Non-gynaecological cytology - Monday to Wednesday 5-7 June 2017

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Cytological material can be exfoliative cytological material or material from fine needle aspirates and is used for screening and diagnostic purposes, to predict outcome or for targeted treatment.

To succeed in the FNA cytology diagnosis all the steps of the procedure should be performed in an optimal way.

**Sampling**

Cells detached spontaneously or scraped, brushed from the surface of organs/lesions, represent exfoliative cytology samples.

Fine needle aspiration (FNA) is used to analyze cells from palpable or visible masses.

Different techniques are used to obtain cells from different anatomic sites. Non-aspiratory sampling can be performed usually on smaller lesion mostly on children. The most often used technique is aspiration sampling by palpation. Non-diagnostic ultrasound guided aspiration sampling is used more and more.

Other type of aspiration samplings are performed together with radiologist under ultrasound or DT guidance, from deeper anatomic sites or bone, intracranial lesions. Under EUS or EBUS guidance mediastinal or intraabdominal lesion may be accessible for FNA.

In these cases usually on site evaluation is important in order to obtain representative material for diagnosis or treatment.

When sampling some specimen-related issues are critical for right diagnosis. One of the most important one is the representativity of the material, which requires right palpation and adequate sampling technique. In case of insufficient material the FNA can be repeated a few times. The quality of material is also important for right interpretation of the sample: ex. bloody samples,
contamination of material with ultrasound gel a.s.o. can make the diagnosis impossible in some cases.

**Preparation**

The obtained material can be prepared in different ways dependent on the clinical question to be answered.

All samples are first cytomorphologically described. Even though different methods can be used for slide preparation, the diagnostic principles are the same.

All cytological material used for morphological diagnosis or immuncytology must be fixated either by different wet fixation techniques or air-dried. The fixation technique and different preparation steps are chosen depending on the purpose of the sampling: ex. for morphological diagnosis air dried May-Grunwald-Giemsa (MGG) stained smears or wet-fixed Papanicolau (PAP) stained smears can be used. Different fixation techniques are used even as pre-treatment before ancillary techniques: ex. immunological analysis in order to define the primary site of a metastasis.

Other fixation methods are used for cells sampled in ThinPrep or cells used for cellblock preparation.

**Analysis**

Cytomorphology is the most important and necessary step in cytology. Even though in the last years more and more molecular analysis are required not only for exact diagnosis but for targeted therapies.

Before submitting the sample for molecular analysis, representativity must be assessed as well as the amount of tumor cells must be documented. The amount of tumor cells is a necessary information for adequate interpretation of the results from different molecular analysis.

Part of the aspirates may be sent for other different analysis like microbiology (ex. bacteria, fungus analysis) or chemistry (ex. PTH…).

Ancillary techniques as immunocytochemistry are based on visualizing different cell component or products by means of monoclonal or polyclonal antibodies and other reagents.

Different materials can be used for immunocytochemistry: ex. Smears, liquid based cytological material or cell blocks.
Sometimes even previously stained slides can be rehybridized with different antibodies.

An important issue is the adequate use of negative and positive controls.

Part of material can be used mostly in hematological malignancies for FACS (flow cytometry analysis) where it can be quantified the expression of several different markers on the same cell.

**Molecular analysis**

Different methods have been developed and used in clinical settings for diagnostic purposes and to predict outcome or to define personalized treatments.

One of the first applied techniques is FISH (fluorescent in situ hybridization) based on specific fluorescent probes that bind to complementary DNA-sites. It can visualize numerical and structural aberrations of chromosomes.

Lately some FISH methods as HER2 amplification detection is based on chromogenic in-situ hybridization(CISH or SISH), when the slides can be evaluated with conventional light microscopes with high magnification.

PCR (polymerase chain reaction) based methods are also used for targeted analysis of different DNA or RNA sequences in order to detect fusion genes, specific mutations, losses or gains of specific DNA sequences, expression of different genes, a.s.o.

NGS (next generation sequencing) is a rapidly emerging technique which is based on targeted massive parallel sequencing. It is much faster than conventional Sanger sequencing. It can give fast results on relative small amounts of aspirate, being a sensitive DNA/RNA analysis method. But be aware of false positives. It creates enormous amounts of data, where it is crucial to have information on tumor cell proportion in the sample in order to select representative changes from the background. It can give information about fusion genes, specific mutations, losses or gains, gene expression, a.s.o.
Fine needle aspiration (FNA) cytology has become a widely used primary method for diagnosis of reactive, and infective lymphadenopathy as well as for lymphoma and metastatic disease. The cytologic interpretation of smears from lymph node aspirates differs in several respects from that of other organs. The cytologic diagnosis of most of solid neoplasms is based on the atypia shown by the tumor cells as compared to their normal counterpart. In contrast low grade non-Hodgkin lymphomas in a majority of cases show little or no cellular atypia and the tumor cells can not with certainty be differentiated from their benign counterparts. Instead the cytologic diagnosis is based on the overrepresentation of one or several cell types in the smear. Obviously such an evaluation can only be made if the spectrum of variation of reactive lymph nodes is fully known. However, even the most experienced cytopathologist cannot reliably diagnose and separate some reactive lymphoid populations from variants of low-grade non-Hodgkin lymphoma on routine smears but also requires ancillary techniques.

In some high grade lymphomas, smears are dominated by blastic cells which may show only mild cellular atypia. Again the lymphoma diagnosis rests on the overrepresentation of the blastic cells as compared to a reactive lesion, but immunophenotyping should always be used for a conclusive diagnosis and subtyping. Not infrequently however the smears from high grade lymphomas show a highly atypical cell population which on routine smears can only be diagnosed as a high-grade malignant tumor n.o.s.

Finally some lymphomas are dominated by benign lymphoid cells, granulocytes or histiocytes. Examples are T-cell rich B-cell lymphomas, variants of follicular lymphomas, nodular lymphocyte predominant Hodgkin lymphoma and some cases of classical Hodgkin lymphoma. A correct diagnosis of these variants rests on the identification and cytologic evaluation of only few tumor cells. An immunological evaluation is necessary to reveal the origin of these tumor cells and a correct antibody panel should be selected on the basis of a tentative cytological diagnosis.
When the morphological assessment of the needle aspiration smear is accompanied by an immunological work up, the diagnostic accuracy is distinctly improved.

This approach has over the last 30 years been used routinely in our laboratory at the Karolinska Hospital. The approach allows the conclusive diagnosis in cases which are difficult to diagnose on cytomorphology alone. Such cases include reactive hyperplasia versus follicle center lymphoma, small cell undifferentiated carcinoma versus high grade malignant lymphoma, small round cell tumours in children versus lymphoma and anaplastic large cell lymphomas versus metastasis of carcinoma and melanoma. In addition the subclassification of most subtypes of non Hodgkin’s lymphoma can be achieved as well as identification of the primary location/site of the lymph node metastases in cases of tumors of unknown origin.

Aspirates from lymph nodes are divided and one part is used for conventional smears and one part is suspended in buffered saline offer an excellent material for an immunological characterization. Routine FNA sampling yields material enough for numerous analyses. Direct smears can be used for immunocytochemistry with nuclear staining but it should not be used for cytoplasmic staining since such material will often have high background staining which can be detrimental to a correct immunological evaluation.

Immunophenotyping can be performed using flow cytometry or immunocytochemistry on cytospin preparations. Flow cytometry is a rapid and accurate technique for immunological characterization of lymphoid cells and is the method of choice in diagnosing most reactive lymphadenopathies as well as low grade lymphomas. However, blastic lymphomas are often fragile and may be difficult to evaluate using flow cytometry. Moreover, Hodgkin lymphomas, anaplastic large cell lymphomas, T-cell rich B-cell lymphomas and non-lymphoid tumors can not be diagnosed using this technique.

Cytospin preparations allow an immunological evaluation of aspirates from both lymphoid and non-lymphoid lesions of various types. In addition, the equipment used for this technique is available to most cytology laboratories. The preparation of cytospin material and immunological staining is however more time consuming, which limits the number of cases that can be processed. In situ hybridisation and in situ amplification techniques are of importance in both diagnosis and subclassification of some lymphomas. Both techniques are readily applicable to cytologic specimens. FNA provides perfect material for analysis by both FISH and PCR techniques.
Technical procedure

Aspirations are performed using 27 to 25 gauge (0.4 to 0.6 mm) needles attached to 10 ml syringes held in a metal pistol as described by Zajicek.

One part of the aspirated material is smeared and stained for morphological analysis by MGG and Papanicolaou technique respectively. A second part of the material is suspended in 1.5 ml PBS and the cell concentration is adjusted to 1-2 million cells/ml. Several cytospin preparations are performed with 60-90 ul of the suspension. Air dried cytospins can be kept at room temperature for up to 5 days.

Immediately before the three step alkaline phosphatase staining is performed the cytospins are fixed in cold acetone. Commercially available monoclonal antibodies are used.

Aspirated cells can also be immunologically characterized by flow cytometry. At the moment eight colour FC is standard in immunophenotyping of lymphomas. Evaluation of scattering light allows elimination of dead cells and granulocytes. Flow cytometry is a rapid and sensitive technique which can detect small abnormal cell populations in a reactive background. Since flow cytometry does not allow an evaluation of cytomorphology it is of importance that the results are correlated to cytomorphology on routinely stained smears. Lymphomas with large cells are often fragile and such cells are destroyed during flow cytometric analysis. A close cooperation between the FC laboratory and the cytopathologist is therefore strongly recommended.

Cytomorphology and immunocytochemical analysis

**Benign lymphadenopathy:** lymph nodes respond to many different agents by enlarging and becoming more active. Depending on the type of stimulus, a node may react with one of three basic histological and cytological patterns: reactive hyperplasia, suppurative lymphadenitis or granulomatous lymphadenitis.

In some cases it is impossible to differentiate reactive follicular hyperplasia from the follicular B-cell non Hodgkin’s lymphoma based only on cytomorphology. Lymphoma of B-cell type is based on demonstrating monoclonality utilising immunoglobulin light chain restriction, either for kappa or lambda (kappa:lambda >6:1 or lambda:kappa ratio 4:1). Polyclonal subpopulations of B-cells composed of a mixture of cells will contain kappa and lambda with a kappa:lambda ratio 2:1. Expression of immunoglobulin light
chains with values intermediate between that acceptable for monoclonality and polyclonality are best regarded as inconclusive for monoclonality. Such expression may be due to atypical lymphoid hyperplasia or partial involvement of the lymph node by lymphoma, and should be reported as suspicious and a repeat FNA is recommended.

