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Practical Surgical Neuropathology: Foundational Principles and Diagnostic Challenges

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Introduction

The brain and spinal cord are complex organs composed of a wide variety of distinctive cellular constituents that convey susceptibility to a large number of disease processes, many of which are unique to the nervous system. The combination of morphologic complexity and special nosologic vulnerabilities imparts the possibility of diagnostic error stemming from a number of sources. We will review the foundational principles of sound diagnosis, followed by a discussion of the most common problematic issues encountered by the general surgical pathologist who deals with surgical specimens from the central nervous system, and suggest appropriate strategies for averting diagnostic misadventure!

First Things First: Foundational Principles

The foundation for competent and confident diagnosis of central nervous system lesions consists of three knowledge pillars: *normal morphology*, *disease morphology* and *clinical information*. Each of these plays a critical role in facilitating accurate diagnosis and avoiding common diagnostic pitfalls.

Normal morphology. *Identification of the abnormal is predicated on knowledge of the normal.* The normal microscopic morphology of the central nervous system includes the H&E, immunophenotypic, and ultrastructural features of the diverse cellular constituents and the regional specializations of the CNS. Such specialized regions of clinical relevance include the meninges, pituitary gland, pineal gland, choroid plexus, optic nerve and meningeal covering, olfactory bulbs & tracts, lumbar cistern (conus medullaris, filum terminale, cauda equina, leptomeninges), circumventricular organs, and cerebello-pontine angle. Several good reference works are available for a review of normal CNS histology (see References).

Disease morphology. The second pillar is familiarity with the pathologic conditions that affect the nervous system, including rare and newly described diseases. This pillar includes not only the different entities but also the *spectrum of morphology* within each entity. Probably the most well known example of the latter is meningioma, for which there are 13 WHO-codified morphologic subtypes (WHO 2007). Nine of these are benign (WHO grade I): syncytial, fibrous, transitional, lymphoplasmacyte-rich, secretory, microcystic, angiomatous, metaplastic and psammomatous; two are atypical (WHO grade II): clear cell and chordoid; and two are anaplastic (WHO grade III): rhabdoid and papillary.

The general surgical pathologist is less likely to be familiar with the various *morphologic subtypes of glioblastoma* that can be encountered. Only two of these are formally recognized in the WHO classification (giant cell glioblastoma and gliosarcoma), but there are *at least 12 different GBM morphologic patterns* with which the surgical pathologist

should be conversant, including giant cell, small cell, granular cell, spindle cell, bland cell (my term - a GM that does not show much pleomorphism but rather appears relatively bland and uniform), epithelioid, rhabdoid, lipidized, inflammatory, metaplastic, myxoid and gliosarcoma.

12 Morphologic Subtypes of Glioblastoma

Giant cell
Small cell
Granular cell
Spindle cell
Bland cell
Epithelioid
Rhabdoid
Lipidized
Inflammatory
Myxoid
Metaplastic
Gliosarcoma

Clinical Information is Critical! Before considering common diagnostic pitfalls, one point cannot be emphasized enough: *Knowledge of the patient's clinical background is absolutely essential to avoid the deleterious consequences of misdiagnosis!*

The key features to know are the patient's age, location of the lesion (MRI and/or CT scans), relevant clinical history (previous surgery, predisposing conditions, etc.) and the duration and nature of the presenting signs and symptoms. The latter can be simplified to "recent onset" versus "long history." In general, a long history, particularly an extended past history of medically intractable seizures, suggests a more indolent disease process or a low-grade tumor. The value of knowing the patient's age, the anatomic location and MRI features of the lesion, relevant medical and surgical history, and the duration and nature of the presenting symptoms cannot be stressed enough.

CRITICAL CLINICAL INFORMATION

Age
Location
MRI Features
Relevant Clinical History
Type and Duration of Symptoms

Clinical information facilitates formulation of the differential diagnosis. For example, the differential diagnosis of a contrast-enhancing mass in a child is very different from that in a 65-year-old: the two most common brain tumors in children are pilocytic astrocytoma and medulloblastoma; in an older adult, the principal entities are metastatic carcinoma, glioblastoma, and primary CNS lymphoma.

The anatomic location of the lesion is equally useful. For example, a solitary mass in the lumbar cistern is most likely one of five tumors: schwannoma, meningioma, myxopapillary ependymoma, paraganglioma of the filum terminale, or a solitary metastasis. Similarly, there are specific tumors that comprise the list of most likely possibilities for a mass in the lateral ventricle, cerebello-pontine angle, etc. The anatomic relationship to various intracranial structural compartments can also be very helpful. For example, the differential diagnosis for a dura-based mass includes specific mesenchymal and hematopoietic tumors as well as a number of non-neoplastic entities.

Lumbar Cistern Solitary Mass: MRI Differential Diagnosis

Schwannoma
Meningioma
Myxopapillary Ependymoma
Paraganglioma of the Filum Terminale
Metastasis (Hematogenous or CSF “Drop” Metastasis)

Lateral Ventricle Mass: MRI Differential Diagnosis

Choroid Plexus papilloma / carcinoma / meningioma / metastasis /
xanthogranuloma
Ependymoma
Subependymoma
Subependymal Giant Cell Astrocytoma
Central Neurocytoma

Cerebello-Pontine Angle Mass: MRI Differential Diagnosis

Schwannoma (“Acoustic Neuroma”)
Meningioma
Lipoma

Epidermoid Cyst
Choroid Plexus Papilloma
Ependymoma
Exophytic Bone/Cartilage Tumor
Exophytic CNS Parenchymal Tumor
Exophytic Papillary Endolymphatic Sac Tumor

Dura-Based Mass: MRI Differential Diagnosis

Meningioma
Solitary Fibrous Tumor/Hemangiopericytoma Family Tumor
Dural Sarcoma
Plasmacytoma
Granulocytic Sarcoma (“Chloroma”)
Solitary Dural Metastasis
CNS Rosai-Dorfman Disease
Castleman Disease
Calcifying Pseudotumor of the Neuraxis (Fibro-osseous Lesion)
Inflammatory Pseudotumor
Plasma Cell Granuloma
Idiopathic Hypertrophic Pachymeningitis
Sarcoidosis

MRI Features. At least a rudimentary knowledge of the patient’s MRI results can be very helpful to the pathologist. In a nutshell, there are three anatomic planes of MRI section (coronal, sagittal and frontal) and four major sequences (T1, T1-post contrast, T2 and FLAIR). In many situations, a quick look at the FLAIR and T1-post contrast sequences will convey an overall picture of the lesion.

Information Gained From Quick Review of MRI Scan/Report

Number of Lesions
Anatomic Location of the Lesion(s)
Lesion / Brain Interface (Circumscribed vs. Diffuse)
Presence or Absence of Contrast Enhancement
Pattern of Contrast Enhancement if Present

Problematic Issues in Surgical Neuropathology: Intraoperative Consultation (IOC) Issues

Preparation for intraoperative consultation (IOC) begins before the page to frozen section arrives with a brief review of the patient's clinical history as described above, with the addition of two additional factors that are especially relevant to IOC: the type of surgical procedure that is being performed (e.g., stereotactic biopsy, open resection, etc.), and the critical question "what is the essential information that the surgeon need to learn from the IOC in order to complete the surgical procedure in an appropriate fashion."

Pre-IOC Preparation List

AGE of the patient
ANATOMIC LOCATION of the lesion
IMAGING CHARACTERISTICS of the lesion
PAST MEDICAL and SURGICAL HISTORY of the patient
TYPE and DURATION of presenting signs & symptoms
TYPE of SURGICAL PROCEDURE that is being performed
WHAT WILL THE SURGEON NEED TO KNOW?

Common Intra-IOC Problematic Issues

The specimen is non-representative
The specimen is very small
The specimen is *seriously* small
The specimen is extensively cauterized
The specimen is necrotic
The specimen is bony

Practical Approach to Intraoperative Consultation (IOC)

Intra-IOC Principles: Summary

Cytology and Architecture are Complementary – USE BOTH!
Don't freeze all of the tissue if possible
Plan for tomorrow!

Plan for tomorrow. There are two priorities that must be addressed at the time of intraoperative consultation: 1) to give the surgeon the necessary information that they need

to complete the operation, and 2) to ensure insofar as possible that the final diagnosis will be as accurate as possible. The latter is largely dependent on having adequate representative material to assess morphologic features and any needed ancillary studies (immunostains, organism stains, special stains, FISH, flow cytometry, molecular testing, etc.). This may pose a challenge in some situations and there are steps that the surgical pathologist can take to help ensure a satisfactory result. As an example, some biopsy specimens are quite small (e.g., endoscopic biopsy, stereotactic biopsy, open biopsy of spinal cord). There are a number of things the pathologist can do to ensure adequate material for workup, including preparing unstained touch preparations, cutting additional unstained slides from the frozen section block before removing it from the cryostat, and ordering additional unstained paraffin sections be cut at the same time as the initial screening H&E to avoid refacing a block with very scant tissue. The most common issues of this nature encountered at frozen section are discussed below.

