-fast bacilli are present in a cell block section (ZN, x 1000).

Rosai-Dorfman’s disease is an uncommon disease involving mainly children and adolescents. It is characterized by bilateral, painless cervical lymphadenopathy associated with fever, night sweats and weight loss. In FNA small lymphocytes and histiocytes with emperipolesis (phagocytosis of lymphocytes by histiocytes) are noted (Figure 3.6).

Figure 3.6. FNA of lymph node with Rosai-Dorfman’s disease showing large histiocytic cells with emperipolesis (Diff-Quik, x 400). (Courtesy of Dr. Thomas A. Thomson, Vancouver, BC, Canada).

Kuchiki’s lymphadenitis. The disease is self-limited and occurs primarily in Asian countries. This is a necrotizing lymphadenitis affecting cervical lymph nodes with atypical peripheral lymphocytosis. The FNA of an affected lymph node reveals necrotic debris, phagocytic histiocytes with sharply angulated nuclei and increased number of immunoblasts. Neutrophils are absent.
Infectious mononucleosis is caused by the Epstein-Barr virus and spreads by person-to-person contact. Clinically, it is characterized by fever, malaise, pharyngitis, skin rash, peripheral lymphadenopathy and splenomegaly. Peripheral atypical lymphocytosis and a positive heterophil (Monospot) test are almost always present and diagnostic of the infection. By FNA, numerous immunoblasts, abundant plasmacytoid lymphocytes and plasma cells are present (Figure 3.7). Binucleated immunoblasts may mimic Reed-Sternberg cells and large immunoblasts may be mistaken for cells derived from a large cell lymphoma. In difficult cases, an excisional biopsy of the affected lymph node should be done for histologic diagnosis.

Figure 3.7. Abundant immunoblasts in a case of mononucleosis (Diff-Quik, x 400).

LYMPHOMA

As palpable cervical lymph nodes can be easily biopsied under local anesthesia for histologic evaluation with extensive ancillary studies, it is the opinion of most authorities in lymphoma diagnosis that FNA has severe limitations in making an initial diagnosis of Non-Hodgkin lymphoma (NHL) and that it should not replace tissue biopsy for this purpose. FNA is an acceptable alternative diagnostic procedure for suspected intrathoracic, intraabdominal and retroperitoneal lymphomas in debilitated patients who are unable to undergo a thoracotomy, mediastinoscopy or laparotomy for lymph node biopsy.

Hodgkin’s disease is a common cause of malignant lymphadenopathy. A classic Hodgkin’s lymphoma yields in FNA small lymphocytes, eosinophils and Reed-Sternberg cells, classic and mononuclear variants. Reed-Sternberg cells account for 0.1 to 10% of the total cell population, depending on the disease subtypes, being lowest in lymphocytic-rich type.

Non-Hodgkin Lymphomas are classified into B- and T-cell tumors, according to the recent WHO classification. About 90% of NHLs in the United States are B-cell neoplasms and about 10% are T-cell NHLs while null-cell NHLs are very rare. A NHL
lymphoma is suspected if a monomorphic lymphoid cell population with or without abnormal forms is present in FNA(Figures 3.8 to 3-10). When a NHL is suspected cytologically, the enlarged lymph node should be surgically biopsied for histologic, cell marker and/or molecular studies for a firm lymphoma diagnosis. The reader is referred to Chapter 5 in the author's monograph on “Essentials of Abdominal Fine Needle Aspiration Cytology” for a more comprehensive discussion on cytodiagnosis of NHL.

Figure 3.8. Monomorphic lymphoid cells showing round or convoluted nuclei, conspicuous nuclei and scant cytoplasm are suggestive of a NHL (Pap, x 500).

Figure 3.9. Monomorphic lymphoid cells with round nuclei and conspicuous nucleoli suggesting a NHL (Pap, x 400).
METASTATIC CANCERS

Carcinomas, sarcomas, melanomas arising from any body site may metastasize to cervical lymph nodes. Among these tumors, carcinomas are the most common ones. In most instances, the diagnosis of metastatic tumor to lymph nodes by FNA is straightforward because of the ease in differentiating “alien” cells from lymphocytes. Epithelial cells are usually seen in clusters or sheets with more cellular cohesiveness, as opposed to the dissociated pattern of arrangement of lymphoid cells. Typing of a metastatic cancer to a lymph node is not problematic in a patient with a known primary malignant tumor, as a comparative study of the needle aspirate with the histologic sections of the primary tumor will be useful. However, when a metastatic cancer to a cervical node represents the initial manifestation of a clinically occult cancer, a careful cytologic evaluation of the nodal FNA with or without adjunctive immunocytochemical studies of the aspirated tumor cells may be helpful in localizing the primary tumor. Many metastatic cancers display specific cytologic manifestations permitting a correct tumor typing. Since immunocytochemical findings may be aberrant, interpretation of these staining results should be made in the context of cytomorphology.