**Reactive hyperplasia:** cytologically it is characterized by mixed lymphatic cells with predominance of small mature lymphocytes. If enlarged germinal centres were aspirated numerous centroblasts and centrocytes, mitoses and macrophages containing tingible bodies are seen (fig 1). If there is an expansion of the interfollicular tissue the cytological component can be predominantly composed of lymphoplasmacytoid cells, plasma cells and immunoblasts.

![Fig 1 Reactive hyperplasia](image)

**Suppurative lymphadenitis (non tuberculous mycobacterial):** occurs often in children, in the cervical region, without sign of infection and fever. Cytology shows cell debris, neutrophils, macrophages and few lymphocytes. Some epithelioid cells can be found. There is no need for immunocytochemistry but mycobacterial culture and/or PCR analysis should be performed for identification of the etiological agent and confirming the diagnosis.

**Granulomatous lymphadenitis:** in tropical areas and in patients with immunodeficiency are tuberculous or fungal infection common etiologies. In developed countries sarcoidosis is the most common cause. Cytologically is characterized by granulomas composed of epithelioid histiocytes and
multinucleated giant cells in variable number (fig 2). Necrosis may be found in tuberculosis and fungal infections.

Fig 2. Granulomatous lymphadenitis - sarcoidosis

**Mononucleosis, viral and postvaccinial lymphadenitis:** These conditions cause intense reactivity in the interfollicular tissue which presents with a prominent immunoblastic proliferation in addition to lymphoplasmacytoid cells and plasma cells. Fig 3. The cytology can be mistaken for lymphoma.

Fig 3. Monocleosis lymphadenitis

**Dermatopathic lymphadenopathy:** This is a special variant of reactive lymphadenitis which is observed in patients with chronic skin disorders such as psoriasis or dermatitis. The germinal centres are hyperplastic and the interfollicular tissue is expanded by cells of histiocytic appearance. Smears from
such lymph nodes show numerous small lymphocytes, plasma cells, eosinophils and occasional blast cells. There are numerous histiocyte-like cells, also known as interdigitating reticulum cells, with pale indistinct cytoplasm. Macrophages containing brown melanin pigment from the damaged skin are always present.

**Sinus histiocytosis:** is a very common finding in reactive lymph nodes and often associated with follicular hyperplasia but may also be seen in its absence. Characteristic is dilatation of subcapsular and trabecular sinuses, which are partially or completely filled with histiocytes/macrophages. This type of hyperplasia is observed in lymph nodes which drain areas with cancer as well as inflammatory lesions but in many cases the cause is unknown. The sinus histiocytosis is characterized by mixture dominated by small lymphocytes, some blasts and numerous, sometime multinucleated, macrophages with abundant foamy cytoplasm and round, oval or kidney shaped nuclei.

**Sinus histiocytosis with massive lymphadenopathy:** This is a rare, extreme form of sinus histiocytosis that was first described by Rosai and Dorfman in 1969. The disorder is seen most often in black children and adolescents. Most patients are in good health and develop massive bilateral non-tender enlargement of the cervical lymph nodes followed by fever. Extra nodal involvement has also been described. The cause is unknown but the disorder has a prolonged course and spontaneous regression of the nodes usually takes place. Cytologically there are numerous lymphocytes and large pale histiocytes which have vesicular nuclei with small nucleoli and an abundant vacuolated cytoplasm. The histiocytes often have well preserved lymphocytes in the cytoplasm which is referred to as lymphocytophagocytosis or emperipolesis.

**Histiocytic necrotizing lymphadenitis:** is a rare well defined clinical entity which was first described by Kikuchi and Fujimoto at al. in 1972. It affects chiefly young women presenting with fever and enlargement of one or more cervical nodes. It is a benign, self limiting disease and its aetiology is still unknown. FNA shows numerous foamy macrophages as well as “tingible body” macrophages containing karyorrhectic debris in a background of necrotic material. Small lymphocytes, as well as activated lymphocytes are found. Neutrophils, epitheloid cells and plasma cells, when present, are in few numbers.
**Foreign body granulomas:** Talc, silicone or beryllium can induce massive lymphadenopathy which clinically is impossible to differentiate from metastatic lymph node disease. The aspirated material consists mainly of giant cells containing foreign body particles, together with lymphocytes of mature type and mononuclear histiocytes. Antibodies to vimentin, and epithelial, lymphoid, melanocytic and myogenic differentiation markers should be used to corroborate the diagnosis.

**Hodgkin’s lymphoma:** affects cervical, mediastinal and axillary nodes of teenagers, young adults and elderly. The smears present a mixed cell population of lymphocytes, histiocytes, eosinophils, plasma cells and variable number of large mononuclear tumor cells of Hodgkin type and binucleated Reed Sternberg cells. Immunologically the tumor cells are CD30+, CD15+, MUM1+, Pax5+, CD45-, EMA-.

**Non Hodgkin’s lymphomas:** Most of the non Hodgkin’s lymphomas affect middle aged and elderly person with the exception of the precursor T- and B-cell lymphoma/leukaemia. They present cytologically with a monomorphic cell population and for correct subtyping the immunological study is needed. Fig 4.

Diffuse large B-cell lymphoma consists of large cells predominated by immature lymphoid cells of variable morphology (Fig 5).

![Fig 4. Non Hodgkin’s lymphoma (flow cytometry showed phenotype as mantle cell lymphoma)](image-url)
Fig 5. Diffuse large B-cell lymphoma: centroblastic and multilobated variants

Fig 5. Diffuse large B-cell lymphoma: immunoblastic and anaplastic variants.

<table>
<thead>
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<th>B-cell lymphomas (WHO classification)</th>
<th>immunology</th>
<th>Cytogenet</th>
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<tr>
<td>B-CLL/small cell lymphoma</td>
<td>CD5+, CD23+, CD43+, CD10-</td>
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<tr>
<td>Lymphoplasmacytic/immunocytoma</td>
<td>CD5-, CD43+/-, CD10-</td>
<td></td>
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<tr>
<td>Mantle cell lymphoma</td>
<td>CD5+, CD23-, CD43+, CD10-, SOX11</td>
<td>t (11,14)</td>
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<tr>
<td>Follicular lymphoma</td>
<td>CD5-, CD23-, CD43-, CD10+, Bcl2+</td>
<td>t (14,18)</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>CD5-, CD23-, CD43-, CD10-</td>
<td></td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>CD5-/+ , CD10-/+</td>
<td></td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>CD5-, CD23-, CD10+</td>
<td></td>
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<tr>
<td>Anaplastic large cell (Ki-1)</td>
<td>CD45+, CD30+, CD15-, EMA+</td>
<td>t (2,5)</td>
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<td>T-cell lymphoma</td>
<td>CD2+, CD3+, CD4+, CD5+, CD7+</td>
<td></td>
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<tr>
<td>Langerhans cell histiocytosis</td>
<td>CD1a, CD68, S100, Langerin</td>
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**Metastasis:** in the lymph node the origin of metastasis can be from epithelial tumors, melanoma or sarcomas. The epithelial tumors can be squamous, urothelial, glandular or undifferentiated. Most of the metastatic conditions are possible to diagnose without immunological studies, but for identification of the unknown primary location of the tumor there are several markers available and will help in identifying the most probable origin.

**Metastasis of unknown primary - panels suggestive of**

<table>
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<th>Tumor Type</th>
<th>Markers</th>
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<tr>
<td>Breast carcinoma</td>
<td>CK7+, CK20-, ER+, PR+, GATA3</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>CK7-, CK20-, PSA+, AR+, ERG+</td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>CK7+, CK20+, Uroplakin+, P63+, GATA3</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>CK7-, CK20+, CEA+, Villin+, CDX2+</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>TTF1+, Thyrgl+, CK19+, HBME-1+, PAX8+</td>
</tr>
<tr>
<td>Medullary thyroid carcinoma</td>
<td>Calcitonin+, CEA+, Syn+, Chromog+. TTF-1+</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>TTF1+, Surf A+, Surf B+, CK7+</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>CA125+, ER+/-,PR+/-, PAX8+</td>
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**Metastasis of lymphoma “look alikes”**

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<th>Tumor Type</th>
<th>Markers</th>
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<tbody>
<tr>
<td>Oat cell carcinoma</td>
<td>CK+, CK7-, CK20-, TTF-1+, Syn+, chromogr+</td>
</tr>
<tr>
<td>Merkel cell carcinoma</td>
<td>CK+, CK7-, CK20+, EMA+, Syn+, chromogr+</td>
</tr>
<tr>
<td>Melanoma</td>
<td>CK-, Vim +, SOX10, HMB45+/-, S100++, melanA+</td>
</tr>
<tr>
<td>Ewing/PNET</td>
<td>CK-, Vim+, LCA-, CD99+, NSE+ t(11,22)</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>CK-, Vim+, LCA-, CD99-/-, Desm+, Actin+</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>CK-, Vim-/-, LCA-, CD99-, Desm-, NB84+, NFP+</td>
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The choice of the immunological markers depend on the **cytomorphology and its differential diagnosis**.

The final diagnosis should be always based on a triplet of: 1) clinical findings, 2) cytomorphology and 3) immunocytochemistry.


References (from the Karolinska experience)


Skoog L, Tani E, Svedmyr E, Johansson B: Growth Fraction in non Hodgkin’s


Introduction

The main indication for Fine-needle aspiration (FNA) cytology of the liver is the evaluation of mass-forming lesions, either symptomatic or incidentally discovered. FNA procedure with high diagnostic accuracy and low risk for complications is generally accepted as a first-line diagnostic modality in the examination of primary and metastatic neoplasms in the liver [1-4]. FNA cytology is less useful in evaluating benign liver lesions, especially inflammatory conditions that do not form masses. However, FNAC has been used to examine the condition of hepatic transplants because it can detect acute cellular rejection. Liver FNA is usually performed with radiologic guidance, the on-site presence of the pathologist or cytopathologist allows checking the adequacy of the specimen.