Intraoperative Consultation: Issues

- Specimen is non-representative
- Specimen is very small
- Specimen is *seriously* small!
- Specimen is extensively cauterized
- Specimen is extensively necrotic
- Specimen is extensively bony

12 Common CNS Surgical Pathology Challenges

- Gliosis vs Glioma
- Diffuse Glioma Classification
- Diffuse Glioma Grading
- Recurrent GBM vs Radiation Necrosis
- Meningioma Grading
- Non-Representative Biopsy
- Necrotic Malignant Brain Tumor
- Demyelinating Pseudotumor vs Diffuse Glioma
- Non-Diagnostic Pituitary Resection
- Colloid Cyst / Rathke Cyst – Cyst Contents without Epithelium
- RARE Tumors
- NEW Tumors

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**Cartilaginous Tumors of Bone and
Giant Cell Tumors of Bone**

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Histologic diagnosis of hydatidiform mole and its problems

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With the increased use of ultrasound hydatidiform mole (HM) is being diagnosed at increasingly early stages of gestation. As villous edema is not fully developed, we can not make a diagnosis of HM by macroscopic observation as indicated by the Japanese criteria for molar pregnancy (diameter of villous > 2mm). Furthermore, microscopically the classic features of complete mole (CM) may be lacking and CM can be easily misdiagnosed as partial mole (PM) or hydropic abortion (HA).

The incidence of cases of PM have been increasing lately, however, it may be partly due to underdiagnosis of early CM as PM. Many PMs have been still misdiagnosed as HA. In our previous study, significant interobserver and intraobserver variability in the diagnosis of molar pregnancy was observed even among placental pathologists. There were most frequently problems of differentiating PM from HA. In a substantial number of cases, it was extremely difficult to distinguish PM from HA on histology alone and p57 immunostaining is not useful to distinguish between them. Edema of the placenta villi, although useful in drawing attention to specimens, may be a secondary phenomenon associated with a variety of condition, such as HA. I consider evaluation of trophoblastic hyperplasia should be more weighted on histologic diagnosis of HM. Hydatidiform mole is characterized by grossly evident hydropic swelling of villi, but histologic examination is essential in a making diagnosis of HM.

Since the risk of persistent disease is 10 to 15% in CMs and 1 to 2% in PMs, and no serious consequences are observed in the majority of patients with PM, practically, a correct diagnosis of early CM is most important. Following are histologic features of early CM; diffuse or focal hydropic change of villi, bulbous or polypoid villi, focal or circumference trophoblastic hyperplasia, cellular villous stroma, network of capillaries, karyorrhexis in villous stroma, prominent placental site intermediate trophoblasts, and absence of embryo. Criteria of PM are; two populations of villi, normal sized villi and edematous villi, irregular villous outlines, focal mild syncytiotrophoblastic hyperplasia, central cistern, trophoblastic inclusion, the presence of an embryo or fetus. p57 immunostaining is useful for differential diagnosis between CM and PM. Villous cytotrophoblasts and stromal cells are negative for p57 in CM.

Presentation of GYN Pathology 2009

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During this conference, we are going to discuss the changes proposed in the new FIGO staging and some new developments in GYN Pathology.

FIGO (International Federation of Gynecologic & Obstetricians) is composed of six members and for this staging system review they have an enlarged committee, including members from AJCC, GCIG, ISGyP, IGCC and SGO.

VULVA

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The proposed changes for vulva are easily understood if we move the previous staging system one step up, so a case that was stage II is now Ib, for example, and there are more details regarding the lymph nodes.

Tumors larger than 2 cm are now stage Ib; tumors extending to lower urethra, vagina or anus are stage II; and in stage III the changes are based on the lymph nodes involved where IIIa is one lymph node larger than 5 mm, IIIb is two or more lymph nodes, and IIIc is extracapsular involvement. Stage IV includes pelvic lymph nodes. I believe that it is important for pathologists to correct the tumor

size after the microscopic slides are reviewed because a 2.5 cm mass composed of more than 50% of fibrosis has only 1.7 cm tumor.

Another important study regarding the vulva is that according to the size of the lymph node metastasis are the possibilities of getting additional metastases. For example, if the metastasis contained one to ten cells, there was a 5% possibility of additional metastases; if the size of the metastasis was one to five mm, then there were 10% possibilities of additional metastases, and if the size of the metastasis was larger than 5mm, there were 40% possibilities of additional metastases.

CERVIX

The changes proposed for the cervix are mainly for stage IIa, where it is divided into IIa1 and IIa2 according to the size of the tumor. If the tumor is smaller than 4 cm it's IIa1 and if it's larger than 4cm it's IIa2. There was an important discussion about whether cervix should be clinically or surgically staged. The people that proposed to continue with the clinical stage based this on the fact that carcinoma of the cervix has a high incidence in poor countries; therefore, most cases are not operated on and because of this, we should continue with clinical stage. In more developed countries, surgical stage would be acceptable.

Because of this problem between clinical and surgical staging of cervix, it could be controversial to stage an endocervical adenocarcinoma metastatic to ovaries. If we are going to use clinical staging, since most probably the metastasis is not going to be identified clinically, the tumor should be stage I. However, using surgical stage, at least it would be classified as a stage III because there is involvement of an organ in the pelvis; however, some gynecologists would prefer to classify the tumor as a stage IV because it is a metastasis and not direct extension.

Current tests to identify HPV are the hybrid capture and in situ hybridization. Hybrid capture is a test used in cytology specimens; however, it cannot be applied to paraffin embedded tissue. New tests which are useful in paraffin embedded tissue are the invader and the complete care. The invader has different probes which identify HPV 16, 18 and high risk types, but not 16 or 18. This is an excellent test because we are interested in identifying high risk HPV types which are or are not 16 -18. The complete care identifies each one of the different fifteen HPV types; however, it is not as useful because there is no need to separate some of the hybridous types that are not 16 - 18. The in situ hybridization today can be done with a third generation probe which is more sensitive and specific than previous probes.

As a general rule, it would be important to remember that 30% of the HPV infections in the cervix and the vulva are due to multiple types, 30% are due to unusual types, and 30% of the high risk which are not 16 -18 do not progress. Because of this latter statement, the American Society for Colposcopy and Cervical Pathology recommended that in cases where a patient is positive for high-risk HPV, but is not 16 -18, to repeat the HPV and cytology after 12 months. If the HPV high risk is 16 -18, they need to do immediate colposcopy.

Cervical dysplasias

The identification of cervical dysplasias in pathology is not difficult because of hypercellularity in the epithelium, cytologic atypia and numerous mitoses. If necessary, immunostains for p16, Ki67 and ProEx will also confirm the diagnosis of dysplasia. We have found an unusual type of dysplasia which does not have significant hypercellularity or atypia and mitotic figures are absent or rare. These lesions simulate metaplastic changes; however, they are diffusely positive for Ki67, ProEx and p16. We refer to these lesions as deceiving dysplasias of cervix, and what is extremely interesting is that 87% of these dysplasias are due to high risk HPV, not 16-18. We are uncertain whether this is a type of dysplasia that continues during the entire evolution with a high risk, not 16-18 type, or if this

is an early classic dysplasia that starts with a high risk HPV, not 16-18, and later on, the type of HPV changes to 16-18.

Most carcinomas of the cervix are related to HPV; however, the frequency of HPV is different according to the type of carcinoma. In 98% of the squamous carcinomas HPV virus will be found, but only in 90% of adenocarcinoma HPV types are found. This probably can be explained by the fact that 10% of the endocervical adenocarcinoma is most probably related to hormonal changes, similar to the endometrial tumors.

Cervical adenocarcinoma

The recurrent rate of cervical adenocarcinoma after a cone biopsy of cervix depends on two important parameters, the status of the margins and the number of fragments involved by the adenocarcinoma. Currently it appears that residual carcinoma after a cone biopsy is associated with involvement of more than half of the tissue in the cone biopsy and that this could be a more important parameter than the status of the margin. Therefore, it is important for pathologists to include in the report the extension of the disease in the cone biopsy.

A current pathology issue is how to submit the recession margins of a trachelectomy specimen. The examination of margins of resection parallel to the lumen or the cervix will be able to determine the distance of the tumor to the margin of the resection; however, it will examine less than 10% of the entire surface. On the other hand, an examination of the resection perpendicular to the lumen will examine the entire surface, but it will not be informative regarding the distance of the tumor to the margin. It is highly recommend to use the enface examination, which is a section perpendicular to the lumen, and if it is positive it would be necessary to obtain another margin, but if it negative, the block can be flipped over and examined on the other side. This would mean that there is a safe margin of at least 5 mm which most probably is sufficient for carcinoma of cervix.

Neuroendocrine carcinomas

It is important to distinguish in the neuroendocrine carcinomas the small cell versus large cell type. This became accepted in different parts of the body including respiratory and digestive areas. These differences are necessary because currently there are new protocols for well differentiated and moderately differentiated neuroendocrine carcinomas. In the cervix the situation is different because all neuroendocrine carcinomas are of high grade and small cell carcinoma and large cell neuroendocrine carcinoma are treated similarly.

New entities in cervix – mucoepidermoid carcinoma

Mucoepidermoid carcinoma of the cervix is a tumor characterized by squamous differentiation with areas having mucin in the cytoplasm, but glands are not seen. Because there are no glands the tumor is not an adenosquamous carcinoma. What is interesting about this entity is that it has been found that this type of tumor has the same translocation (11; 19) as mucoepidermoid carcinomas of the head and neck area. This translocation is related to the CRTC1-MAML2 gene fusion. This area is a potential target for therapy and this is the main reason why mucoepidermoid carcinoma should be recognized as a new “entity” in the cervix.