Metastatic Squamous Cell Carcinoma. The cytologic manifestations of a well- and poorly differentiated squamous cell carcinoma are different. A metastatic well-differentiated squamous carcinoma yields in FNA, large keratinizing tumor cells with hyperchromatic nuclei, dense eosinophilic or orangeophilic cytoplasm with sharp cytoplasmic border. Many single tumor cells are pleomorphic with polygonal, “tadpole” and fiber configurations and epithelial pearls may be seen. Tumor with extensive necrosis shows necrotic debris admixed with abundant polymorphonuclear leukocytes and rare tumor cells (Figure 3.11).
Figure 3.11. Well-differentiated squamous cell cancer showing in FNA keratinizing tumor cells with "hard" orangeophilic cytoplasm (Pap, A x 400 and B, x 200).

FNA of a poorly differentiated squamous carcinoma reveals non-keratinizing tumor cells that tend to occur in loose clusters or syncytia with crowded, overlapping nuclei. They are less pleomorphic than those of a keratinizing carcinoma and display inconspicuous nucleoli (Figure 3.12).
Cytodiagnosis of a metastatic squamous cell carcinoma is not always straightforward:

1. In some cases of metastatic well-differentiated squamous cell carcinoma, the tumor cells may closely resemble benign squamous cells and the anucleated squames may mimic material from an epidermal inclusion cyst or branchial cleft cyst. The presence of hyperchromatic, atypical nuclei and abnormal cell shaped is helpful for a more accurate diagnosis of a squamous carcinoma.

2. Extensive necrosis in a metastatic squamous cell carcinoma constitutes a diagnostic pitfall. The necrotic debris must be distinguished from that of tuberculous lymphadenitis. In the latter instance, necrotic material is granular and amorphous and may show fibroblasts, lymphocytes and epithelioid cells, whereas in metastatic carcinomas the necrotic material may contain many ghost tumor cells and rare viable tumor cells. A cell block or a repeat FNA is of diagnostic help in this situation (Figure 3.13).
Metastatic Adenocarcinomas display in FNA certain characteristic cell patterns: monolayered sheets; 3-dimensional clusters; papillary and acinar or glandular formations. The individual tumor cells are cuboidal or columnar, with central or basal nuclei, single or multiple prominent nucleoli and pale or vacuolated cytoplasm (Figures 3.14).

Metastasis from a specific site can be recognized in some cases because of certain characteristic cytologic features. Papillary tissue fragments with or without psammoma bodies are most often associated with a thyroid or ovarian carcinoma (Figure 3.15). A metastatic thyroid papillary carcinoma may produce a large amount of thick colloid containing scantly tumor epithelial fragments with nuclear grooves and intranuclear cytoplasmic inclusions.
Columnar cells with basal nuclei in “picket-fence” arrangement or arranged radially around a space are most commonly seen in a metastatic colonic adenocarcinoma. These tumor cells are CK7 negative and CK20 and CDX2 positive (Figures 3.16). Malignant cells distended by a large intracytoplasmic mucinous vacuole with a nucleus displaced to the periphery are typically associated with a gastric linitis plastica.
Cells from a metastatic *conventional renal cell carcinoma* have a clear or granular cytoplasm and are seen singly and in cohesive sheets. They react positively with cytokeratin, vimentin and renal cell carcinoma antibodies (Figures 3.17).

**Metastatic Anaplastic Carcinoma** consists of 2 histologic types: large cell and small cell types. FNA from a *large cell carcinoma* reveals large neoplastic cells with a variable amount of cytoplasm and single or multiple prominent nucleoli. In some cases, multinucleated giant cells and spindled cells are seen (Figure 3.18).
Figure 3.18. Metastatic lung large cell carcinoma showing in FNA single large tumor cells with some cells displaying multiple nuclei with prominent nucleoli (Pap, x 500).