Sampling Technique, Complications, and Diagnostic Accuracy of FNA

Most liver FNAs are performed percutaneously and almost always guided by either ultrasound (US) or computed tomography (CT). Ultrasound offers the advantage of real-time visualization of the needle tip. An endoscopic transesophageal approach may be an option in selected cases for an endoscopic ultrasound (EUS)–FNA of the liver.

Recent studies report good sensitivity of FNA for liver malignancy, ranging from 67 to 100 %, and specificity of 100 % or close to it. False-positive diagnoses are rare and positive predictive values approach 100 % [1-3].

Reasons for false negatives include paucity of tumor cells and failure to recognize tumor cells with subtle malignant features.

Liver FNAC is a safe technique with a low rate of serious complications, mostly bleeding or infection [2-4]. Absolute contraindications for liver FNAC are markedly abnormal coagulation parameters, and relative contraindications include the location of focal lesions in the liver parenchyma that cannot be accessed safely because they are close to large vascular structures.
Normal Elements

Hepatocytes occur in smears as dispersed single cells and small-to-medium-sized (occasionally large) sheets or clusters of round and polygonal cells with abundant granular cytoplasm and low N/C ratio. Hepatocytes contain one or two round to oval regular nuclei with visible nucleoli of variable size and occasional intranuclear pseudoinclusions. Hepatocytes commonly contain intracytoplasmic lipofuscin and occasionally bile pigment (abundant in cases of cholestasis) and less commonly, hemosiderin. The presence of endothelium cells within the sheets of hepatocytes is rare. Other components of normal liver FNA include bile duct epithelium and mesothelial cells. Kupffer cells are rarely seen in FNA of the liver.

Smears showing normal hepatocytes:

- Hepatic adenoma
- Focal nodular hyperplasia
- Regenerative nodules- Cirrhosis

Non-neoplastic Entities

Hepatitis

Acute hepatitis with liver abscesses can be confirmed by FNAC. Abscesses typically yield cellular specimens consisting of neutrophils and histiocytes in a background of necrotic debris.

Chronic hepatitis and its specific active and persistent forms cannot be definitely diagnosed by cytologic examinations.

Alcoholic hepatitis is characterized by variable presence of fibrotic tissue and hepatocytes with fatty change and occasionally with Mallory bodies.

Granulomatous hepatitis presents a broad differential diagnosis, including infectious agents (tuberculosis, parasites) and other causes including sarcoidosis, foreign body reaction, and drug related or autoimmune disease.

Fatty Change

Macrovesicular or microvesicular fatty change is defined as presence of macrovacuoles or microvacuoles within the cytoplasm of hepatocytes. These microscopic findings are nonspecific and may occur in conditions such as alcohol abuse, drug injury, obesity, malnutrition, and diabetes.
Liver Cirrhosis
Smears from cirrhotic liver most often show reactive hepatocytes adjacent to fibrous tissue, bile stasis, and an increased number of duct epithelial cells. Hepatocytes obtained from cirrhotic liver may show reactive atypia and some features that may be confused with hepatocellular carcinoma.

Parasitic Infection
Several parasitic organisms are known to infest liver, and some of them may cause mass-forming lesions. *Echinococcosis (hydatid cyst)* is caused by infestation by the larval stage of animal tapeworms of the genus *Echinococcus*, of either the species *granulosus* (forming unilocular, sometimes large, cystic lesions) or *multilocularis* (forming smaller, multilocular cysts, tumor-simulating masses, or both). Intentional FNAC of a hydatid cyst is generally avoided because of the substantial risk of an anaphylactic reaction; the diagnosis is confirmed serologically. Unintentional FNAC of *E. multilocularis* lesions does happen, however, when the masses resemble malignancy.

Simple Cysts
A simple, *solitary cyst* is most often a congenital unilocular cyst lined by a single layer of cuboidal cells that resembles bile duct epithelium. Aspiration from such cysts comprises fluid containing macrophages and occasionally small clusters of benign epithelial cells. Uncommon *foregut cysts* lined by ciliated epithelium similar to respiratory epithelium contain mucinous fluid.

Extramedullary Hematopoiesis (Myeloid Metaplasia)
In rare patients, mostly those with hematologic diseases, extramedullary hematopoiesis may form focal lesions simulating neoplasia. FNA smears from these lesions contain hematopoietic elements, including megakaryocytes.

Benign Neoplasms

Hemangioma
Hemangioma is the most common benign tumor of the liver. It is usually
asymptomatic and detected incidentally. The imaging characteristic is diagnostic in most patients, so that biopsies are generally considered not necessary and are not done. If sampled, the aspirate comprises blood with occasional endothelial cells and sparse connective tissue fragments.

**Focal Nodular Hyperplasia**
Focal nodular hyperplasia (FNH) is after hemangioma, the second most frequent benign tumor of the liver usually incidentally discovered in patients of both sexes and all ages. FNH is characterized by a central area of fibrosis, which may already have been clearly seen as a central stellate scar in the imaging. On FNAC, the presence of normal hepatocytes and ductal cells differentiates FNH from hepatic adenomas and hepatocellular carcinoma.

**Hepatic Adenoma**
Hepatic adenoma occurs in a non-cirrhotic liver and consists of a solid, benign proliferation of normal hepatocytes, which are not accompanied by portal spaces or ductal structures. Adenomas usually occur in young women in relation to contraceptive hormone use; they also may be related to the use of androgenic-anabolic steroids. Cytologically, normal hepatocytes can be found, with lack of ductal structures.

**Angiomyolipoma**
Angiomyolipoma belongs to the HMB45-expressing group of tumors of perivascular epithelioid cells and may rarely occur in the liver. It consists of a variable admixture of spindled or epithelioid cells showing smooth muscle phenotype with adipose tissue. An aggressive clinical course or malignant transformation is rare.

**Malignant Neoplasms**

**Hepatocellular Carcinoma**
HCC is a most common primary malignancy of the liver, frequently affecting older patients. This tumor typically arises as a solitary mass but may present as a diffuse process or multiple liver nodules mimicking metastasis.
Well-Differentiated HCC

The cytoarchitectural pattern of smears is very helpful to determine whether the aspirate is representative of well-differentiated HCC or benign liver lesions [5,6]. Smears are often hypercellular and tend to maintain cohesive nests and a thick trabecular arrangement with smooth edges and endothelial cells wrapping around the tumor nests and trabeculae. Other components of the smears include tubules, balls, and sheets of tumor cells, as well as isolated cells and naked nuclei. Malignant hepatocytes are rather monomorphic, showing only a slightly increased N/C ratio and nuclei often with distinct macronucleoli. Occasionally, bile-pigment thrombi are visible in smears from HCC.

Cytologic features:

- Hypercellular smears
- Cohesive nests and thick trabeculae with smooth edges
- Endothelial cells wrapping around the tumor nests and trabeculae
- Rather monomorphic hepatocytes with slightly increased N/C ratio
- Often nuclei with distinct macronucleoli

Differential diagnosis:

- Regenerating nodule in cirrhosis
- Focal benign lesions such as FNH or liver-cell adenoma

Moderately and Poorly Differentiated HCC

The main architectural features of a moderately differentiated HCC are similar to the features of better-differentiated tumors, but the proportion of the cell dispersion shifts toward isolated cells and the cells are larger, with stronger pleomorphism and occasional mitotic figures. In poorly differentiated HCC, the cellular pleomorphism is pronounced, with large nucleoli and numerous mitotic figures, including atypical forms. The hepatocellular differentiation can be confirmed immunohistochemically using the HepPar1 antibody [7].

The fibrolamellar variant and clear-cell variant of HCC represent rare subtypes of HCC. The fibrolamellar variant of HCC occurs in noncirrhotic livers of young patients and is generally associated with better prognosis. The clear-cell variant of HCC consists of cells with clear cytoplasm, which results from the loss of glycogen and lipids during processing.

Cholangiocarcinoma
Cholangiocarcinomas constitute approximately 10–20% of primary malignancies of the liver and are, tumors of biliary epithelial origin that are not associated with elevated alpha-fetoprotein (AFP) serum levels. The risk factors for cholangiocarcinoma such as bile stones, inflammatory diseases (e.g., primary sclerosing cholangitis), or parasitic infection are related to chronic bile stasis. Rare multinodular presentation of a cholangiocarcinoma may lead to difficulties in differentiating it from metastatic disease on imaging. Cytologically, cholangiocarcinomas resemble adenocarcinomas of pancreas showing a variety of morphologic subtypes and range of differentiation. FNA smears display a variable number of irregular clusters of cuboid to columnar cells with nuclei varying in size and showing membrane irregularities. Because there are no specific markers, the diagnosis of a primary intrahepatic cholangiocarcinoma is mostly a diagnosis of exclusion.

**Cytologic features:**

- Smears of variable cellularity
- Irregular groups of malignant columnar cells of variable size
- Distortion of the cell-group architecture with loss of honeycomb pattern
- Fine to coarse chromatin and irregularities of nuclear membrane
- Glandular differentiation
- Rare normal hepatocytes

**Differential diagnosis:**

- Metastases of extrahepatic adenocarcinoma
- Hepatocellular carcinoma

**Hepatoblastoma**

Hepatoblastoma is the most common primary tumor of the liver in children younger than 5 years of age in more than 90% of cases associated with marked elevation of AFP serum levels. The tumors consist of variable amounts of epithelial and stromal elements, variably admixed in individual tumors [8].

**Malignant Lymphoma**

Primary hepatic or hepatosplenic lymphoma is much less common than secondary lymphoma manifestations and corresponds most commonly to B-cell
non-Hodgkin lymphomas of a diffuse large-cell type. Post-transplant lymphoproliferative disease (PTLD) may be observed in the context of organ transplantation. On FNAC, the lymphatic nature of the cells is mostly obvious, but ancillary methods such as flow cytometry and immunohistochemistry or molecular methods are necessary for precise subtyping.

**Epithelioid Hemangioendothelioma**

Epithelioid hemangioendothelioma (EH) is an uncommon intermediate malignant tumor with endothelial differentiation which presents often as a multinodular mass in the liver. The cells of EH typically contain erythrocytes in intracytoplasmic lumina and express endothelial markers (CD31, CD34, Flt-1, ERG), express cytokeratins (focally) in approximately 30% of cases.