UTERUS

For a long time, it has been recognized that there are multiple problems with the staging of uterine carcinomas. These problems include: lack of agreement of the superficial invasion of the myometrium, the significance of cervical involvement, peritoneal cytology, and lymph nodes involvement. Therefore, there are numerous changes in the staging of endometrial carcinomas. In stage I cases, the proposed changes will include Ia and Ib for tumors that involve less than half of the myometrium or more than half of the myometrium. In stage II there is going to be only cases that

involve the stroma of the cervix. Cases with involvement of only the glands in the cervix will be classified as stage I. In the stage III cases, peritoneal cytology has been eliminated from staging and positive peritoneal cytology will not change the stage.

Biomarkers predicting endometrial cancer stage

It has been found that six different genes induced by estrogen are related to endometrial carcinoma stage. The lower expression of these genes is associated with stage Ic or higher. The study of these genes has been done in patients undergoing hysterectomy and it appears that the determination of these genes is equal or better than frozen section to determine the depth of invasion of the tumor. Similarly different genes can separate endometrioid from non-endometrioid cases. This is a very important study because it demonstrates that some carcinomas are high grade from the beginning and they do not evolve from hyperplasia to low grade tumors to high grade tumors. This is similar to the known fact that in ovarian carcinomas tumor size is not directly related to the aggressiveness of the tumor. A smaller tumor can be more aggressive than larger tumors, supporting the possibility that some tumors are aggressive from the beginning of their development.

Endometrium-microsatellite instability (MSI)

It is a common practice today to request stains to study MSI in patients younger than 50 years, or the ones that have tumors with abundant lymphocytes, or with family history of similar tumors in the ovary, colon, or breast. It is also known that 12% of the carcinomas in the lower uterine segment can have MSI compared to only 5% for tumors in the corpus. The interpretation of the stains is clear when they are totally negative or diffusely positive; however, there is some disagreement about heterogeneous staining. Newer studies are showing that the presence of heterogeneous staining, meaning areas positive mixed with areas negative is equivalent to negative staining when molecular studies are done.

Menorrhagia

Most cases of menorrhagia can be explained by endometrial breakdown, or disordered proliferative endometrium, or leiomyomas, or polyps. Recent studies suggest the possibility that in some cases menorrhagia can be explained by changes in the uterine vasculature. The uterus has a very rich vasculature which starts on the plexus arquatus on the outer third of the myometrium. These arteries are connected to smaller vessels that eventually will get to the endometrium. A very unusual feature in the uterus is the presence of vessels within vessels. Arteries are seen within the veins. We have found that some cases of menorrhagia can be explained by the presence of numerous vessels within vessels close to the endometrium and also the presence of group of vessels within one low power field of the endometrium. The uterine vasculature has not been well studied in cases of menorrhagia; however, due to the fact that in uterine pregnancy the uterus is enlarged many times changes in the vasculature could be the cause of menorrhagia.

Staging for uterine sarcomas

FIGO is proposing a new stage for uterine sarcomas including leiomyosarcomas and endometrial stroma sarcomas. There is a difference in the stage I of these different entities. For leiomyosarcoma the tumors are going to be divided in Ia when the tumor is less than 5cm in larger dimension and Ib when it's larger than 5 cm. For endometrial stroma sarcoma Ia is going to be a tumor in the endometrium or the cervix, Ib is a tumor with less than half of myometrial invasion, and Ic with more than half of myometrial invasion. Stage II, III and IV are similar for both entities. In stage II the tumors are in the pelvis, in stage III in abdominal tissues, and in stage IV they are distant metastases. It is suggested that since 70% of leiomyosarcomas arise in association with leiomyomas, and because tumor size is an important factor, pathologists should correct the final tumor size after the entire lesion is examined. A 10cm leiomyosarcoma, arising in association with leiomyoma could represent a stage Ia or Ib depending on the percentage of each component.

Trophoblast

The diagnosis of hydatidiform moles depends on histologic examination and studies of the DNA; however, histologic features are not clear in all cases and sometimes interpretation of the DNA is difficult, therefore it has been proposed that genotyping will be the most important and specific type of study for classifying moles. Genotyping of the hydatidiform moles is based on the comparison of 16 different polymorphic foci, which are well-known typical for each individual, between decidua and the possible mole. This study can be performed on paraffin embedded tissue. A biparental diploid and a monoanthro-diploid would be an abortion. A diandric triploid is a partial mole and diandriatric diploid is a complete mole. In addition, by PCR and short tandem repeats, it has been found that a complete mole which is homozygous is going to be less aggressive than a complete mole that is heterozygous. Some large studies from many different countries found that less than 1% of patients with a complete mole died of disease, 10% of patients with choriocarcinoma died of disease, and approximately 16% of patients with tumors of intermediate trophoblast died of disease.

OVARY

Recent studies have shown that trying to induce ovarian-type of carcinomas immortalizing the cells with telomerase and infecting them with HER-2/neu can develop different tumors whether the cells are implanted in the subcutaneous tissue or in the peritoneum. In the subcutaneous tissue we will obtain undifferentiated carcinomas and in the peritoneum, we will obtain papillary serous carcinomas.

Ovarian cancer personalized treatment

Currently, it is not unusual to receive a request to study receptors in ovarian tumors in order to determine the treatment of the disease. One of the dilemmas that pathologists have with this approach is whether to study the receptors in the primary lesion or in the metastatic foci, and how to

select the block. Since it is important to determine if the tumors have uniform distribution of the receptors in different areas, several studies have been performed. Current studies have shown that there is a significant variation in the positive reaction of the receptors depending on the examination of different sites within the primary tumor or comparing the primary with different metastases. Therefore, it is recommended that more than one site should be examined to determine the best personalized treatment for the patient.

Currently it is uncertain whether the proliferation of the epithelium in ovarian tumors is directly related to the aggressiveness of the neoplasm. We have demonstrated that the proliferation of the epithelium is not directly related to the invasiveness of the neoplasm. Tumors that are very proliferative do not show significant invasion and vice versa. Based on this study we concluded that most probably epithelial proliferation and invasion follow different pathways.

Recently studies based predominantly on prostatic neoplasms have proposed that it is not necessary to treat aggressively low grade tumors because the progression of low grade tumors to high neoplasm is unusual. Based on these studies a conservative treatment of low grade tumors has been suggested. It is important to recognize that the same situation has not occurred in the GYN tract because there are several examples where low grade tumors in the GYN area progress to high grade neoplasms.

Some of the examples are the following:

- In the endometrium undifferentiated carcinomas are often associated with low grade adenocarcinomas
- 70% of clear cell carcinomas are associated with endometrioid carcinomas
- 50% of low grade serous carcinomas are associated with borderline tumors
- 70% of small cell carcinoma in the cervix are associated with squamous or adenocarcinomas
- 70% of leiomyosarcoma are associated with leiomyomas

Therefore, it is important to recognize that in the GYN tract the progression of low grade to high grade tumors is not unusual and treatment of the low grade tumors is very important.

Pathologists are currently facing an interesting dilemma which is if the classification of tumors should continue being histologic, like it has been for the last 200 years, or if should change to a molecular type of classification. For example, a poorly differentiated carcinoma with squamous areas will probably be classified as a poorly differentiated squamous carcinoma by most pathologists. However, the tumor could be also be within the group of the NUT carcinomas because of the NUT oncogene. The development of new treating modalities directed toward correcting abnormal genes would indicate that probably we should start classifying tumors based on molecular features because this is going to be associated with specific types of treatments.

Presentation of GYN Cases

Elvio G Silva, MD

Case 1:

This is a 40-year-old patient with multiple smooth muscle tumors involving the pelvis, peritoneum and ovaries. Histologically, the tumors had a benign appearance. There was no atypia, mitoses or necrosis and mitotic figures were rare. In this type of situation the usual question is whether the patient had a previous hysterectomy and in this case it is necessary to review the slides of the hysterectomy. Frequently these slides only show benign leiomyomas and the new lesions in the peritoneum and the ovaries are also benign leiomyomas. I believe in this situation the diagnosis should be multiple tumors most probably multiple primaries. Whenever there is a possible “recurrence” in a patient having smooth muscle tumors, the different possibilities are that the first lesion was a leiomyosarcoma, or that the first lesion had vascular invasion, or cases like the one we present here were recurrences are difficult to explain. Some authors believe that if it is difficult to explain the recurrences the cases represent low grade leiomyosarcomas. I reviewed low grade leiomyosarcomas and compared with high grade leiomyosarcomas. The age of the patients with low grade leiomyosarcoma is fifteen years younger, but the most important feature is that all lesions of the low grade leiomyosarcoma “recur” in the peritoneum, while high grade leiomyosarcoma “recur” in the lung. Less than half of the patients with the so-called “low grade leiomyosarcoma” died of disease compared to 90 % of the patients of high grade leiomyosarcoma. Immunohistochemistry shows that most uterine tumors are positive for ER, PR and WT1, and this is in contrast to extrauterine, soft tissue smooth muscle tumors, which are predominately negative for ER, PR and WT1. It is extremely interesting that peritoneal smooth muscle tumors issues are similar to uterine tumors rather than to extrauterine, soft tissue, smooth muscle tumors. This is probably due to the fact that peritoneal lesions are mullerian type of smooth muscle tumors, similar to the uterine tumors.