Small Cell Carcinoma, most commonly originating from the lungs, shows in FNA small cells with rounded or angulated nuclei and scanty, ill-defined, basophilic cytoplasm. When the tumor cells are well preserved, the nuclei show “salt and pepper” chromatin and inconspicuous nucleoli. They tend to occur singly and in clusters with nuclear molding. Linear basophilic nuclear debris is frequently present (Figure 3.19). Cells derived from a lung small cell carcinoma are NSE, chromogranin and TTF1 positive.

Figure 3.19. Metastatic small cell carcinoma showing single and clustered tumor cells with nuclear molding and “salt and pepper” chromatin (Pap, x 600).

Metastatic Melanoma usually shows in FNA a mixture of different cell types and there is a distinct tendency for the tumor cells to be dissociated. The identification of intra-cytoplasmic melanin pigment is a useful aid in diagnosis. In most cases, the epithelioid-type cells constitute the major component. They vary from small to large polygonal or plasmacytoid cells with prominent nucleoli, well-defined cytoplasmic borders and variable amounts of granular cytoplasm. The nuclei are eccentrically located and may show intranuclear cytoplasmic inclusions. The spindle-shaped cells have oval or elongated
nuclei with prominent nucleoli and bipolar, slender cytoplasmic processes. The giant tumor cells are moderately pleomorphic large cells with single, double or multiple nuclei and macronucleoli. The cytoplasm is abundant and has a glassy appearance. Intracytoplasmic melanin pigment granules may be observed in routinely stained melanoma cells. Staining of the aspirated tumor cells with S-100 protein, HMB-45 and melan A antibodies will be helpful for tumor typing. Rarely, tumor cell clusters with vascular transgression may mimic tumor tissue fragments aspirated from a thyroid papillary carcinoma (Figures 3.20 to 3.23).

Figures 3.20. Two examples of metastatic melanoma to lymph nodes: A. Loosely clustered melanoma cells with oval, bland nuclei and intracytoplasmic melanin pigment granules (Diff-Quik, x 500). B. Clustered melanoma cells with prominent nucleoli mimicking malignant glandular cells (Pap, x 500).
Figures 3.21. Two examples of metastatic melanoma:
A. Bizarre large single tumor cells.
B. Isolated spindle malignant cells with prominent nucleoli.
(A and B: Pap, x 500).

Figure 3.22. Large tumor cells with plasmacytoid configuration and prominent nucleoli
(Pap, x 500).
Figures 3.23. Metastatic melanoma:
A and B: Tumor cell clusters with vascular transgression mimicking a papillary carcinoma.
C: Tumor cells with a positive cytoplasmic reaction to HMB-45 antibody.
(Pap, A x100, Bx200; ABC, Cx400).
Metastatic nonepithelial cancers yield in FNA dyshesive, pleomorphic or monomorphic non-epithelial tumor cells.

Olfactory neuroblastoma is a rare tumor arising from the olfactory mucosa and may metastasize to cervical lymph nodes. FNA from a metastatic olfactory neuroblastoma usually shows small tumor cells with nonspecific features. However, when a glial fibrillary background is present a metastatic neuroblastoma can be suggested (Figures 3.24). Cells from an olfactory neuroblastoma are negative for epithelial and positive for neuroendocrine markers.

Figures 3.24. Metastatic olfactory neuroblastoma:
A. Histology of the tumor consisting of small tumor cells (HE, x 250).
B. Tumor FNA showing small tumor cells with scant cytoplasm in a glial fibrillary smear background (Pap, x 400).
VALUE OF IMMUNOHISTOCHEMISTRY IN WORKING UP A POORLY OR UNDIFFERENTIATED CARCINOMA OF UNCERTAIN PRIMARY

Immunohistochemical studies can be performed on routinely stained smears without prior destaining with an acetic acid-ethanol solution. However, best results have been obtained with formalin-fixed and paraffin-embedded minute tumor tissue fragments in a cell block prepared from the lymph node FNA. Fixation of tissue fragments in ethanol may result in an over-expression of some cell makers that may mislead immunohistochemical interpretation. Final diagnosis should be made in conjunction with other clinical findings such as biochemical and diagnostic imaging findings.