**Angiosarcoma**

Angiosarcoma is the most common sarcoma type primarily occurring in the liver. It usually shows a highly malignant clinical course. Most hepatic angiosarcomas are idiopathic, but almost one third are reported to be related to exposure to toxic substances such as polyvinyl chloride, thorium dioxide, or arsenic. Microscopically, obviously highly malignant cells with variably abundant cytoplasm and highly pleomorphic nuclei with irregular chromatin can be seen as loose clusters or in isolation. The endothelial differentiation must be proved immunocytochemically (CD31, CD34, Flt-1, ERG). Keratin (+) in significant subset of epithelioid angiosarcoma.

**Other Tumors**

Rare cases of other entities such as Synovial sarcoma, Hepatobiliary rhabdomyosarcoma, Undifferentiated embryonal sarcoma, Follicular dendritic cell sarcoma, leiomyosarcoma, inflammatory myofibroblastic tumor are known to occur as primary tumors in the liver.

**Metastatic Neoplasms**

Metastases are much more common in the liver than primary tumors and accounts for 70-80% of malignant liver tumors. They are almost never found in cirrhotic organs. In many cases, the primary tumor of the suspected metastasis is known and FNAC is performed to confirm the metastatic nature of the liver.
mass. Most metastases correspond to carcinomas, often demonstrating features typical of primary tumors on the background of normal hepatocytes.

Any subtype of lung carcinoma may metastasize to the liver. Metastases from adenocarcinoma of the colon show typically extensive necrosis and palisading of the nuclei in acinar structures. Breast carcinoma commonly metastasizes to the liver. The FNA of the liver may be used in the determination of markers relevant to therapy, such as hormone receptor expression of Her2 gene amplification status. The differentiation of metastatic pancreatic ductal adenocarcinoma and cholangiocarcinoma is nearly impossible without clinical correlation. Carcinomas of virtually any other organ such as the kidney, adrenal, or bladder may spread to the liver and be encountered as a liver mass. Neuroendocrine tumors of the pancreas, intestine, and lung regularly form liver metastases. The FNA slides are cellular and contain numerous relatively monomorphic cells with nuclei with typical coarse chromatin and eccentric, granular eosinophilic cytoplasm.

Less often found to be the origins of metastatic disease are malignant melanomas, including ocular primaries, GISTs, germ cell tumors, sarcomas, and lymphomas.

**Primary HCC versus metastatic adenocarcinoma [9,10]:**

- **AFP:** Positive staining supports a diagnosis of HCC but negative staining does not rule it out
- **HepPar-1:** highly specific for hepatic differentiation but staining positivity decreases with decreasing differentiation
- **Glypican-3:** a specific (96%) marker for hepatic differentiation
- **CEA (polyclonal):** Stains positively up to 80% HCC in a "canalicular" pattern
- **Arginase-1:** Stains HCC with more sensitivity and specificity than HepPar1
- **MOC-31:** adenocarcinoma including cholangiocarcinoma, bladder, renal and neuroendocrine tumors (80-90%); negative in HCC
References


Cytology of the pancreato-biliary system

Anders Hjerpe MD, PhD, Prof em
F49, Department of Clinical Pathology and Cytology, Karolinska University Hospital, SE14186 Huddinge, Sweden
Email: anders.hjerpe@sll.se

This handout is intended as a short introduction to what will be discussed during the lecture and in the workshop.

Introduction

Cytology of the pancreato-biliary tract has become increasingly important for mainly two reasons. The first is the development of new imaging techniques such as ultra sound guidance, which enables fine needle aspiration from defined pathological structures in this region. The second is the possibility to obtain endoscopic brush samples from the biliary and pancreatic ducts, this in combination with the possibility to successfully treat bile duct carcinomas by liver transplantation, improving the 5 year survival from previous 5% to 30-50%. Pancreatic carcinoma, on the other hand, is still associated with a poor prognosis. Curative treatment efforts include major surgery; still the survival rates are in the order of only 12% for the most favourable clinical stages.

Since diagnostic biopsies can be difficult to obtain from these organs, cytology is often the only pathological material on which handling of the patient can be based. With the therapeutic options available for tumours in these organs, the importance of a correct diagnosis cannot be overestimated.

Sampling for bile duct cytology

Cells covering the bile ducts will exfoliate into the bile, which can be collected for cytology. Bile, however, contains bile acids, which are strong detergents that may be deleterious to cell membranes and cell morphology. Bile can be a source for diagnostic material, but this is of inferior quality compared to brush samples obtained at endoscopy (endoscopic retrograde cholangio-pancreatography, ERCP).
Most patients examined in this way have jaundice due to a constriction somewhere in their bile ducts. A particularly important group are those with primary sclerosing cholangitis (PSC) with a high risk to develop malignancy.

When obtaining brush samples, these should be suspended in a LBC fixative without delay. Attempts to make direct air-dried smears often resulted in poorly preserved cells, because of the bile detergents. The use of LBC based techniques improve the cell morphology greatly and allows ancillary analyses to be performed, thereby making cytology a sensitive way of diagnosing these tumours.

**Sampling for pancreatic cytology**

Cytologic sampling is of particular importance in diagnosing pancreatic neoplasms, since coarse needle tissue sampling is associated with significant risks for complications. One way to obtain material for a cytological diagnosis of pancreatic carcinoma is an ultrasound guided fine needle aspiration biopsy obtained during endoscopy (EUS). Since most pancreatic cancers develop from the pancreatic ducts, brush samples can also be obtained during ERCP. The first symptom of a pancreatic carcinoma is often jaundice due to overgrowth and obstruction of the choledocus. In these cases diagnostic cells can sometimes be obtained as a brush sample from the bile ducts. This, however, requires that the tumour penetrates the duct mucosa to allow cells to be collected from the lumen, and the sensitivity is therefore for detecting pancreatic cancer in this way.

**Cytomorphology of pancreato-biliary cancers**

The vast majority of pancreato-biliary tumours constitute various forms of adenocarcinoma or, less commonly, some different endocrine cancers. The cytological criteria are those normally associated with malignancy. The following seven findings are useful to register:

- Architectonical atypia
  - Crowding and overlapping of nuclei, irregular distribution of nuclei within cell groups (“drunken honeycomb”), sometimes forming pseudoglandular acini or rosettes.

- Cellular
  - High nuclear : cytoplasmic ratio, large nuclei
  - Dissociation of atypical cells
• Nuclear
  o Hyperchromasia, polymorphism
  o Atypical nuclear envelopes (coarse and uneven nuclear membrane, "rat bites")
  o Atypical nucleoli (multiple, prominent and polymorphic)
  o Chromatin clumps ("salt & pepper")

When only 1-2 of these criteria can be noticed, the findings are in most cases of benign reactive nature. Such reactive stimulation of the bile duct epithelium may result in a substantial cellular atypia. On the other hand, when 6-7 of the above criteria were present, all cases turned out to be malignant. This leaves a number of inconclusive cases. Today the best way to resolve these inconclusive cases is to do an ancillary analysis of ploidy by FISH[1, 2] (see below).

The less common pancreatic endocrine neoplasms (PEN) occur as high- or low-grade tumours. Their endocrine nature can be shown by immunocytochemistry (CD56, synaptophysin, chromogranin or other hormonal markers). Precancerous lesions such as BiIN, PanIN and intraductal carcinomas are difficult to distinguish from established invasive tumours based on cytology alone. The demonstration of high-grade precancerous epithelial lesions in the bile ducts may, however, be sufficient to motivate even a liver transplantation.

The cytological diagnosis of aspirates from pancreatic cysts is of particular importance. These cysts may represent serous cystadenomas, mucinous cystic neoplasms or pseudocysts. While the serous cysts and pseudocysts are benign conditions, the mucinous ones represent neoplasms with a malignant potential. Of importance for this diagnosis is the finding of mucin, which can be demonstrated histochemically both intra- and extracellularly (PAS-diestas, Alcian Blue). An immunological MUC profile may also help, distinguishing the neoplastic cells (MUC 5+) from contaminations of intestinal mucosa.

**Ploidy analysis by FISH**

The ancillary use of ploidy analyses using the UroVysion FISH kit has increased the sensitivity of bile duct cytology to >80%. Three centromeric probes labels those three chromosomes that are most commonly gained in solid tumours (chromosomes 3, 7 and 17) while a fourth probe set labels the 9p21 band, locus for the p16 gene. The basic algorithm for diagnosing aneuploidy associated with malignancy is either the finding that at least 4 out of 25 nuclei analysed should
present with increased numbers of two centromeric probe signals, alternatively that 12 cells show homozygous deletion of the 9p21 region (loss of both signals). However, a general tetraploidization (3-4 signals for all labelled chromosomes) can be the effect of a reactive proliferation, and should not be considered aneuploid. A noticeable finding is that of general gain (triploidization or polyploidization) in only one gene. This may indicate a clonal proliferation that is remarkable together with cellular atypia, but it is not on its own considered sufficient for a malignant diagnosis.

Take home message

Use ploidy analysis by FISH to verify malignancy whenever you are uncertain of the diagnosis. Have the FISH results evaluated by people with cytology training.

References


**Serous Effusions (Malignant Mesothelioma and Metastases)**

**Anders Hjerpe** MD, PhD, Prof em

F49, Department of Clinical Pathology and Cytology, Karolinska University Hospital, SE14186 Huddinge, Sweden

Email: anders.hjerpe@sll.se

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This handout is intended as a short introduction to what will be discussed during the lecture and in the workshop.

**Introduction**

Effusion cytology is associated with diagnostic challenges. The effusion fluid contains exfoliated cells and is often the first, sometimes the only biological material available for diagnosing a malignant condition in the serous cavities. The cytopathologist’s primary challenge is to recognize a malignant condition and to distinguish this from a reactive mesothelial proliferation. Adjuvant analyses developed during the last decades make it possible also to provide further clinically-relevant information, such as identifying the primary site of the tumour or predicting the best therapeutic regimen.

Once a malignancy is shown, the differential diagnoses include metastatic carcinoma and primary malignant mesothelioma. Metastatic carcinomas are in most centres the most common malignancy of the serous cavities. They are in most cases adenocarcinomas, while small cell lung cancer and squamous carcinomas for some reason are less apt to spread to the serosal cavities. Also other tumours, such as lymphomas, malignant melanomas and others, are occasionally present in the effusion.