When we receive probable “recurrences” of uterine smooth muscle tumors, and they occur in the peritoneum, it is important to determine if the lesions are identical in the peritoneum and the uterus, and if the immunohistochemistry is similar or different because if there are differences the cases most probably represent independent primaries. There are patients having multiple smooth muscle tumors in different areas of soft tissue, for example patients with immunodeficiencies, like transplants or AIDS, and in these cases they have EBV viruses in the tumor cells. We are all also very familiar with an entity called peritoneal leiomyomatosis, which is associated with pregnancy or contraceptives and endometriosis. I believe that the presence of multiple smooth muscle tumors in the peritoneum is similar to peritoneal leiomyomatosis with the exception that the lesions are not very small but larger size tumors.

Case 2:

This is a 35-year-old patient who has a known serous borderline tumor in the ovaries. We received a biopsy of the peritoneum which shows the presence of a serous tumor sitting on top of the peritoneum. My diagnosis here is low grade serous carcinoma. I believe it is extremely important to separate cases of non-invasive implants with cases of invasive implants because the recurrence rate in 10 years is very different. 30% of patients with non-invasive implants recur and 70% of these as low grade serous carcinoma, while 65% of patients with invasive implants recur and all the patients recur as low grade serous carcinoma. When we render a diagnosis of a serous borderline tumor, there are three features that are extremely important and they should be included in the report, the presence of exophytic pattern, multiple sites of microinvasion, and micropapillary pattern. These features are associated with high stage disease. However, the amount of micropapillary pattern is important because if it represents less than 10% of the entire tumor there's no significance, while if it represents more than 10% of the entire tumor it will behave as a noninvasive serous carcinoma. I believe that today we can diagnose a tumor as serous carcinoma in a borderline neoplasm when there is invasion without desmoplasia, but larger than 3 mm in maximum dimension, or when there is

invasion with desmoplasia, regardless of the size of invasion, and also when there is extensive micropapillary pattern larger than 10% of the entire tumor. In the latter situation, we would call the tumor a noninvasive serous carcinoma. We make diagnosis of invasion when we see tumor cells in the spaces that are not lined by epithelial cells and then we call it NELS (nonepithelial lined spaces), regardless of the size of the area or the location of the lesion. It is also possible to diagnose invasion based on the distribution of haphazard glands which do not have spaces around them. These glands are different than endosalpingiosis because they do not have tubal metaplasia and they are always in a haphazard distribution with significant fibrosis around them.

Case 3:

This is a 40-year-old patient who had an ovarian tumor that is predominantly papillary and the papillae are lined by few epithelial cells. The low power appearance is that of a borderline tumor; however, the significant cytological atypia and numerous mitotic figures are diagnostic of a high grade papillary serous carcinoma. We divide papillary serous carcinoma into high grade and low grade based on the presence or absence of atypia and mitoses per 10 high power fields. In addition, low grade serous carcinoma have calcifications in all cases, and in 70% of the cases mucin is present. The recurrence rate and the survival of low grade serous carcinoma and high grade serous carcinoma is similar; however, the survival time is different between the two entities because invasive low grade serous carcinoma will progress and eventually will be lethal in more than 10 years, while usually patients with high grade serous carcinoma died of disease within 5 years. The diagnosis of low grade serous carcinoma is made when there is invasion by NELS, or well-differentiated glands, or orphan papillae. We call it orphan papillae to structures that do not have attachment to the border of the epithelium or to other papillae. Often from these areas with orphan papillae areas of invasion of the stroma can be seen. We are uncertain whether low grade and high grade serous carcinoma are two different types of tumors or two different grades of one tumor. Most probably they represent two different types because there is no transformation of the low grade into the high grade. It is important

to remember that all, or most high grade serous carcinomas, are papillary but not all papillary carcinomas are serous type. High grade serous carcinoma needs to be distinguished from transitional cell carcinoma, microcystic carcinoma, and clear cell carcinoma. This is easily made because the terminal papillae in high grade serous carcinoma do not have stroma and the papillae in all the other tumors including transitional, clear cell and endometrioid have stroma. We believe that serous carcinoma is papillary in most cases and if we see only sheets of cells we make a diagnosis undifferentiated carcinoma, not papillary serous carcinoma. Microcystic carcinoma is an important type of tumor because it has signet-ring cells mixed with the solid component of the tumor and frequently it is associated with areas of high papillary serous carcinoma.

Case 4:

This 24 year-old patient was seen at M. D. Anderson Cancer Center because she had endocervical polyp which showed a proliferation of spindle cells with some atypical features and rare mitotic figures. There was no cambium layer or myxoid areas. Immunostains showed that the proliferation of spindle cells was positive for desmin but negative for smooth muscle actin. Other stains for skeletal muscle were all negative. The diagnosis of this case is superficial cervicovaginal myofibroblastoma and the differential diagnosis of this lesion is embryonal rhabdomyosarcoma; however, in the latter case there is cambium layer, frequent mitoses, significant atypia, and the cells are positive for desmin and also for myoglobin. We have performed a recent study where we are showing that the stromal cells of the cervix are different in the ectocervix and endocervix. In the ectocervix they are positive for desmin and negative for smooth muscle actin; however, in the endocervix the opposite occurs, they are positive for smooth muscle actin but negative for desmin.

Case 5:

This is a 45 year-old patient who presented with vaginal bleeding and an endometrial curettage showed an adenocarcinoma. The histologic examination of the adenocarcinoma showed

areas of a well-differentiated tumor and areas of completely undifferentiated neoplasm. In the undifferentiated component, there were foci with rhabdoid cells, which had eosinophilic cytoplasm but they were negative for desmin. Based on presence of these two components, the diagnosis is dedifferentiated endometrioid adenocarcinoma, which represents an undifferentiated carcinoma associated with areas of well-differentiated carcinoma was made. In this type of situation sometimes it is difficult to confirm the epithelial nature of the solid component because keratin is frequently negative or only very focally positive. Keratin 18 and EMA sometimes are more positive than regular keratin cocktail. It is extremely important to separate areas of solid tumor in grade 2, and grade 3 carcinomas from areas of undifferentiated carcinoma. In the latter situation there are no glands in the solid component while in the former situation there are glands in the solid area. The 5 years survival of undifferentiated carcinoma or dedifferentiated endometrioid carcinoma is worse than the survival of grade 3 endometrioid carcinoma, 25% versus 75%.

Case 6:

This is a 35-year-old patient presented with an ovarian mass. Histologically this is a proliferation of luteinized cells without any specific pattern. Clinically the patient has several similar nodules in the ovary and she has been recently pregnant. With that histologic appearance and the clinical information received, we made the diagnosis of pregnancy luteoma and the significance of this case is that it needs to be differentiated from juvenile granulosa cell tumor, which is a tumor with similar amount of luteinized cells; however, they have different patterns including well demarcated groups, mucinous areas and cords. In addition, granulosa cell tumors are not multiple and they are not bilateral. The differential diagnosis is extremely important because juvenile granulosa cell tumor needs to be resected and pregnancy luteoma does not.

Case 7:

This is a 45-year-old patient who had a 10 cm ovarian mass. Histologic examination of the tumor showed only very well formed glands; however, they did not have mitosis, mucin or squamous differentiation. In addition there were small groups of spindle cells in close relationship to the glands. This type of lesion can be seen in metastasis to the ovaries, primary carcinomas (predominantly endometrioid) and Sertoli-Leydig cell tumors. The main difference between these three tumors is that in metastasis there are deposits of tumor on the capsule and they never have squamous areas. Primary endometrioid carcinoma frequently has squamous areas and in Sertoli-Leydig tumors squamous and mucin are never seen. In addition in Sertoli-Leydig tumors, mitoses are rare in the glands, and small groups of spindle cells are seen associated with the glands. The diagnosis of these types of lesions is difficult and frequently immunohistochemistry is necessary. Probably the main marker that separates metastasis, primary, and Sertoli cell tumor is EMA, which is positive in the first two lesions and negative in the Sertoli-Leydig tumors. In order to see a positive reaction in Sertoli-Leydig tumors, we would need to stain the tumor for calretinin and inhibin. Sertoli-Leydig tumors are characterized by uniform histology, absence of squamous or mucin and foci of spindle cells. Sertoli-Leydig tumors are divided in well-differentiated, intermediate and poorly differentiated tumors. Sertoli-Leydig tumors may have heterologous components and because of this appear to be a different type of lesion. There are four well-known types of heterologous metaplasia in Sertoli-Leydig tumors including mucinous, carcinoid, skeletal muscle and cartilage. The last two are associated with less differentiated tumors while the first two are associated with better differentiated tumors. Whenever we see very well formed glands but without mitoses and squamous metaplasia, we need to think about the possibility of Sertoli-Leydig tumors and look for other features of the sex-cord tumors including cords and areas of spindle cells. The retiform variant of Sertoli-Leydig is seen in young patients and it can be confused with serous borderline tumors; however, it does not have tubal differentiation and cell detachments are rare.

The 2009 Diagnostic Pathology Course

Discovery of Disease and Development of Diagnostics

Lymphomatoid Gastropathy

ALK-positive Lung Cancer

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Lymphomatoid Gastropathy

Introduction

In the WHO classification of tumors of hematopoietic and lymphoid tissues, more than 60 types of lymphomas are listed up. In relation to these overt lymphomas, several reactive or borderline lesions mimicking lymphoma are well known: infectious mononucleosis, drug-induced lymphadenitis (especially related to anticonvulsants), histiocytic/subacute necrotizing lymphadenitis (Kikuchi-Fujimoto disease), etc.