Since malignant cells aspirated from a poorly differentiated lymphoma may mimic those from a poorly differentiated/undifferentiated melanoma, carcinoma, sarcoma and mesothelioma, staining of the tumor cells with some antibodies, such as leukocyte common antigen, S-100 protein, melan A, HMB-45, AE1/AE3, CAM5.2, calretinin, MOC31 and vimentin antibodies, proves to be useful in classifying them into 5 broad categories: lymphoma cells, melanoma cells, sarcoma cells, mesothelioma cells and carcinoma cells. When carcinoma cells are identified, a coordinate staining of those cells with CK7 and CK20 antibodies will further classify them into different cell lines and additional expressions of other cell markers may further confirm the anatomic sites of the primary carcinomas, according to Dabbs:

1. **CK7+/CK20+ cells may derive from an urothelial or ovarian mucinous carcinoma:**
   - Urothelial tumor cells express uroplakin III (UROIII), P63 and thrombomodulin.
   - Mucinous ovarian carcinoma cells may express WT-1.

2. **CK7+/CK20- cells may derive from a lung, breast, endometrium, endocrine and thyroid carcinoma or a germ cell tumor.**
   - Bronchogenic carcinoma cells express TTF-1 and CEA.
   - Breast carcinoma cells express ER, PR and Gross cystic disease fluid protein fraction 15.
   - Serous ovarian carcinoma cells express WT-1 and Ber-Ep4.
   - Endometrial carcinoma cells express vimentin and ER.
   - Endocrine carcinoma cells express chromogranin, NSE and synaptophysin.
   - Germ cell tumor cells are negative for EMA and positive for alphafetoprotein (endodermal sinus tumor), beta-HCG (choriocarcinoma), CD30 and OCT4 (embryonal carcinoma).
   - Thyroid carcinoma cells are positive for TTF-1 and negative for CEA (exception: medullary carcinoma cells express CEA).
   - Epithelial mesothelioma cells express WT-1, CK5/6, calretinin and mesothelin.

3. **CK7-/CK20- cells may derived from a squamous cell, prostatic or renal cell carcinoma.**
   - Squamous cell carcinoma cells are positive for CK5/6 and P63.
Prostatic carcinoma cells are positive for PSA.
Renal cell carcinoma cells react positively with vimentin and renal cell carcinoma antibodies.

4. CK7-/CK20+ cells may derive from a colorectal or Merkel cell cancer:
- Colorectal carcinoma cells are positive for CDX2, CEA and villi.
- Merkel cell tumor cells express chromogranin and synaptophysin.

DIAGNOSTIC ACCURACY

For lymph node FNA a non-diagnostic/unsatisfactory rate of 5-15% has been reported. In a 10-year review of the literature Volmar et al. found that lymph node FNA for NHL had a modest to high sensitivity rate (66-100%, mostly >80%), high specificity rate (58-100%, mostly >90%) and high diagnostic accuracy rate (50-100%, mostly >85%).

The higher diagnostic sensitivity, specificity and accuracy rates of NHLs were reported in more recent series using advanced ancillary diagnostic methods such as cell marker studies and/or molecular genetics techniques. For Hodgkin disease FNA has a lower sensitivity rate (48-86%) and a higher specificity rate (98-100%). FNA of metastatic cancers has a high sensitivity rate (91-98%), high specificity rate (95-99%) and high overall accuracy rate (94-97%).

SUMMARY

FNA cytology of cervical lymph nodes is highly accurate in identifying metastatic cancers. It can be used to diagnose Hodgkin disease and a recurrent or transformation of a known low-grade to a high-grade NHL. Its value in the primary diagnosis of NHLs is still controversial.
REFERENCES

Chapter 4

INTRACRANIAL TUMORS

Gia-Khanh Nguyen and Edward S. Johnson

Cytologic evaluation of crushed tissue samples from brain lesions was originally used by Cushing in the early years of 1930s to diagnose brain tumors during the course of his operations (1). Since that time, this diagnostic procedure has been sporadically practiced by pathologists as well as neurosurgeons, and has gradually been recognized as a useful adjunct to the frozen section diagnosis of brain tumors (2-6). With the current widespread use of stereotactic brain biopsies to obtain morphologic evidence of deeply located and unresectable brain tumors prior to the institution of appropriate radiotherapy and chemotherapy, the crush preparation diagnostic method has become a routine adjunct to quick tissue diagnosis (7-16). The small size of specimens procured by stereotactic biopsy makes their histologic interpretation by frozen section challenging, and cytologic examination of crush preparations of minute brain tissue fragments that are unsuitable for frozen section preparation, has greatly increased the diagnostic accuracy of most intracranial neoplasms (7-16).