**Cytomorphology of malignancy**

On the cellular level the typical diagnostic criteria are the same as for other exfoliative cytology, i.e., polymorphism, hyperchromasia, prominent nucleoli, granular chromatin (“salt and pepper”) and increased nuclear : cytoplasmic ratio. Presence of a malignant condition can be hidden in cases with simultaneous extensive admixture of inflammatory cells, these cells displacing the diagnostic tumour components.
The differentiation from reactive mesothelial proliferation ("mesotheliosis") can be difficult in peritoneal and pericardial fluids. Proliferation in the peritoneal mesothelium is common, particularly in the pelvic region, and these papillary groups tend to develop an epithelial immunophenotype in spite of their benign nature. Such cells, however, have a blend nuclear morphology. Inflammatory pericardial effusions may present a greater diagnostic problem, with prominent cellular atypia. Malignancy must in these cases be verified with caution by ancillary methods using ICC and possibly other techniques such as analysis of ploidy by FISH (see below).

**Metastatic carcinomas**

The presence of cell groups is a particularly important finding in effusions with epithelial malignancy. These groups may show architectonical atypia with uneven distribution of nuclei ("drunken honeycomb"), sometimes forming pseudoglandular structures. Cellular atypia is often prominent.

**Malignant Mesothelioma**

Routine cytology has long been deemed insufficient for an accurate mesothelioma diagnosis. Modern clinical cytology, however, combines basic morphology with information from adjuvant analyses, and a conclusive diagnosis of a mesothelioma can in a majority of cases be based on effusion cytology alone (positive predictive value 100%). International guidelines are now published[1]. The cytology can in this way enable an earlier diagnosis, earlier onset of therapy and improved outcome, and efforts should be made to establish or rule out the diagnosis whenever an effusion is rich in mesothelial-like cells. Sarcomatoid mesotheliomas regularly don’t shed diagnostic cells into the effusion.

The typical findings include cellular atypia as described for metastatic carcinomas and often an extensive richness in papillary cell groups that vary considerable in size. An additional finding is a fine vacuolization of mesothelial cells close to the nucleus, and when located above the nucleus, these vacuoles punch holes through the nucleus. The cell groups show less cohesiveness with gaps ("windows") and sometimes they include metachromatic connective tissue matrix cores. The production of hyaluronan (a polysaccharide tumour marker for malignant mesothelioma) can in the MGG stained specimen be seen as a red or pink granular background and a pink haze outside the cell membrane. This is not seen in the Pap stained slides, since the compound is water soluble and
effectively washed away during staining. The diagnosis of a malignant mesothelioma should always be supported by ancillary analyses such as immunocytochemistry (ICC).

**Sarcomas**

Sarcomatoid tumors rarely exfoliate into the serous cavity, and are hence not diagnosed by cytology. Hematopoetically derived malignant conditions are characterized by extensive dissociation. Chronic lymphocytic leukaemia and malignant lymphoma may present as a lymphocytic effusion that can be difficult to recognize if nucleoli are not obvious. Repeated lymphocytic effusions should motivate further ICC or analysis by FACS.

**Differential diagnoses.**

The main differential diagnosis to a malignant condition is mesothelial changes of reactive nature. ICC can often elucidate this and in case ICC fails ploidy analysis by FACS (see below) is a helpful complement. In case of malignant mesothelioma the challenge is to distinguish this tumour from metastatic adenocarcinoma, which in most cases can be done with ICC.

**Adjuvant analyses**

ICC will in most cases solve diagnostic dilemmas in effusion cytology. Other techniques that can be used include, electron microscopy, fluorescence in-situ hybridization, and biomarker testing in serum or effusion fluid. Genomic analyses are useful in predicting outcomes of targeted therapies for lung cancers. Tumour cells from effusions are excellent for such analyses, today comprising EGFR mutational analysis and translocations of the ALK-1 and ROS-1 genes.

**ICC**

Effusion material can be prepared for ICC either using cell blocks or cytospin. The conditions for ICC are differently optimized depending on preparation technique. ICC may in most cases distinguish malignant from benign and epithelial from mesenchymal, and it is a valuable tool to identify the tumour primary. A large number of immunological reagents are helpful in diagnosing tumour involvement of the serosal cavities. It can therefore be recommended to
do the immunological phenotyping stepwise. Below is the routine used at the Karolinska University Laboratory in Huddinge, Sweden.

1. In highly cellular cases we start with two double stains: Calretinin/BerEp4 and EMA/Desmin. The calretinin indicates mesothelial cells and BerEp4, labelling the EPCAM complex, epithelial/metastatic cells. Desmin will demonstrate benign mesothelial cells, while general reactivity to EMA (should be clone E29, DAKO) indicate malignancy. When unequivocal calretinin+/BerEp4- and EMA+/desmin- this is indicates a mesothelioma.

2. If epithelial malignancy is indicated a second battery is used, selected according to serous cavity and sex:

<table>
<thead>
<tr>
<th>Pleura, pericardium</th>
<th>Man</th>
<th>TTF1/Napsin A (double staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman</td>
<td>TTF1/NapsinA (double staining)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PAX8/Ca125 (double staining)</td>
<td></td>
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<tr>
<td></td>
<td>Estrogen receptor</td>
<td></td>
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<tr>
<td></td>
<td>GATA3</td>
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<td></td>
<td>p53</td>
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</table>

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<tr>
<th>Ascites</th>
<th>Man</th>
<th>Ca19-9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDX2</td>
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<tr>
<td></td>
<td>Cytokeratin 20</td>
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<tr>
<td></td>
<td>Ca19-9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDX2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytokeratin 20</td>
<td></td>
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<tr>
<td></td>
<td>PAX8</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Woman</th>
<th>PAX8/Ca125 (double staining)</th>
</tr>
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<tbody>
<tr>
<td>Ca19-9</td>
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<tr>
<td>CDX2</td>
<td></td>
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<tr>
<td>Cytokeratin 20</td>
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<tr>
<td>Estrogen receptor</td>
<td></td>
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</tr>
<tr>
<td>GATA3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In case epithelial malignancy is probable already based on the first routine cytomorphology this second battery is directly combined with the first step described above. This latter reaction set-up may indicate and distinguish metastases from lung, breast, intestinal tract and ovaries. Note the importance of p53 in combination with ovarian carcinomas (PAX8+/Ca125+), distinguishing high and low grade tumours. Additional antibodies to find the tumour primary can be used as a third step. However, always be observant on anamnestic information on previously diagnosed malignancies.

Optional ancillary analyses
Fluorescent-in-situ-hybridization (FISH) can in many cases be helpful in effusion cytology[2]. The commercial UroVysion kit, containing three centromeric probes to the chromosomes most commonly gained in solid tumors (chromosomes 3, 7 and 17) and one probe for the 9p21 band, locus for the p16 gene. This FISH is an accurate and sensitive way of establishing malignancy. Homozygous deletion of the 9p21 band is a common finding in mesothelioma. Fresh effusions contain many live cells with well-preserved nucleic acids, which make better material for molecular biology analyses than paraffin-embedded samples. FISH analyses of lung adenocarcinoma cells can be used in predictive tests, when demonstrating translocations of the ALK-1 and ROS-1 genes.

Electron microscopy was for long the gold standard for a mesothelioma diagnosis. It can be performed on effusion cell groups and tumour fragments within a cell pellet that is directly fixed in glutar aldehyde. When properly fixed, electron microscopy is another way to establish malignancy, but the draw back is the time needed for handling, often exceeding 2-3 weeks.

ICC makes it possible to distinguish biomarkers attached to the tumour cells. Other diagnostic biomarkers can be found in the effusion supernatant, either as secreted products or as a result of tumour cell decay. Such compounds used diagnostically today are hyaluronan and mesothelin[3, 4]. Hyaluronan (previously named hyaluronic acid) is a polysaccharide synthesized in the cell membrane and secreted. It has a high predictive value for malignant mesothelioma when present in high concentration. Mesothelin is more of a tumour marker with only moderate specificity for mesothelioma, since high levels also can be found in ovarian and pancreatic carcinomas. Today these biomarkers can be combined and measured by ELISA, evaluating the joint results by a logistic model[4].
Take home message

Learn to recognize the highly cellular effusion as a sign of malignancy. Have a liberal attitude towards using ICC. Use FISH-based aneuploidy tests when uncertain of malignancy. Follow up and learn from your cases, particularly when your diagnosis is incongruent with the outcome; remember, the only one who doesn’t make an error is the one who doesn’t do anything at all.

References


Cytology in Salivary Tumors
Jerzy Klijanienko MD PhD, MIAC
Ass. Professor, Institut Curie, 26 rue d’Ulm, 75248 Paris cedex 05, France
Email: jerzy.klijanienko@curie.fr

General consideration

The head and neck area is complex anatomical structure where various tissues and organs are present in a narrow space. This variability gives a large spectrum of different tumors arising from these tissues. Moreover, the presence of a rich blood and lymphatic vascularization associated to the presence of numerous groups of lymph nodes draining different areas, add a supplementary degree of difficulty in the diagnosis of head and neck tumors. Clearly some anatomical sites and related tumors groups may be individualized, but their radiological, clinical and even morphological presentation may overlap. Their sites/groups are head and neck mucosal tumors, skin tumors, orbital tumors, head and neck cavities tumors, salivary tumors, thyroid tumors, enlarged lymph nodes, soft tissue and bone tumors and neck masses. Additionally the distinction between primary and metastatic tumors is additional responsible challenging in both surgical and fine-needle aspiration (FNA) diagnoses.

The group of salivary gland tumors is heterogeneous. Numerous benign and malignant conditions including pseudotumors and metastases constitute its content. The clinico-morphological differential diagnosis among the group of salivary tumors, the discrimination between malignant or being, primary of secondary character may be challenging. In many medical centers without possibility to perform preoperative cytological diagnosis, the solution is peroperative frozen sections analysis knowing that the performance may be low and the decision of type of surgery may be thus delayed.