We recently identified a “histologically malignant” disorder of an unrecognized NK-cell proliferation in the stomach, and designated it as lymphomatoid gastropathy (LyGa). Based on conventional histopathological criteria, the lesion is readily diagnosed as lymphoma, especially extranodal NK/T-cell lymphoma of nasal-type. However, considering its clinical behavior, LyGa is recognized as a benign process. It vanishes spontaneously without any treatment.

Case summary

The ten patients were comprised of 5 males and 5 females. The age of patients ranged from 46 to 75. They had no gastric symptoms at the time of gastroscopy. Three patients received gastroscopy for a follow-up study for gastric cancer, and the others had the examination for a secondary check up because abnormal shadows were pointed out by gastric X-ray screening for cancer. The gastroscopy procedures showed approximately 1 cm-sized ulcerative or elevated lesion(s) in the stomach. In 7 of the 10 cases, the initial pathology diagnosis to the biopsied sample was extranodal NK/T-cell lymphoma or suspicion for it. However, all of the lesions spontaneously regressed. All patients have been followed up (for 10 years at most) without chemotherapy and found to have no recurrences.

Histopathology

The atypical cells infiltrated diffusely in the lamina propria and occasionally into the glandular epithelium. The cells were medium-sized to large with moderate amount of clear or slightly eosinophilic cytoplasm. Interestingly, most cases contained a variable portion of cells with eosinophilic granules in the cytoplasm. The atypical cells are CD2- or variably+, CD3+ (cytoplasmic), CD4-, CD5-, CD7+, CD8-, CD16-, CD20-, CD45+, CD56+, CD117-, and cytotoxic molecule-related proteins+ (TIA1+, GranzymeB+, Perforin+) and EBER-.

Differential diagnosis

With its NK-lineage immunophenotype, the most important differential diagnosis of LyGa is extranodal NK/T-cell lymphoma of nasal-type. LyGa fortunately has several characteristic features which are inconsistent with extranodal NK/T-cell lymphoma. First, stomach is not a common site for NK/T-cell lymphoma to originate from. Second, necrosis was seen in some cases of LyGa but angiocentric or angiodestructive growth patterns or prominent apoptotic bodies are not observed, which are the very common features of extranodal NK/T-cell lymphoma. Third, the cytomorphology of LyGa is atypical for this type of lymphoma. Although the cytological spectrum of extranodal NK/T-cell lymphoma is broad, to the best of our knowledge, large eosinophilic cytoplasmic granules observed in the atypical cells of LyGa have never been found in the histopathology section of extranodal NK/T-cell lymphoma. Lastly, EBER in situ hybridization that is almost always positive in NK/T-cell lymphoma of nasal-type is exclusively negative in LyGa.

Conclusion

LyGa should be regarded as a distinctive clinicopathologic entity and be observed without treatment. If not well recognized, LyGa is likely to be misdiagnosed as lymphoma histopathologically, and might be treated with radical therapeutic procedures. Fortunately, LyGa shows highly conserved and characteristic features in terms of morphology, immunophenotype and clinical presentation. Thus, as long as recalled as a differential diagnosis, LyGa would never be misdiagnosed for malignancy.

ALK-positive Lung Cancer

Introduction

ALK is a receptor tyrosine kinase, which was discovered as a fusion with NPM in anaplastic large cell lymphoma (ALCL). In addition to ALCL (fused to *NPM*, *TPM3*, *TPM4*, *ATIC*, *TFG*, *CLTC*, *MSN*, *MYH9* or *ALO17*), *ALK* is found in a fusion form in inflammatory myofibroblastic tumor (*TPM3*, *TPM4*, *CLTC*, *CARS*, *RANBP2*, *ATIC* or *SEC31L1*), ALK+large B-cell lymphoma (*NPM* or *CLTC*) and ALK+histiocytosis (*TPM3*). In 2007, a recurrent chromosome translocation, *inv(2)(p21p23)*, was identified in lung cancer that results in the production of an EML4-ALK fusion-type protein tyrosine kinase(1).

Development of diagnostic analyses and discovery of novel fusions

In order to detect *EML4-ALK*, RT-PCR, immunohistochemistry (IHC) for ALK and FISH may be raised as candidate techniques. However, there are some difficulties in each technique, which are derived from the characteristics of *EML4-ALK*. The fusion point of *EML4-ALK* varies from case to case, and therefore primers for RT-PCR must be carefully designed. To solve this problem, we developed a multiplex RT-PCR system, which enabled all theoretically-possible in-frame fusion to be detected(2). The RT-PCR system not only successfully detected the four known fusion variants(1, 3) but identified five unrecognized variants(2, 4). IHC is a relatively easy diagnostic technique and is commonly used in diagnostic pathology. In contrast to the efficient detection of above-mentioned ALK fusion proteins, however, many researchers have encountered difficulty in detecting *EML4-ALK* fusion proteins by immunohistochemical analysis, possibly as a result of weak transcriptional activity of the promoter-enhancer region of *EML4* that drives the expression of *EML4-ALK* compared with that of the *NPM* promoter. We have established a sensitive immunohistochemistry-based screening system for ALK fusion protein-positive tumors, designated as iAEP (intercalated antibody-enhanced polymer) method(4). Furthermore, with this approach, we discovered a novel *ALK* fusion gene, *KIF5B-ALK*, in lung adenocarcinoma(4).

Clinicopathological features

These analyses are identifying new cases and are unveiling the clinicopathological features of ALK+lung cancer. Briefly, ALK+lung cancer is younger onset, unrelated to smoking habit and histologically often shows acinar structure with prominent mucin production. *ALK* fusions occur mutually exclusive to both *EGFR* and *KRAS* mutations(5, 6). In terms of therapy, a small molecule inhibitor of ALK proved effective to lung cancers generated in the *EML4-ALK* transgenic mice(7). Moreover, a clinical trial is ongoing for PF-02341066, a c-MET/ALK inhibitor (ASCO 2009).

Conclusion

Epithelial tumors, especially major carcinomas, have been considered to less frequently have pathological gene fusions than leukemias and sarcomas. Recent discovery of the fusions in prostate and lung cancers has changed the situation. As shown in leukemia and sarcoma, gene fusions are very useful markers for accurate diagnosis. In molecular targeted therapy, selecting the patients who benefit from the therapy is most important, and it is the pathology diagnosis that can precisely show the presence of the target. Developing a new diagnostic tool and making use of it appropriately in daily pathology diagnostic service will be required more, and these ceaseless efforts themselves will make the field of diagnostic pathology broader, brighter and more indispensable in the future.

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AN UPDATE ON HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS

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NEW ANTIBODIES AVAILABLE FOR ANALYSIS OF HISTIOCYTIC/ DENDRITIC CELL NEOPLASMS

CD163: New histiocytic marker

CD163 is a hemoglobin scavenger receptor. All the various types of histiocytes and monocytes are positive, except tingible-body macrophages of follicle centers (including splenic white pulp), epithelioid histiocytes and multinucleated giant cells of granulomas. Dendritic cells, including plasmacytoid dendritic cells, are negative.

Main advantage over CD68/PGM1 immunostaining: cell bodies of histiocytes are better defined.

Main applications of CD163 antibody: (1) diagnosis of histiocytic sarcoma; (2) diagnosis of acute monocytic leukemia. Dendritic cell tumors are typically negative. Epithelial and mesenchymal tumors are negative except a subset of fibrous histiocytoma.

Langerin (CD207)

CD207 is a surrogate marker for Birbeck granules. The antibody reacts against a C-type lectin localized in Birbeck granules. Langerin is expressed in Langerhans cells. It is an excellent marker for Langerhans cell histiocytosis (100% positive) – *now a defining feature of this entity*. Other types of histiocytic proliferations are negative.

Unexpected staining results: (1) lymph node: some sinus-lining cells are positive (Langerin+, CD1a-); (2) liver: some sinusoid-lining cells are positive.

SELECTED PROBLEMS IN DIAGNOSIS OF HISTIOCYTIC/ DENDRITIC CELL NEOPLASMS

Acute monocytic/ monoblastic leukemia or histiocytic sarcoma ?

Immunophenotypically these two entities are indistinguishable. Acute monocytic/ monoblastic leukemia shows a predilection to infiltrate skin and oral mucosa. In general, monocytic sarcoma (tissue infiltration by acute monocytic/ monoblastic leukemia) is characterized by the following features which differ from those of histiocytic sarcoma:

- (1) Much smaller cells and less cytoplasm
- (2) Cells often appear monotonous
- (3) Often single file pattern of infiltration in some areas
- (4) Peripheral blood involvement (if present)

Broadened morphologic spectrum of follicular dendritic cell sarcoma

Follicular dendritic cell (FDC) sarcoma is a neoplasm showing FDC differentiation. It can occur in a wide variety of sites (lymph nodes and extranodal sites). It is a low to intermediate grade malignancy. This tumor has a broad morphologic spectrum – and is still an underrecognized entity.