BIOPSY TECHNIQUE, SMEAR PREPARATION AND INTERPRETATION

Deeply located mass lesions of the brain are usually sampled by computed tomography or magnetic resonance imaging-guided stereotatactic biopsy. No general anesthesia is necessary (17). A frame is commonly used to guide a cup forceps, spinal-type needle, side-cutting needle and 14-gauge core-needle (18). At the University of Alberta Hospital, a side-cutting needle and a large syringe are used to aspirate the brain tissue samples. Tissue from the central parts and at the periphery of the mass are sampled. A few cylindrical tissue fragments measuring about 0.1 cm in diameter and up to 1.5 cm in length and several minute tissue fragments measuring less than 0.05 cm in greatest dimension are usually obtained. These tissue fragments are put in normal saline and submitted to the pathology laboratory for frozen section diagnosis. A small tissue fragment measuring about 1 cubic mm excised from a larger tissue fragment or 2 to 3 minute tissue fragments measuring less than 0.5 mm in greatest dimension are gently crushed and smeared between two glass slides. The prepared smears are immediately fixed in 95% ethanol or formalin and stained with hematoxylin and eosin at the same time with the frozen tissue sections (10,11). For firm tissue fragments, touch or imprint

* This chapter is an updated and slightly modified version of the authors' paper on "Value of crush preparation cytology to frozen section diagnosis of brain tumors sampled by stereotactic biopsy. Russian News of Clinical Cytology. 2004; 8: 23". The permission was kindly granted by Pr. Naum Shapiro, Editor-in-Chief of the journal, Moscow, Russia.
preparations are made and stained with hematoxylin and eosin or by the Diff-Quik technique for cytologic evaluation.

Cytologic interpretation of crush preparations of brain lesions is challenging and requires not only a knowledge of brain tumor histopathology and cytology but also clinical information of the patient under investigation. A direct communication with the neurosurgeon who operates on the patient, in difficult cases, will provide the pathologist with useful information to avoid errors in cytologic and histologic interpretations of brain lesions.

DIAGNOSTIC ACCURACY AND DIFFICULTY

The diagnostic accuracy of brain lesions obtained by stereotactic biopsy depends largely on the team efforts of a neurosurgeon who biopsies the brain lesions and the pathologist who examines the tissue and cell samples. In general, the cytodiagnostic efficacy of crush preparations is higher than that of frozen section (84% versus 75%) (12). Reported sensitivity rates of intraoperative cytologic technique varied from 76.6 to 96% (10,11,13,14). A correct cytohistologic correlation is obtained in over 90% of the cases (13). On several occasions a high-grade astrocytoma or oligodendroglioma has been correctly diagnosed by crush preparation alone, as the biopsied tissue fragments are inadequate for frozen section diagnosis (11). Diagnostic difficulties are commonly encountered in cases of low-grade astrocytoma that display a smear pattern mimicking a reactive gliosis (10,11).

NORMAL BRAIN

Normal brain tissue smears smoothly and evenly. The deep white matter of the cerebrum is characterized by a low cellularity, finely granular eosinophilic smear background, thin capillary blood vessels, thicker muscular vessels, scattered astrocytes and oligodendrocytes (11). These cells are easily identifiable as astrocytic nuclei are oval and show a finely granular chromatin and those of oligodendrocytes are smaller, round and hyperchromatic. The cytoplasm of both astrocytes and oligodendrocytes is ill-defined but fibrillar. The cerebral cortex, basal ganglia and hypothalamus are more cellular than the normal white matter and numerous neurons and glial cells are seen. Neurons commonly appear as large oval, naked nuclei with prominent nucleoli. An intact neuron has a triangular configuration and abundant cytoplasm. Corpora amylacea may be seen in material from elderly patients (11). Smears from cerebellar cortex contain numerous cells from internal granular layer admixed with Purkinje cells. The former shows round, small hyperchromatic nuclei and scant, ill-defined cytoplasm, and the later displays abundant cytoplasm with long cytoplasmic processes, large nuclei and prominent nucleoli. The cerebellar subcortical tissue is similar to the white cerebral matter cytologically (Figures 4.1 to 4.3) (11).
Figure 4.1. Normal brain showing neurons, glial cells and thick branching blood vessels (HE, x 100).