The FNA technique was introduced in 1954 in our Institut. This allowed us to collect many thousands of salivary gland tumors which were analyzed and diagnosed using FNA. We also dispose of complete surgical and clinical follow-up of these patients. Based on this performance we strongly believe that FNA in salivary tumors may be of great accuracy and clinical utility. Additionally, we have elaborated a simplified cytological classification which makes salivary cytology comprehensive and handful (1).
FNA classification of salivary gland tumors

Smears from salivary tumors are usual cell-rich and stroma-rich. The correct recognition of both, cellular and stromal tumor components is fundamental for accurate tumor diagnosis. To rich the level of high accuracy, the pathologist should use a simplified cytological classification of tumors as previously published. It consists on recognition of:

- myoepithelial,
- oncocyctic,
- basal cell
- squamous,
- mucosecreting,
- acinar cell, and
- non-specific/poorly differentiated cell predominance for carcinomas.

Tumors with myoepithelial cells predominance

Pleomorphic adenoma, adenoid cystic carcinoma, polymorphous low grade adenocarcinoma and epi-myoepithelial carcinoma belong to this group of tumors. Cytology smears are cells-rich and stroma-rich and composed mainly of myoepithelial cells. Pleomorphic adenoma exhibits distinctive morphological characteristics, whereas all three carcinomas may exhibit overlapping patterns. Intra-capsular carcinoma in pleomorphic adenoma (“in situ” carcinoma ex pleomorphic adenoma) is difficult to be detected and is a source of numerous false negative results. The most important clues to the differential diagnosis are summarized in the Table 1.

Table 1. The most important clues to the differential diagnosis of tumors with myoepithelial cells predominance

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmacytoid cells</td>
<td>Characteristic for pleomorphic adenoma excluding other malignancies</td>
</tr>
<tr>
<td>Chondromyxoid stroma or fibrillary matrix with badly delimited margins</td>
<td>Characteristic for pleomorphic adenoma, excluding other malignancies</td>
</tr>
<tr>
<td><strong>Plasmacytoid cells and chondromyxoid stroma or fibrillary matrix with badly delimited margins associated to mucus</strong></td>
<td>Possible pattern in classical pleomorphic adenoma. Exclude a possibility of mucoepidermoid carcinoma arising in pleomorphic adenoma. If this carcinoma remains underdiagnosed there are no clinical consequences to the patients (quasi benign behaviour of “intracapsular” carcinomas).</td>
</tr>
<tr>
<td><strong>Hyaline globules</strong></td>
<td>Hyaline globules are not only suggestive of adenoid cystic carcinoma and are also commonly seen in myoepithelial-predominant neoplasms (polymorphous low grade adenocarcinoma, adenoid cystic carcinoma and epi-myoeptihelial carcinoma) as well as in basaloid tumors. In polymorphous low grade adenocarcinoma the nuclei are larger, rounder and uniform and usually are clarified. Adenoid cystic carcinoma show characteristic tubular and finger-like structures which are absent in polymorphous low grade adenocarcinoma. Epi-myoeptihelial carcinoma, when is well differentiated (clear cell carcinoma), show the presence of double population: darker (ductal) and clarified (myoepithelial) cells. However, they may be occasionally seen in cellular pleomorphic adenoma – source of false positive diagnoses. They are also common in extrasalivary malignancies such as basaloid squamous cell carcinoma and skin appendix tumors.</td>
</tr>
<tr>
<td><strong>Cylindrical structures, finger-like structures, hyaline globules associated to darker cells</strong></td>
<td>Strongly suggestive of adenoid cystic carcinoma</td>
</tr>
<tr>
<td><strong>Elongated and clarified nuclei</strong></td>
<td>Strongly suggestive of polymorphous low grade adenocarcinoma</td>
</tr>
<tr>
<td><strong>Hard palate tumor</strong></td>
<td>Clinically suggestive of polymorphous low grade adenocarcinoma or pleomorphic adenoma</td>
</tr>
<tr>
<td><strong>Patterns of pleomorphic adenoma and signs of sarcoma</strong></td>
<td>Possibility of carcinosarcoma</td>
</tr>
</tbody>
</table>
Patterns of adenoid cystic carcinoma with clarified cells

Possibility of epi-myoepithelial carcinoma

**Tumors with oncocytic cells predominance**

When oncocytic cells are predominant on smears the diagnosis of benign salivary tumor should be rendered. Two benign entities should be investigated: Warthin’s adenoma and oncocytic adenoma. Some malignant entities like poorly differentiated acinic cell carcinoma and mucoepidermoid carcinoma may also show epithelial cells with large cytoplasms mimicking true oncocyes. The most important clues to the differential diagnosis are summarized in the Table 2.

**Table 2.** The most important clues to the differential diagnosis of tumors with oncocytic cells predominance

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncocytic cells</td>
<td>Characteristic for Warthin’s tumor and oncocytic adenoma excluding any malignancy.</td>
</tr>
<tr>
<td>Mast cells within oncocytic clusters</td>
<td>Characteristic for Warthin’s adenoma, but may be also seen in mucoepidermoid carcinoma.</td>
</tr>
<tr>
<td>Abundant lymphocytic background</td>
<td>Characteristic for Warthin’s adenoma. When oncocytic cells are absent, possible misdiagnosis with stimulated lymph node</td>
</tr>
<tr>
<td>Abundant pseudonecrotic background</td>
<td>Characteristic for Warthin’s adenoma. When oncocytic cells are absent, possible misdiagnosis with low-grade mucoepidermoid carcinoma, necrotic squamous cell carcinoma or necrotic adenitis. Some cysts may also exhibit similar morphology.</td>
</tr>
<tr>
<td>Oncocytic cells without lymphocytes or pseudonecrosis</td>
<td>Strongly suggestive of oncocytic adenoma or oncocytosis</td>
</tr>
<tr>
<td>Oncocytic cells and chondromyxoid background</td>
<td>Strongly suggestive of metaplastic pleomorphic adenoma</td>
</tr>
</tbody>
</table>
Tumors with basal cell predominance

This is a heterogeneous group of tumors. Seven different entities belong to this group: basal cell and canalicual adenomas, basal cell adenocarcinoma, as well as solid variant of adenoid cystic carcinoma, neuroendocrine (small cell) salivary carcinoma and extra-salivary basaloid carcinomas. Despite the variability of clinical presentation and variable behaviour, the common point is that these tumors are composed of basaloid, roundish and dark cells. Morphologic resemblance of basaloid tumors necessitates the mutual differential diagnosis. Surprisingly, differentiation of basaloid tumors seems to be relatively easier on cytology smears than on surgical biopsies. In fact, only canalicual and basal cell adenomas are morphologically similar, other entities differ significantly on smears. Extrasalivary basaloid carcinomas, such as basaloid squamous cell carcinoma or basal cell cutaneous carcinoma may be excluded on the basis of clinical presentation (Table 3).

Table 3 The most important clues to the differential diagnosis of basal cell tumors

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cells, narrow cytoplasm, 3-D clusters, palisading, naked nuclei, non specific connective debris</td>
<td>Characteristic for basal cell adenocarcinoma if necrosis and mitotic figures are found. Necrosis and mitotic figures are absent in basal cell or canalicual adenomas.</td>
</tr>
<tr>
<td>Squamous cells</td>
<td>Differentiate with basaloid squamous cell carcinoma of head and neck area or with squamous component of basal cell adenocarcinoma / basal cell adenomas</td>
</tr>
<tr>
<td>Hyaline globules, tubular and finger-like structures</td>
<td>Usual pattern in adenoid cystic carcinoma. Basal cell adenocarcinoma do not show tubular and finger-like structures</td>
</tr>
<tr>
<td>Necrosis, mitotic figures</td>
<td>Sign of malignancy. Predominant in neuroendocrine carcinoma</td>
</tr>
</tbody>
</table>
**Tumors with squamous cell predominance**

All tumors showing squamous predominant cells are malignant. Usually it concerns primary or metastatic squamous cell carcinoma. Occasionally, benign squamous metaplastic cells in pleomorphic adenoma, Warthin’s adenoma or basal cell tumors, may also be detected, but this component is not predominant. Rare squamous cells are present in high-grade mucoepidermoid carcinoma. The most important clues to the differential diagnosis are summarized in the Table 4.

**Table 4.** The most important clues to the differential diagnosis of tumors with squamous cells predominance

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant squamous cells without background mucin</td>
<td>Characteristic squamous cell carcinoma or high-grade mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Malignant squamous cells with background mucin</td>
<td>Characteristic for high-grade mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Cell resembling oncocyes</td>
<td>May be a pattern of high-grade mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Abundant necrotic background</td>
<td>Characteristic for squamous cell carcinoma or high-grade mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Malignant squamous cells without background mucin</td>
<td>Exclusion of primary site on the scalp, eyelid or head and neck area</td>
</tr>
<tr>
<td>Squamous or keratin debris associated with chondromyxoid, myoepithelial or basal cells</td>
<td>Suggestive of metaplastic pleomorphic adenoma, squamous metaplasia in basal cell tumors, squamous metaplasia in Warthin’s tumor.</td>
</tr>
</tbody>
</table>

**Tumors with mucosecretion**

Mucus is common in aspirates from salivary glands tumors. When abundant it is indicative of mucoepidermoid carcinoma. Occasionally, mucus may be seen also in pleomorphic adenoma, Warthin’s adenoma and salivary cysts. Inflammatory conditions may also show mucin. It is important to differentiate mucin from chondromyxoid background and necrosis.
The most important clues to the differential diagnosis of tumors with mucosecretion are summarized in the Table 5.