Classical histology:

- Well circumscribed or invasive
- Pushing rather than permeative borders
- Growth pattern varied:
- Storiform (commonest; mimicking malignant fibrous histiocytoma)
- Fascicular
- Whorled (mimicking meningioma)
- Diffuse
- Follicular / Nodular
- Spindly, ovoid or polygonal cells
- Cell borders often poorly defined; cytoplasm can show fibrillary quality
- Round/ovoid nuclei; thin nuclear membrane; clear or granular nucleoplasm
- Nuclei tend to show irregular clustering
- Scattered multinucleated giant cells
- Lymphocytes: Sprinkled +/- perivascular cuffing

Cytologic variants:

- Polygonal cells with abundant hyaline cytoplasm (mimicking melanoma)
- Tumor cells with distinct cell borders
- Oncocytic (mimicking various oncocytic tumors)
- Clear cells
- Pleomorphic/anaplastic variant (mimicking anaplastic carcinoma)

Growth pattern variations:

- Plexiform pattern
- Formation of tumor islands, mimicking lymphoepithelial carcinoma
- Dispersed tumor cells, mimicking Hodgkin lymphoma

Uncommon stromal features:

- Prominent fibrous septa/lobulation, mimicking thymoma or CASTLE
- Prominent vasculature, mimicking neuroendocrine tumor
- Pseudovascular spaces, mimicking vascular tumors
- Myxoid change, mimicking myxoid chondrosarcoma or other myxoid sarcomas

Unusual reactive cellular background:

- Prominent plasma cell infiltration
- Interspersed reactive osteoclastic giant cells
- Nodular B-cell-rich variant (mimicking nodular lymphocyte predominant Hodgkin lymphoma)
- Inflammatory pseudotumor-like FDC tumor: variant with distinctive clinicopathologic features (women; liver and spleen; sometimes weight loss and fever; indolent relapsing course)

Inflammatory pseudotumor-like FDC tumor: Further deceptive morphology

- Numerous eosinophils masking the neoplastic cells
- Numerous epithelioid granulomas masking the neoplastic cells

How to confirm diagnosis of FDC sarcoma?

- Cytokeratin: –
- Traditional FDC markers: CD21, CD35 (can be difficult to demonstrate if FDCs are dispersed)
- Newer markers (but which are not entirely specific):
 - Clusterin
 - D2-40 / podoplanin
 - CXCL13

Difficulties in diagnosis of interdigitating and fibroblastic dendritic cell sarcoma

In contrast to FDC sarcoma, which has specific immunohistochemical markers (such as CD21, CD35), immunohistochemical markers for interdigitating and fibroblastic dendritic cell tumors are rather nonspecific. Thus their diagnoses are often only presumptive or tentative.

Interdigitating dendritic cell sarcoma

The immunophenotypic profile of interdigitating dendritic cell sarcoma is: S100 ++; CD68 +/-; CD1a -; Langerin -; FDC markers - (by definition). In the S100 immunostain, one should look for presence of long/branched dendritic processes. One should also obtain further support of diagnosis by demonstrating additional hemolymphoid markers, e.g. CD45, CD43, CD4. Many cases originally thought to represent nodal interdigitating dendritic cell sarcoma turn out to be metastatic melanoma, thus it is helpful also to stain for melanoma markers (for exclusion). Electron microscopy is also helpful for looking for the dendritic cell processes.

Fibroblastic dendritic cell tumor

This is a rare nodal spindle cell neoplasm resembling follicular dendritic cell or interdigitating dendritic cell tumor, but lacking follicular dendritic cell markers and S100 protein. A finely collagenous background is often present. The immunophenotype is variable: actin+/-, desmin+/-, CK+/-, CD68+/- . Thus it is extremely difficult to render this diagnosis with great confidence.

Difficulties in classifying some histiocytic/dendritic cell tumors

Some cases may show overlap phenotypes, rendering it difficult even to determine the tumor is of histiocytic or dendritic cell type. Thus there can be hybrid tumors, or still uncharacterized entities besides those currently recognized in the 2008 WHO Classification:

MACROPHAGE

- Histiocytic sarcoma
- Disseminated juvenile xanthogranuloma (?dermal dendritic cell tumor)

DENDRITIC CELL

- Langerhans cell histiocytosis / sarcoma
- Follicular dendritic cell sarcoma

- Interdigitating dendritic cell sarcoma
 - Other rare dendritic cell tumors (fibroblastic, Indeterminate cell)
-

NEW ENTITIES

Indeterminate dendritic cell tumor

Indeterminate dendritic cell tumor is also known as “indeterminate cell histiocytosis”. Single case reports and small series are available in the literature since late 1980’s. Included as an entity in the 2008 WHO Classification of hematolymphoid neoplasms.

Sites of disease: skin (commonest), lymph node, or other sites. Morphology is similar to Langerhans cell sarcoma. While S100 and CD1a are positive, Langerin is negative. It shows a highly variable clinical course, but cutaneous cases tend to be indolent.

ALK+ histiocytosis

ALK+ histiocytosis is rare, recently characterized form of systemic histiocytic proliferation affecting infants. The patients present with pallor, massive hepatosplenomegaly and failure to thrive. Blood counts reveal anemia and thrombocytopenia.

Liver biopsy shows infiltration of the sinusoids (+/- portal tract) by very large histiocytes with irregularly folded nuclei and fine chromatin. Some of these histiocytes show phagocytosis of blood cells or debris. The marrow commonly shows subtle involvement (demonstrable by immunohistochemistry).

The disease resolves slowly (spontaneously or after chemotherapy), but can be life-threatening during active disease

Immunophenotypic features:

- CD68 +
 - CD163 +
 - S100 + (heterogeneous)
 - CD1a –
 - Langerin –
 - ALK1 + (membranous)
-

NEW CONCEPTS ON CELLULAR LINEAGE OF HISTIOCYTIC/ DENDRITIC CELL NEOPLASMS

Histiocytic and dendritic cell sarcomas

WHO Classification 2001 description for histiocytic sarcoma: “The more recent precise phenotypic definition of histiocytic sarcoma requires the absence of clonal immunoglobulin and T-cell receptor genes for the definition of this neoplastic disease”.

This has been modified in the WHO Classification 2008: “Histiocytic sarcomas usually lack clonal *IGH* or *TCR* rearrangements, but rare cases have been reported to show antigen receptor gene rearrangements.”

The current truth: “A substantial proportion of histiocytic sarcomas and dendritic cell tumors exhibit clonal *IGH* rearrangements, and rarely *TCR* rearrangement.” Various histiocytic/dendritic cell neoplasms (e.g. Langerhans cell tumor, histiocytic sarcoma) may develop subsequent to: (1) lymphoblastic leukemia/ lymphoma (B or T), or (2) low grade B-cell lymphoma, especially follicular lymphoma. In such circumstances, the histiocytic/dendritic cell neoplasm often shares the clonal markers of the previous leukemia/lymphoma (such as *IGH* rearrangement, and in the case of follicular lymphoma *BCL2* rearrangement).

Blastic plasmacytoid dendritic cell neoplasm

This new terminology of “blastic plasmacytoid dendritic cell neoplasm” replaces the previous term “blastic NK cell lymphoma”. This entity has also been known as “CD4+ CD56+ hematodermic malignancy”. This entity is placed under the group of “Acute myeloid leukemia and related precursor neoplasms” in the 2008 WHO classification.

So-called blastic NK cell lymphoma actually shows no definite evidence of NK cell differentiation. Recent studies have shown this to represent a tumor of precursor plasmacytoid dendritic cells.

Most patients present with skin lesions. May have simultaneous lymph node, bone marrow or peripheral blood involvement. The disease pursues a relentless course, with frequent relapses despite initial response to chemotherapy (survival only 12-14 months).

Immunophenotype:

- Surface CD3 –
- Cytoplasmic CD3ε -/+
- CD56 +
- CD4 +
- CD123 +
- Myeloid markers -
- TdT + in 60%
- Cytotoxic markers -
- BDCA-2/CD303 +, TCL1 +, CLA +

IgG4-related sclerosing disease: A syndrome with evolving clinicopathologic observations

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WHAT IS IgG4?

IgG4 is the least common (3-6%) of the four subclasses of IgG (IgG1, IgG2, IgG3 and IgG4). The major differences of the four subclasses lie in the composition and structure of the hinge region. The length and flexibility of the hinge region influences the antigen binding and effector functions of the immunoglobulin molecules, consequently determining the different functional characteristics of each IgG subclass, such that IgG4 does not activate complement and has a low affinity for target antigens. The normal physiologic function of IgG4 is still unclear, but it appears to play a significant role in various allergic reactions such as atopic eczema, bronchial asthma and bullous skin lesions. On the other hand, IgG4 may act as a protective blocking antibody to avoid allergen-induced, IgE-mediated effector cell triggering in parasitic infestation.

IgG4 has not attracted much attention of the surgical pathologists until the recent recognition of IgG4-related sclerosing disease as a distinct syndrome. The saga of the IgG4-related sclerosing disease began with autoimmune pancreatitis.

THE CONCEPT OF AUTOIMMUNE PANCREATITIS

The concept that some case of chronic pancreatitis had an autoimmune etiology was proposed by Sarles *et al* in 1961, and Yoshida *et al* subsequently refined the concept and proposed the term “autoimmune pancreatitis”. Since then autoimmune pancreatitis has been increasingly recognized as a distinct entity, characterized by tumor-like mass lesion of the pancreas with narrowing of the pancreatic ducts, clinical manifestation of painless obstructive jaundice, and favorable response to steroid therapy.