Figure 4.2. Normal brain tissue showing 2 large neuron nuclei with prominent nucleoli at right upper corner, larger naked, round nuclei of astrocytes and small round nuclei of oligodendrocytes (HE, x 400).

Figure 4.3. Normal cerebellum showing two Purkinje cells and several naked nuclei of inner layer cells (HE, x 400).
INTRACRANIAL TUMORS

A. Tumors of Neuroglia and Choroid Plexus Epithelium

Astrocytomas account for 75-80% of all intracranial tumors and about 60% of all gliomas (20). Histologically, these tumors may be graded into 4 grades with grade I and grade II tumors being well-differentiated astrocytomas. Grade III tumor is an anaplastic astrocytoma and grade IV tumor or glioblastoma multiforme is the most dedifferentiated tumor of the group (10,11). Grade I and II astrocytomas account for 25-30% and grade III and IV tumors for 50% of all astrocytomas, respectively (20).

In crush preparations, a correct diagnosis of a well-differentiated astrocytoma is difficult. Grade I astrocytomas are seen mainly in children and young adults and have the most favorable prognosis of all astrocytomas. The tumors resemble normal white matter cytologically. Grade II tumors have a diffuse growth pattern and patients with grade II tumors have an average life expectancy of 5 years. Grade II astrocytomas yield a more cellular material and may show slightly thick-walled blood vessels with marginated slightly atypical astrocytes (10,11) (Figures 4.4). Pilocytic astrocytoma is a morphologic variant of grade I astrocytoma. It often occurs in the posterior fossa and is characterized cytologically by the presence of abundant bipolar cells with "hair-like" cytoplasmic extensions, Rosenthal fibers and, less commonly, eosinophilic granular bodies (Figure 4.5).
Figures 4.4. Grade II astrocytoma showing abundant monomorphic tumor cells with scant cytoplasm and oval nuclei marginated around a thin-walled blood vessel. Note the glial granular and fibrillary background (HE, A x 100, B, x 400).

Figure 4.5. Pilocytic astrocytoma showing monomorphic cells with hair-like cytoplasmic extensions and oval nuclei. An irregular, eosinophilic and granular body is noted at the lower right part of the figure (HE, x 400).

*Grade III or anaplastic astrocytoma* is a rapidly growing neoplasm and patients with this tumor have an average life expectancy of 2-3 years. The tumor affects cerebral hemispheres most commonly. It is easily identified cytologically. Smears prepared from the tumor tissue are hypercellular and show abundant pleomorphic malignant cells with many bizarre multinucleated large cells (Figure 4.6). Proliferated, branching thick-walled blood vessels with marginated aggregates of tumor cells are commonly found (10,11).
Figure 4.6. A and B. Anaplastic astrocytoma showing in crush preparation smear pleomorphic malignant cells in a glial fibrillary background (HE, x 400).

*Grade IV astrocytoma or Glioblastoma multiforme* accounts for about 40% of all primary CNS tumors and affects most commonly patients over the age of 40 years. The tumor is highly aggressive and the patient's life expectancy is only 18 months on average. It is characterized by large, pleomorphic, bizarre tumor cells with several multinucleated giant tumor cells, numerous mitotic figures and proliferated vessels (Figure 4.7).
Figures 4.7. Cytology of a glioblastoma multiforme showing in:
A. and B. Pleomorphic tumor cells with the larger ones showing multiple nuclei. A tripolar mitotic figure is seen in B.
C. A proliferated tortuous capillary blood vessel.
(Diff-Quik, A & B, x 1000; C x 400). (Courtesy of Dr. Yuri K. Batoroev, Irskutsk, Russia).
The **gemistocytic astrocytoma** is a high-grade astrocytoma. It shows in crush preparations tumor cells with thick, abundant granular cytoplasm and eccentrically located nuclei (11) (Figure 4.7).

![Figure 4.7. Gemistocytic astrocytoma showing large tumor cells with abundant, granular cytoplasm, cytoplasmic extensions and eccentrically located nuclei in a glial fibrillary background (HE, x 400).](image)

2. **Oligodendrogliomas** account for 5-8% of all intracranial gliomas with a peak incidence in the 4th and 5th decades of life (20). In crush preparations of a low-grade oligodendroglioma abundant rounded tumor cells with slightly pleomorphic nuclei and scant cytoplasm are seen, and tumor cells arranged around round empty spaces may be observed. Perinuclear halo, as seen in tissue sections, may also be observed in cytologic preparations (10,11,19) (Figure 4.8). A high-grade oligodendroglioma shows more pleomorphic cells with variable and wispy cytoplasm (19) (Figure 4.9).