**Table 5.** The most important clues to the differential diagnosis of tumors with mucosecretion

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant squamous cells with background mucin</td>
<td>Characteristic for mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Intermediated cells with abundant background mucin</td>
<td>Characteristic for mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Oncocytic or clarified cells</td>
<td>May be present in low grade mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Necrotic background with mucin</td>
<td>Differentiate with Warthin’s tumor and Küttner tumor (at submandibular localisation). Possibility of diagnostic error!</td>
</tr>
<tr>
<td>Background mucin and numerous “microvacuolated macrophages”</td>
<td>Possibility of low grade mucoepidermoid carcinoma. True salivary cysts rarely contain macrophages or mucin. Major source of diagnostic error!</td>
</tr>
<tr>
<td>Mucin and pleomorphic adenoma patterns</td>
<td>Two possibilities: mucoepidermoic carcinoma arising in pleomorphic adenoma or pleomorphic adenoma exhibiting mucosecretion. Histological control may be necessary.</td>
</tr>
</tbody>
</table>

**Tumors with acinic cell predominance**

Acic cells are present on smears in three distinctive settings, in sialadenosis, in mammary-analogue salivary carcinoma and in well differentiated acinic cell carcinoma. The most important clues to the differential diagnosis of acinic cell predominance are summarized in the Table 6.
Table 6. The most important clues to the differential diagnosis of tumors with acinic cell predominance

<table>
<thead>
<tr>
<th>Pattern</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated acinic cells</td>
<td>Common pattern for sialadenosis and well differentiated acinic cell carcinoma. Cells are small and clustered in sialadenosis and isolated or clustered in carcinoma.</td>
</tr>
<tr>
<td>Atypical microvacuolated cells</td>
<td>Usually seen in less differentiated acinic cell carcinomas, or in other adenocarcinomas</td>
</tr>
<tr>
<td>Oncocytic or clarified cells</td>
<td>May be present in acinic cell carcinoma</td>
</tr>
<tr>
<td>Numerous naked nuclei</td>
<td>In favour of acinic cell carcinoma</td>
</tr>
<tr>
<td>Bilateral lesion in patients</td>
<td>In favour of sialadenosis</td>
</tr>
<tr>
<td>Pediatric age</td>
<td>In favour of acinic cell carcinoma</td>
</tr>
</tbody>
</table>

**Tumors with non-specific/poorly differentiated cell predominance.**

- Salivary duct carcinoma is a high-grade malignancy related morphologically to mammary ductal carcinoma. This entity represents approximately 3% of all salivary tumors but it is an entity more and more diagnosed.

- Undifferentiated carcinoma is a high grade carcinoma with similar morphology to undifferentiated carcinoma of nasopharyngeal type occurring in EBV endemic areas. At salivary localisation this tumor is relatively common in Greenland.

- Metastatic tumors to the major salivary gland are common. They represent the most frequent salivary malignancy in our Institute. The most common primary sites are malignant melanoma, skin squamous cell carcinoma, breast adenocarcinoma and lung small cell carcinoma. In patients with a history of previous malignant disease, metastastatic tumors are easy on cytology smears. The most important clues to the differential diagnosis of tumors with non-specific/poorly differentiated cell predominance is shown in Table 7.
Table 7 The most important clues to the differential diagnosis of tumors with non-specific/poorly differentiated cell predominance

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Clues</th>
</tr>
</thead>
<tbody>
<tr>
<td>High grade carcinoma pattern</td>
<td>The most pertinent clue is to differentiate between salivary primary high grade malignancy and metastatic tumor, so exclude secondary nature at salivary localisation. The exact diagnosis may be difficult, and a diagnosis of high grade carcinoma is usually sufficient. The presence of squamous cells and mucus-secreting cells are strongly in favour of high-grade mucoepidermoid carcinoma. Some high grade mucoepidermoid carcinomas may be not characteristic and mimic salivary duct carcinoma.</td>
</tr>
<tr>
<td>Oncocytic malignant cells</td>
<td>Differentiate between mucoepidermoid carcinoma, poorly differentiated acinic cell carcinoma, metastatic carcinoma and oncocytic primary salivary carcinoma</td>
</tr>
<tr>
<td>Comedonecrosis</td>
<td>The differential diagnosis may be difficult. Abundant granular cytoplasm, nuclear atypia, coarse chromatin are usually seen in oncocytic carcinoma and necrosis is usually absent or scant. Comedonecrosis is common in salivary duct carcinoma</td>
</tr>
<tr>
<td>Know history of previous malignant disease</td>
<td>Metastatic tumor should always be differentiated from a primary salivary carcinoma</td>
</tr>
</tbody>
</table>

References:

Cytology in Mammary Tumors
Jerzy Klijianenko MD PhD, MIAC
Associate Professor, Institut Curie, 26 rue d’Ulm, 75248 Paris cedex 05, France
Email: jerzy.klijianenko@curie.fr

General consideration
Mammary tumors are common. Clinically, they may be divided into two major groups: palpable and non-palpable tumors. This division is important in the management and choice of diagnostic method. After clinical and radiological examination breast tumor may be sampled using palpation-guided or radio-guided technique. Sampling consists in cytological and/or histological material.

Over the past three decades, detection of non-palpable breast lesions (NPBLs) has significantly increased due to breast cancer screening programs, based on breast imaging. To better standardize breast imaging reports and define patient management, the Breast-Imaging Reporting and Data System (BI-RADS) lexicons of the American College of Radiology (ACR) have been introduced. Each category is defined by a range of positive predictive values (PPV) for cancer. Accordingly, it is recommended that BI-RADS categories 2 and 3, with low PPVs (0% and <2% respectively), only warrant imaging follow-up whereas higher BI-RADS categories (from 4 to 5) warrant pathological evaluation. The number of NPBLs requiring pathological diagnosis has significantly increased as well. Percutaneous imaging-guided sampling techniques have been developed to sample NPBLs, namely fine-needle aspiration (FNA), core-needle biopsy (CNB) and vacuum-assisted biopsy (VAB). Stereotactic, ultrasound (US) or MRI guidance have been successively developed to target NPBLs and coexist nowadays. Patient management was improved using these techniques and avoided unnecessary surgical biopsies for benign lesions.

Regarding FNA, stereotactic-guidance has been demonstrated to show inadequate diagnostic accuracy and is therefore not recommended. US-guided FNA (USFNA) is a valuable procedure because it is rapid, economical, simple and safe in comparison to CNB and VAB. Several studies have assessed its diagnostic performance, and found variable results.

Our institution has a long experience in breast cytology dating back to its early development by Prof. Antoine Zajdela in the mid-20th century (1). Since 1992, we introduced USFNA as a diagnostic tool for NPBLs. One particular feature of USFNA in our institution is the “four-hand procedure” where the radiologist
selects the lesion and keeps track of the target with the US probe while the pathologist performs the aspiration. Improved diagnostic performance has been reported when experienced pathologists are involved in the sampling procedure; yet, this has not been analyzed in a large study. From our experience, we also think that this “four-hand procedure” further improves the diagnostic performance of USFNA.

FNA was demonstrated as very powerful and accurate technique. Using USFNA in 2601 histologically verified mammary non-palpable tumors, we have obtained excellent results where sensitivity was 62.6, specificity was 96.8 and inadequate rate was 0.9% (2). For palpable lesions the results are similar (3,4).

The choice when sampling should consist on cytology and when should consist on histology is clearly codified. Initial and unique tumors should always be histologically verified before any therapeutic investigation. Multiple, recurrent or metastatic lesions should be sampled by cytology. Cell block may also be performed for immunohistochemistry.

Moreover, we believe, that association of cytology with histology should be performed in all cases, because FNA has superior or equal accuracy comparing to core-needle biopsy in many cases. This was observed, whatever tumor, size in our and others authors experience. Combination of both tests significantly minimizes missing cases as false negative (4).

The combination of sampling practice in the same clinical consultation has a positive impact on sample accuracy. Core needle biopsy may be performed in the same consultation, immediately after FNA. Paraffin-embedded material should be used for accurate histological diagnosis and immunohistochemistry.

The practice of cell block is recommended in metastatic, recurrent or “clinically difficult” tumors. Usually, the quality of immunostaining is of the same quality that in core-needle biopsies.

Cytological and histological material may be also used for molecular diagnosis and therapeutic strategies.

Finally, the knowledge of N status is helpful in the choice of treatment. FNA of sentinel lymph node is of great accuracy and allows upgrading the TNM staging in a large number of patients.

In conclusion, FNA of breast lesions is a powerful diagnostic technique in experienced hands. This practice may be completed by histological sampling, in the same clinical consultation. This dual technique seems to be the optimal clinical diagnostic method in patients with breast lesions.
References:


Thyroid Fine Needle Aspiration

Beatrix Cochand-Priollet, MD, PhD

Department of Pathology, Cochin Hospital, University Paris Centre Sorbonne, France.

Email: beatrix.cochand-priollet@aphp.fr

Thyroid FNA is a long story now; the first report dates from 1944: 70 years ago. But the principles and the morphological criteria for thyroid FNA have been published during the 1970th by some Swedish cytopathologists. During the next decade famous cytopathologists from the USA, Sweden and Australia published a lot about this subject and have shown how much thyroid FNA may be useful and safe. In France during the same period thyroid FNA has been introduced by J Zajicek.

Thyroid nodules are common (7% of the population) but are malignant in only 5-10% of cases. International experts agree that a preliminary cytological examination by fine needle aspiration (FNA) is the most efficient test to detect cancer. As it is a screening test, its sensitivity is high -usually more than 90%- but its specificity is lower -usually 50 to 65%- (1) So during 25 years everyone was satisfied with thyroid FNA results which allowed to avoid many unnecessary surgical controls. But 10 years ago this technique appeared as unsatisfying; the clinicians asked for more specific diagnoses; assessment of risk malignancy and diagnosis linked with patient’s management.

They asked for that because our results were inadequate : of course we answered all benign or malignant but for the « indeterminate » cases our terms were not similar and the prognosis for the patients remained unclear. Some official terminologies were already published but the terms were still different allowing no comparison for the « indeterminate cases »; moreover these terminologies were often used in the USA but not in Europe where « individual classifications were used »; The aim of this lecture will be 1) to present the different available terminologies 2) to describe shortly the cytomorphological features observed on thyroid FNAs; 3) and finally to introduce the ancillary techniques that may help to increase the diagnostic accuracy of our cytological diagnosis.

Concerning the terminologies it appeared at the beginning of the 21st century that a harmonization of the practices, performed for the thyroid nodules
investigations, was urgently required; the Bethesda conference was organized in this context. It concerned all the aspects of thyroid investigations; one session was devoted to Terminology.

In the Bethesda system (BTS) (2) 6 categories have been defined; the originality of the BTS was that the diagnostic categories are linked with a risk of malignancy based on an extensive literature and therefore with relevant guidelines for the patients management. Furthermore a blue book was published including very well described morphological criteria; this is the key for diagnostic reproducibility, for patient management and for national as well as international correlation. Therefore the BTS has been recommended in the USA but also in many European countries (Croatia, Finland, France, Greece; Portugal, Spain etc…..) and adopted by the ATA in 2015 for the new guidelines (1).