Some early reports suggested that some patients with autoimmune pancreatitis are associated with other autoimmune diseases such as Sjogren syndrome, sclerosing cholangitis, primary biliary cirrhosis and inflammatory bowel disease. Serum autoantibodies such as antinuclear antibody (43%-75%), rheumatoid factor (13%-30%), carbonic anhydrase II (30-59%) and lactoferrin (50-76%) are commonly detected. Autoimmune pancreatitis has been shown to be associated with HLA DRB1*0405-DQB1*0401 haplotype in the Japanese population.

It soon became evident that what has been known as “autoimmune pancreatitis” is probably not a homogeneous entity, but includes at least two broad clinicopathologic subtypes.

(1) One subtype, referred to as lymphoplasmacytic sclerosing pancreatitis or predominantly lobular autoimmune pancreatitis, mainly affects elderly male (mean age 63.4 years) and is commonly associated with salivary gland enlargement. It is characterized by dense lymphoplasmacytic infiltrates and sclerosis involving all compartments of the pancreas (including ducts, lobules, connective tissue, blood vessels and common bile duct) as well as obliterative phlebitis.

(2) The other subtype, referred to as idiopathic duct-centric chronic pancreatitis, predominantly ductal autoimmune pancreatitis or autoimmune pancreatitis with granulocytic

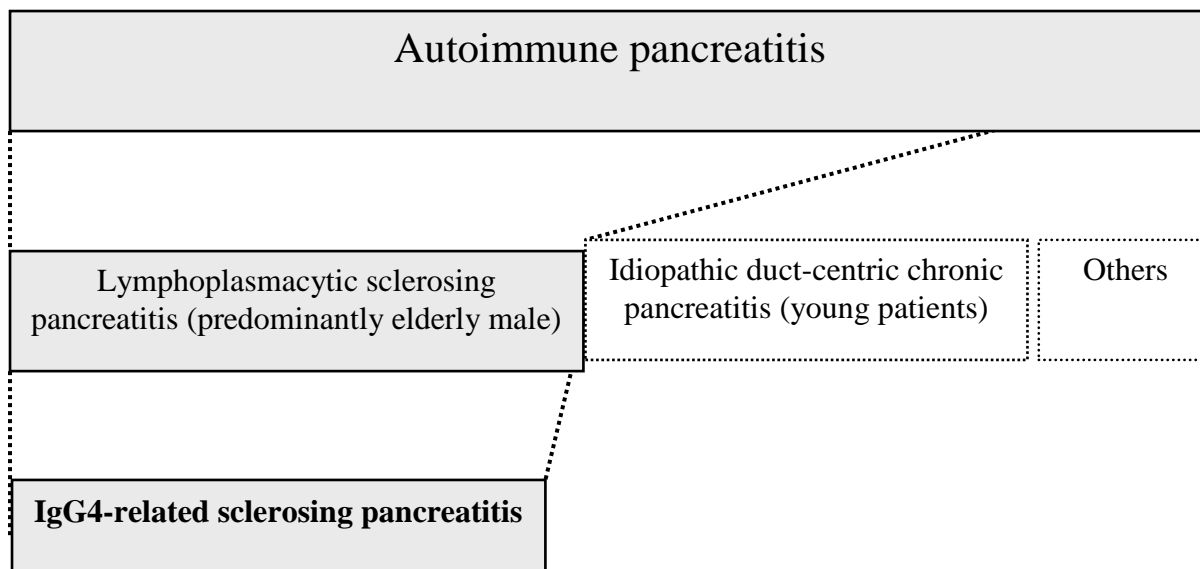
epithelial lesion, affects younger patients (mean age 48 years) with no gender predilection, and is associated with inflammatory bowel disease. The inflammatory infiltrate comprises abundant chronic inflammatory cells and neutrophils, predominantly involving the pancreatic lobules rather than interlobular septa, and shows prominent epithelial destruction, while phlebitis is not seen.

Nonetheless, the autoimmune etiology of “autoimmune pancreatitis” is far from settled. The autoantibodies detected in these patients are mostly not disease-specific, and their role in the pathogenesis remains speculative. It is also possible that antibodies against carbonic anhydrase and lactoferrin may develop as a consequence of release of these antigens from the pancreatic acinar cells damaged by the inflammatory process rather than playing a pathogenetic role. Furthermore, the predilection for elderly men is most unusual for an autoimmune disease. The dramatic response to steroid therapy further suggests that hypersensitivity reaction may represent a more plausible pathogenetic mechanism.

IgG4-RELATED SCLEROSING PANCREATITIS - A MORE ACCURATE NOMENCLATURE

In 2001, Hamano *et al* reported that serum IgG4 was specifically elevated in patients affected by sclerosing pancreatitis (another term for autoimmune pancreatitis), but not pancreatic carcinoma, non-specific chronic pancreatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Sjogren syndrome and normal subjects. In parallel to the serum IgG4, IgG4-expressing plasma cells in the involved tissues are markedly increased in a subset of autoimmune pancreatitis, histologically corresponding to the lymphoplasmacytic sclerosing pancreatitis type, but not in idiopathic duct-centric chronic pancreatitis, non-specific chronic pancreatitis or pancreatic carcinoma. Thus, IgG4 helps define a clinically and morphologically specific subtype of autoimmune pancreatitis, which we propose the terminology of “IgG4-related sclerosing pancreatitis” since the term “autoimmune pancreatitis” as used in the literature refers to heterogeneous entities and the notion of an autoimmune nature may not be correct.

Evolution of concept of IgG4-related sclerosing pancreatitis



Clinical features

IgG4-related sclerosing pancreatitis is a rare disease mainly affecting elderly men (mean age 59-68 years, M:F ratio 4:1 to 7.5:1). The prevalence rate is 2-11% among patients with chronic pancreatitis, but the disease accounts for 21-34% of pancreatoduodenectomies performed for benign conditions because the disease mimics pancreatic carcinoma clinically and radiologically. The majority of patients present with painless obstructive jaundice and/or pancreatic mass, features closely mimicking pancreatic carcinoma. Disturbed endocrine or exocrine functions of the pancreas manifesting as new-onset type II diabetes mellitus or steatorrhea are seen in some cases. Systemic symptoms such as fever, weight loss or generalized malaise are rare. Computed tomography typically reveals focal or diffuse enlargement of the pancreas, predominantly at the pancreatic head. Endoscopic retrograde cholangiopancreatography typically shows irregular narrowing of the pancreatic duct, often accompanied by stenosis of the common bile duct.

Morphologic features

The involved pancreas is firm with mass-like enlargement and tan-whitish cut surfaces. The histologic triad is (1) dense lymphoplasmacytic infiltrate, (2) fibrosis and (3) obliterative phlebitis. The inflammatory infiltrate comprises mainly plasma cells, small lymphocytes and variable numbers of eosinophils, and tends to accentuate in the periductal region while sparing the ductal epithelium. The lymphoid infiltrate often forms reactive follicles. Periductal and stromal sclerosis is another prominent feature, taking the form of broad fibrous bands or collagenous bundles, although the stroma can be loose with edema in some cases. The ductal lumen, as a result of inflammatory infiltrate and fibrosis, becomes irregularly narrowed giving rise to the characteristic imaging and endoscopic findings. Phlebitis, featuring transmural inflammatory infiltrate of the veins with eventual obliteration of the lumen, is another cardinal feature. The arteries that lie adjacent to the veins are consistently spared, but they are useful for recognizing the accompanying obliterated veins. Elastic stain may be required to highlight the fragmented elastic lamina of the obliterated

veins in the form of arrays of interrupted corrugated fibers. With time, the involved pancreas is replaced by fibrous tissue and the lobular architecture is also lost.

Presence of large numbers of IgG4+ plasma cells represents a specific and defining feature of IgG4-related sclerosing pancreatitis. Assessment of the numbers of IgG4+ plasma cells appears to be even more reliable than serum IgG4 assay (normal < 135mg/dL), since serum IgG4 can be normal in up to 30% of patient with this disease, while it can be mildly elevated in 7-10 % of patients with pancreatic carcinoma. The cut-off number of IgG4+ plasma cells is not universally standardized, but a number greater than 30 per high power field has been reported to be reasonably specific. Alternatively, the proportion of IgG4+/IgG+ cells may be more discriminating than the absolute IgG4+ cell count -- using a cut-off percentage ranging from 10%-47%, the sensitivity and specificity for the diagnosis is 83%-86% and 95-96% respectively.

Diagnostic criteria

An accurate preoperative diagnosis of IgG4-related sclerosing pancreatitis can spare the patient a major operation for this steroid-responsive disease. Since it is difficult to obtain specimens from the pancreas, ampullary biopsy can be a useful alternative, although false negative result is one major issue because the pancreas can be involved in a patchy fashion. Therefore, IgG4-related sclerosing pancreatitis is usually diagnosed by a combination of clinical, laboratory, imaging and histologic features. The diagnostic criteria have undergone frequent revisions and vary in different countries as evidenced by the criteria adopted in Japan, Korea, Italy and United States. In essence, the histologic triad of lymphoplasmacytic infiltrate, sclerosis and phlebitis plus numerous IgG4+ plasma cells represent the consensus gold standard for the diagnosis.