![Figure 4.8. Low-grade oligodendroglioma showing slightly pleomorphic tumor cells with scant cytoplasm arranged around empty spaces in a glial fibrillary background (HE, x 400).](image)
3. **Ependymomas** arise in the ventricular system and account for about 5% of all intracranial gliomas (20). In crush preparation, ependymomas are characterized by cuboidal or columnar epithelial-like cells in monolayered fibrillary sheets or clusters (10,11). A papillary ependymoma with or without myxomatous change occurs almost exclusively in the conus medullaris or around the filum terminale, although rarely in the cerebrum, and is characterized by columnar cells arranged in sheets and clusters in a myxomatous smear background (11) (Figures 4.11).
4. **Medulloblastoma** occurs most commonly in the first decade of life and accounts for about 6% of all intracranial tumors (20). It is most likely arising from cells of the external granular layer of the cerebellum and is located almost exclusively in the cerebellum. It may occur sporadically or may arise in association with Turcot or Gorlin syndrome. In cytologic preparation medulloblastoma is characterized by single and loosely clustered malignant cells with hyperchromatic, pleomorphic nuclei and scanty fibrillary cytoplasm. Tumor cell forming rosettes and nuclear molding may be seen (10,11) (Figure 4.10).

5. **Choroid plexus papillomas and carcinomas** are rare tumors and account for less than 1% of all intracranial gliomas (20). These tumors occur most commonly in the lateral ventricles and are more common in young individuals during the first decade of life (20). In crush preparations, these tumors are characterized by sheets and
tridimensional clusters of oval to cuboidal cells with scant cytoplasm (11) (Figure 4.12). *Choroid plexus carcinomas* are very rare and characterized by single and clustered pleomorphic malignant glandular cells, indistinguishable from those of a metastatic adenocarcinoma (11).

![Figure 4.12. Choroid plexus papilloma showing cuboidal tumor cells with round, bland nuclei predominantly in large tridimensional clusters (HE, x 250).](image)

**B. Metastatic cancers**

Metastatic cancers to the brain account for 15-25% of all intracranial tumors (20). The primary tumor can arise from any anatomic sites with bronchogenic carcinomas being the most common primary cancers, accounting for about 65% of all metastatic cancers to the brain (20). Metastatic tumors to the brain are usually multifocal but may, on rare occasion, be solitary and mimic a primary brain tumor clinically and radiologically. In crush preparations the cytologic manifestations of metastatic cancers resemble those with the same histologic types sampled by FNA (Figures 4.13 A-D).

![Figure 4.13A. Dysesive pleomorphic non-keratinizing squamous cells from a metastatic squamous cell carcinoma (HE, x 400).](image)
Figure 4.13B. A sheet of malignant glandular cells from a metastatic adenocarcinoma (HE, x400).

Figure 4.13C. Metastatic small cell carcinoma showing tumor cells with scant cytoplasm and nuclear molding (HE, x 500).

Figure 4.13D. Dyshesive malignant cells with prominent nucleoli from a metastatic amelanotic melanoma (HE, x 400).
C. Other tumors
1. An intracranial germ-cell tumor, craniopharyngioma, pituitary adenoma, lymphoma and chondroma and chondrosarcoma of the skull base may be mistaken clinically and radiologically with a deeply located glioma. These neoplasms have fairly distinctive cytologic patterns in crush preparations permitting their correct identification in the majority of cases (11). A germ cell tumor is characterized by single and loosely clustered large malignant cells with scant cytoplasm and prominent nucleoli (11) (Figure 4.14).

![Figure 4.14. Germ cell tumor showing isolated tumor cells with ill-defined cytoplasm, large nuclei and prominent nucleoli in a necrotic debris (HE, x 400).](image)

A craniopharyngioma shows in crush preparation fragments of benign squamoid epithelium admixed with cellular debris and squamous pearls (10,11) (Figure 4.15).