Nevertheless the BTS is not the only system; other terminologies have been published either at the same time or more recently; there is the english one (BTA 2009 revised 2014) (3); the italian one, published in 2006 revised in 2015 (4); the japanese and the australian ones (5). All these terminologies are considered to be transposable into the BTS.

We would like to insist about the use of a unique terminology; the applications and consequences are numerous; by using the BTS it is possible: 1) to analyze our results and to compare them with the expected BTS percentages of risk of cancer; 2) to compare our results with those of other series; 3) to analyze the discrepant results, to test the reproducibility and to take in account the epidemiological data.

Due to this increase in terminologies it was decided in 2015, during the ECC in Milan, to organize a symposium called « new classifications and new interpretations »; Differences between these terminologies have been widely shown. It was also underlined that for publication a careful translation of results into the BTS should be considered to facilitate comparisons between different published studies (6).

Concerning the morphology, cytology may be based on conventional slides or on liquid-based cytology (7, 8). The advantages of the thin-layer techniques compared to the conventional preparations are: 1) an optimal cellular preservation due to adequate and rapid fixation and a decrease in the number of red blood cells; 2) available residual material for ancillary techniques; The disadvantages are essentially the cytological artifacts or changes due to the alcoholic fixation requiring long training for cytopathologists who want to switch from one technique to the other. Cytological features of both techniques (conventional and LBC) are briefly described for each category.
NON-DIAGNOSTIC/UNSATISFACTORY CATEGORY

Criteria: this category includes
- Cases with less than six well-preserved follicular cells groups with ten cells each
- Poorly prepared or stained follicular cells
- Cyst fluid with or without histiocytes and fewer than six groups of ten benign follicular cells

For solid nodules with atypical cells, with lymphocytic inflammation or with abundant thick colloid, the minimum number of follicular cells is not required. The FNAs are classified in one of the following category depending on the cellular material observed.

BENIGN CATEGORY

Criteria: this category includes
- Colloid nodules, nodular goiter, hyperplastic/adenomatoid nodules and Grave’s disease nodules
- Thyroiditis (lymphocytic, granulomatous, Riedel, acute thyroiditis)

The following components are observed in varying proportions
- Regular sheets of cells
- No nuclear atypia
- No or very few microfollicles
- Colloid
- Histiocytes and/or fibroblasts
- Inflammatory cells
- Few oncocytes

Notice that on LBC the diagnoses of lymphocytic thyroïditis may be difficult due to the lack of inflammatory cells retained by the technique; the oncocytes are often difficult to recognize due to the fixative

FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE/ATYPIA OF UNDETERMINED SIGNIFICANCE
CATEGORY

Criteria: this category includes many different situations including 9 specific criteria
- Some microfollicules in a scant slide
- Predominance of oncocytes in a scant slide
- Predominance of oncocytes in a context of thyroiditis or a goiter
- Focal nuclear atypia suggestive of papillary carcinoma (chromatin changes or irregular nuclear borders)
- Nuclear atypia due to sample preparation artifacts
- Minor population of atypical cells
- Cyst-lining cells
- Atypical lymphoid population
- Other

FOLLICULAR NEOPLASM/ SUSPICIOUS FOR A FOLLICULAR NEOPLASM CATEGORY

Criteria: this category includes all cases for which it is impossible to distinguish a benign follicular lesion (nodular hyperplasia, follicular adenoma) from a follicular carcinoma. It is a very specific entity. Cellular atypia suspicious for other carcinoma than a follicular carcinoma are excluded from this category.
- Numerous microfollicules
- Nuclear atypia (enlarged nuclei; prominent nucleoli)
- Markedly cellular sample
- Scant colloid

FOLLICULAR NEOPLASM, HÜRTHLE CELL TYPE/ SUSPICIOUS FOR A FOLLICULAR NEOPLASM, HÜRTHLE CELL TYPE CATEGORY

Criteria: this category includes only cases with exclusively or almost exclusively oncocytes. Papillary carcinoma oncocyic variant are excluded.
- Cells highly variable in size with more or less amount of cytoplasm
- Enlarged nuclei often eccentric; prominent nucleoli
- Bi or multi nucleation
- **Granular cytoplasm blue or grey-blue with MGG staining, green with Papanicolaou staining.**  

*Oncocytic cells are not easily recognized on LBC; cytoplasm appears less obviously granular; nuclei are sometimes round and enlarged, sometimes irregular shaped.*

**SUSPICIOUS FOR MALIGNANCY CATEGORY**

**Criteria:** this category includes all malignant cases except follicular and Hürthle cell carcinomas for which the cytological criteria of malignancy are weak for a definitive diagnosis. Therefore this category is divided into several sub categories since it is necessary to specify the type of suspected malignancy (papillary carcinoma or medullary carcinoma or lymphoma or metastases).  

*In this category have to be included cases for which malignancy is considered more likely than not.*

**MALIGNANT CATEGORY**

*This includes all the different subcategories listed above but the cytological criteria of malignancy are sufficient to assess the diagnoses.*

**Criteria:**

- **Papillary carcinomas:** cells in tridimensional clusters or in papillae or in syncitial-like monolayers; more or less enlarged nuclei; irregular shaped sometimes grooved nuclei; powdery chromatin or ground glass nuclei and nuclear cytoplasmic inclusions; psammoma bodies and histiocytic giant cells.

- **Medullary carcinoma.** The most characteristic features are: isolated, cuboidal or polygonal or sometimes elongated cells with rare syncitial-like clusters; nuclei with plasmacytoid nuclear chromatin, better visualized on Papanicolaou staining than on MGG; binucleation and pseudoinclusions. Pink intracytoplasmic granules. In the background, some homogeneous pink droplets, positive for Congo Red staining, corresponding to amyloid material.

- **Poorly differentiated carcinoma** The main criteria include: a high cellularity with ribbons of cells and dyscohesive cells; there are few microfollicules. The cells are small, intermediate sized cells with bland nuclei, fine chromatin; small nucleoli.

- **Lymphoma.** Same cytological criteria as for lymph nodes cytology.
Metastases: features depend on the primary tumor

Ancillary techniques: 70% of thyroid nodules are classified either as benign or malignant; 20-25% of thyroid nodules are «indeterminate»; About 15-30% of patients have a cancer. The aim of clinicians and of the cytopathologists is to reduce the number of these «indeterminate lesions», the number of unnecessary thyroidectomies and to avoid chronic disease. The main histological lesions concerned by the «indeterminate categories» are the follicular neoplasms (adenoma/carcinoma); the Hurthle cell neoplasms (adenoma/carcinoma) and the follicular variant of papillary carcinoma. These “indeterminate categories” exist in all the terminologies whatever their names; therefore the objective is to find the cut off point between cases which need surgery and those which do not need.

They are few published studies about immunocytochemistry (ICC) but all have shown that it may be helpful to classify the indeterminate cases either into the benign or into the malignant categories. In our own study (9) we have shown the negative predictive value of ICC applied with 2 antibodies, CK 19 and HBM E1, which was correlated in all cases with a benign histology; furthermore 42% of the so called «indeterminate cases» have been classified either as benign or malignant. So the indeterminate could be reduced up to 50% with ICC. Other studies have shown similar results (10, 11) but it is necessary to underline that all are using a panel of antibodies and not a single antibody.

Furthermore some specific antibodies are obviously used for diagnoses of medullary thyroid carcinoma, metastases or parathyroid lesions.

The genetics events leading to thyroid cancer initiation and progression are now well known. In the MAPkinase pathway, required for papillary thyroid carcinoma (PTC), a crucial role is held by BRAF V 600E. This gene is highly specific for PTC but unlikely implied in only 60% of the PTC classic variant and in 10% of the PTCFV (PTC follicular variant) which are more difficult to detect on FNA than the classic variant.

The BRAF mutation may be revealed on conventional smear by scratching (12); on LBC or also with a recent available antibody (13). RAS mutations are involved in 40-50% of follicular carcinoma (FC); nevertheless 20-40% of follicular adenoma (FA) have RAS mutations and only 10-20% of PTCFV. So the RAS mutation is less sensitive and less specific than the BRAF and the cut-off between benign and malignant cases is not high enough to be applied on cytology.

Some authors like Nikiforov (14) have suggested new algorithms for the patients’ management based on the mutational analysis. The risk of cancer
differs in the categories, especially when the test is negative; but we should notice that it is really relevant only in the FLUS category. Therefore in 2014 the AACE/ACE (15) has concluded that at present molecular testing is meant to complement and not to replace clinical judgment.

A worthwhile change is occurring in thyroid histological WHO classification. It concerns PTCFV. This entity has been described in 1977 and is characterized by the lack of papillary structures combined with PTC nuclear atypia; it is divided in 2 subgroups: the non encapsulated or invasive PTCFV and the encapsulated one. This encapsulated variant is now called the NIFT-P. During the last 20 years the PTCFV entity increased for 2 or 3 fold; representing finally 20-30% of the PTC in Europe. But its prognosis is excellent with 0% of recurrence as reported from different studies. In June 2016 this NIFT-P was finally published as an indolent tumor (16). Considering the nuclear atypia it will be very challenging for the cytopathologists to recognize this entity; two studies have already been published showing a significant difference in risk of malignancy for each BTS category if NIFT-P considered as a non malignant (17, 18). Considering the lack of BRAF and RET/PTC mutation in the NIFT-P, molecular biology could be helpful to recognize or at least to suggest this entity.

So, many changes occurred during the last 10 years since the BTS first edition; A symposium gathering around 20 cytopathologists has been held in Yokohama during the last ICC in May 2016. After discussion, panelists’ recommendations have been presented. They have been published (XXX) and the second edition of the BTS is foreseen for 2018.

Take home messages

- The Bethesda System has standardized thyroid FNA reporting
- Other terminologies are used but as far as possible a unique terminology should be applied
- The “indeterminate categories” which represent 20-25% of thyroid FNA raises a problem but exist in all terminologies
- The introduction of molecular testing and ICC should reduce around 50% of the IC
- NIFT-P has significantly impacted thyroid cytopathology and has to be further studied.
- An updated BTS edition will be published in 2018
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