![Figure 4.15. A craniopharyngioma showing fragments of benign squamoid epithelium admixed with cellular debris and squamous pearls (HE, x 400).](image)
Figure 4.15. Craniopharyngioma showing large sheets of benign squamous cells (HE, A, x 100 and B, x 250).

A pituitary adenoma shows single cuboidal epithelial cells admixed with naked tumor cell nuclei. No glial fibrillary smear background is noted (17) (Figure 4.16).

Figure 4.16. Pituitary adenoma showing abundant naked, oval tumor cell nuclei and a few cuboidal neoplastic cells with defined cytoplasm (HE, x 400).

A chordoma is characterized by clustered of large, oval cells with intracytoplasmic vacuoles (physallipherous cells) (Figure 4.17).
A chondrosarcoma arising from the skull base is characterized by single and clustered malignant cartilaginous cells with pleomorphic nuclei (Figure 4.18).

2. Meningioma and Schwannoma. Meningiomas accounts for 13-18% and schwannomas arising from the acoustic nerve for about 8% of all intracranial tumors (20). With current diagnostic imaging techniques, a preoperative diagnosis of benign and some malignant meningiomas and schwannoma is possible in the majority of cases. However, in aberrant locations these two tumors may mimic a glioma or a pinealoma clinically or radiologically, and, therefore may be sampled by stereotactic needle biopsy. These tumors show in crush preparations distinctive cytologic features permitting their correct identification in almost all cases. It is important to note that the smears lack a glial fibrillary background, as seen in neuroectodermal tumors (21,22) (Figures 4.19 to 4.24).
Figure 4.19. Meningothelioma showing cells with oval nuclei and abundant, ill-defined cytoplasm in sheets (HE, x 400).

Figure 4.20. Fibromatous meningioma showing loosely clustered “wire-like” cells with elongated nuclei and scant cytoplasm (HE, x 400).

Figure 4.21. Dyshesive plasmacytoid cells from a malignant meningioma (HE, x 400).
Figure 4.22. Imprint preparation from a rhabdoid meningioma showing single and clustered polygonal tumor cells with abundant granular, ill-defined cytoplasm, intracytoplasmic globular body and eccentrically located oval nuclei (Diff-Quik, x 1000). (Courtesy of Dr. Yuri Batoroev, Irkutsk, Russia).

Figure 4.23. Schwannoma showing a thick fragment of tumor tissue with elongated nuclei in vague palisading arrangement (HE, x 400).
DIAGNOSTIC PITFALLS

The majority of commonly encountered intracranial primary tumors display fairly distinctive cytologic features in crush preparations permitting their correct cytologic identification. However, it should be kept in mind that classification of brain tumors is complicated and may require extensive histologic examination with immunohistochemical, electron microscopic and even molecular studies. Common diagnostic pitfalls include:

1. Cells of a medulloblastoma may mimic those of the granular layer of the cerebellum and those of a Non-Hodgkin lymphoma. Cells derived from a Non-Hodgkin lymphoma commonly show nuclear indentation and protrusions that are not usually seen in medulloblastoma cells and possess scant cytoplasm without fibrillary background (11).

2. Pleomorphic malignant cells from a grade IV astrocytoma or glioblastoma multiforme can mimic cells of a metastatic anaplastic carcinoma to the brain (11).

3. Reactive gliosis may occur at the periphery of a neoplastic, inflammatory, vascular or degenerative lesions of the brain including chronic edema (9). Cytologically reactive gliosis is cellular and may show slightly pleomorphic glial cells mimicking a low-grade astrocytoma. The presence of a heterogenous cell population consisting of oligodendrocytes and hypertrophic astrocytes usually indicates a reactive gliosis. Astrocytes with gemistocytic (reactive, hypertrophic) change and inflammatory cells may be present (9) (Figure 4.25).

4. Radiation-induced brain necrosis, cerebral infarct, xanthomatous lesions, sarcoidosis, plasma cell granuloma, collagen vascular diseases and amyloidoma may mimic a brain tumor on CT scan (23). Tissues from these lesions may not smear smoothly and nor show malignant cells. However, reactive glial cells are
commonly found, as well as other acellular or necrotic material. Blood vessels with thick hyalinized walls and hyperplastic endothelial cells may be observed from tissue samples from a radiation-induced necrosis.

Figure 4.25. Reactive gliosis showing a hypercellularity with an admixture of oligodendrocytes and astrocytes (HE, x 400).

REFERENCES


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