IgG4-RELATED SCLEROSING PANCREATITIS IS BUT ONE MANIFESTATION OF SYSTEMIC IgG4-RELATED SCLEROSING DISEASE

IgG4-related sclerosing pancreatitis is frequently associated with extrapancreatic lesions (49%- 80% of cases). Common symptoms include intrahepatic biliary strictures, retroperitoneal fibrosis, salivary gland enlargement, and hilar lymphadenopathy. Traditionally “autoimmune pancreatitis” has been described as being associated with autoimmune diseases such as primary sclerosing cholangitis, Sjogren syndrome, and retroperitoneal fibrosis. However, these “associations” are not genuine but represent systemic manifestations of IgG4-related sclerosing disease. The involved organs show morphologic features similar to those in the pancreas, with the common thread linking these manifestations being tissue infiltration by abundant IgG4+ cells. In addition, increased IgG4+ plasma cells can be found in asymptomatic, apparently uninvolved organs such as the nasopharynx, gastric mucosa, colonic mucosa, and bone marrow in patients with IgG4-related sclerosing disease, supporting the systemic nature of the disease. On the other hand, extrapancreatic lesions with identical morphology and increased tissue IgG4+ cells in the absence of IgG4-related sclerosing pancreatitis are increasingly recognized.

Therefore, IgG4-related pancreatitis represents but one manifestation of a systemic disease sharing similar morphologic features and unified by increased IgG4+ plasma cells. The extrapancreatic lesions can co-exist, precede or develop subsequent to a diagnosis of IgG4-related sclerosing pancreatitis, or without pancreatic disease at all. Multisystem involvement over time is common. In fact, in our experience, lacrimal gland, salivary gland and lymph node involvement are more common than pancreatic involvement.

OTHER (EXTRAPANCREATIC) LESIONS OF IgG4-RELATED SCLEROSING DISEASE

Hepatobiliary system

Sclerosing cholangitis is a heterogeneous entity that may be associated with bile stones, infection, parasitic infestation and drug-induced damage. The term “primary sclerosing cholangitis” is usually reserved for cases without a known cause. It mainly affects male patients in their early adulthood, and is frequently associated with ulcerative colitis. The disease course is progressive, eventually leading to biliary cirrhosis. Steroid therapy is usually ineffective. In contrast, IgG4-related sclerosing cholangitis predominantly affects elderly male, usually involves the extra-hepatic segment of the bile duct, is frequently associated with pancreatic involvement, and demonstrates good response to steroid. The onion-skin type fibrosis typical of primary sclerosing cholangitis is not seen. In retrospect, IgG4-related sclerosing cholangitis probably accounts for at least some cases of “primary sclerosing cholangitis” reported in the past that were associated with pancreatitis and responded to steroid.

The gall bladder can also be affected, so-called acalculous lymphoplasmacytic cholecystitis. There is prominent transmural lymphoplasmacytic infiltration, which may be predominantly extramural.

The liver can be involved in the form of a mass lesion, which has been described as “inflammatory pseudotumor”, but probably more accurately described as an inflammatory fibrosclerosing process. There is accompanying intrahepatic IgG4-related sclerosing cholangitis.

Orbit (including lacrimal gland)

The orbit is commonly involved by IgG4-related sclerosing disease, either centered on lacrimal gland with or without extraglandular extension (also known as IgG4-related chronic sclerosing dacryoadenitis) or the orbital soft tissues. The patient typically presents with bilateral or unilateral painless eye swelling with no significant impairment of visual acuity or keratoconjunctivitis sicca symptoms. Clinically, the disease is indistinguishable from low grade lymphoma. Long-standing disease may lead to optic nerve atrophy and blindness.

Morphologically, lacrimal gland involvement shares the same features of IgG4-related sclerosing disease in other parts of the body, except that obliterative phlebitis is found only rarely. In the early phase, the lacrimal gland commonly shows topographic variations in degrees of involvement by the inflammatory process, that is, while some lobules are markedly involved, some lobules are relatively spared. The interlobular septa are thickened by collagenous tissue. The lobules show atrophy and loss of acini, and moderate to heavy infiltration by lymphocytes and plasma cells, accompanied by variable degrees of sclerosis. The residual ducts commonly exhibit periductal sclerosis. Scattered reactive lymphoid follicles are present. As the disease progresses, lobular atrophy and sclerosis become more prominent, while the inflammatory component may become less prominent. In advanced cases, there are can be extensive areas of dense sclerosis. The fibrosclerosing process can also extend beyond the gland into the orbital soft tissues.

When the disease involves the orbital soft tissues only (often inaccurately referred to as “orbital inflammatory pseudotumor”), the lesion has ill-defined borders and is dominated by sclerosis. There is patchy chronic inflammatory cell infiltration, with or without follicle formation. Phlebitis is a common finding.

Salivary gland

The major salivary glands, which are analogous and morphologically similar to lacrimal glands, are frequently involved by IgG4-related sclerosing disease, especially the

submandibular and parotid glands. The pathologic features are also identical to those seen in the lacrimal glands. Involvement of the submandibular glands may present as unilateral or bilateral hard masses clinically indistinguishable from a neoplasm, clinically known as Kuttner tumor (chronic sclerosing sialadenitis).

Retroperitoneum, mesentery, mediastinum and aorta

Retroperitoneal and mediastinal fibrosis are idiopathic sclerosing mass-forming lesions involving the soft tissues of the retroperitoneum or mediastinum. Chronic periaortitis, a closely-related condition, mainly affects the adventitial layer of the abdominal aorta with or without aneurysm formation and dense adhesion to the adjacent retroperitoneal structures. Sclerosing mesenteritis, also known as mesenteric lipodystrophy, mesenteric panniculitis, retractile mesenteritis or mesenteric fibrosis, is a mass-forming lesion mainly affecting the mesentery of the small bowel, characterized by fibrosis, chronic inflammation and fat necrosis. These entities are considered interrelated, representing different manifestations of an inflammatory fibrosclerosing lesion, characterized histologically by lymphoplasmacytic infiltrate, sclerosis and phlebitis. Other components of this disorder may include sclerosing cholangitis, Riedel thyroiditis, orbital pseudotumor and fibrosis of the salivary glands. It is no wonder therefore retroperitoneal fibrosis, peri-aortitis or inflammatory abdominal aortic aneurysm, mediastinal fibrosis, and sclerosing mesenteritis are recently shown to represent manifestations of IgG4-related sclerosing disease in a substantial proportion of cases.

Thyroid gland

Riedel thyroiditis is an intriguing entity that has become vanishingly rare in the last decade. Involvement of the thyroid gland by IgG4-related sclerosing disease with histologic proof has not been reported so far, although Hamano did report hypothyroidism in 22% of patients with IgG4-related sclerosing pancreatitis and the thyroid function was restored by steroid therapy. Recently, a proportion of cases of Hashimoto thyroiditis are reported to have increased IgG4+ plasma cells, and the relationship of such cases with IgG4-related sclerosing disease, if any, remains to be clarified.

Lymph node

Lymphadenopathy is common in IgG4-related sclerosing disease, and is in fact detectable by imaging in 80% of patients. Usually multiple lymph node groups are involved, the commonest being mediastinal, intra-abdominal, and axillary. The lymph nodes are generally not very large (less than 2 cm in size). Despite the systemic nature of the disease, fever and constitutional symptoms are usually absent, and serum lactate dehydrogenase is not raised or only minimally raised. We have described three morphologic patterns: Type I (multicentric Castleman disease-like), Type II (usual follicular hyperplasia), and Type III (interfollicular expansion). Sato and colleagues classify the cases into two types: interfollicular (corresponding to Types I and III, with the latter type referred to as “atypical lymphoplasmacytic and immunoblastic proliferation-like”) and intrafollicular (corresponding to Type II). We prefer our morphologic subdivision over Sato’s scheme, since it is not that logical to lump Type I and Type III together. In contrast to other organs, fibrosis is practically never seen in the involved lymph nodes.

	Type I (multicentric Castleman disease-like)	Type II (usual reactive follicular hyperplasia)	Type III (paracortical expansion)
Architecture	Intact	Intact	Distorted
Follicles	Hyaline-vascular follicles and usual hyperplastic follicles	Usual hyperplastic follicles	Scanty and often atrophic

Interfollicular zone	Rich in mature plasma cells	Rich in small lymphocytes and/or mature plasma cells	Increased high endothelial venules. A mixture of small lymphocytes, immunoblasts, mature plasma cells and immature plasma cells
CD20 and CD3 immunostains	CD20 immunostaining highlights the follicles, while CD3 the interfollicular zone	CD20 immunostaining highlights the follicles, while CD3 the interfollicular zone	Interfollicular zone comprises a mixture of CD20+ and CD3+ cells (including small cells and large cells)

* Increased IgG4+ cells. Staining for kappa and lambda immunoglobulin light chains shows a polytypic pattern

Lung

Lung involvement by IgG4-related sclerosing disease may be asymptomatic or may present as dyspnea, hemoptysis, pleural effusion or chest discomfort. Vague lung nodules or alveolar consolidation with a peribronchial distribution and perihilar predominance are found on imaging studies. Enlarged mediastinal and hilar lymph nodes are also common. Histologically, lung involvement is characterized by interstitial pneumonia or a localized lung mass rich in IgG4+ plasma cells and lymphocytes. Non-destructive and non-granulomatous inflammation of veins as well as arteries is a prominent and distinctive feature. Prominent lymphatic dilatation with histiocytes showing emperipolesis of lymphocytes are also seen.

Kidney

Patients with kidney involvement present with impaired renal function with or without mild proteinuria. Imaging reveals ill-defined nodular masses in the kidneys, sometimes leading to nephrectomy for exclusion of renal malignancy. Zonal tubulointerstitial nephritis rich in lymphoplasmacytic infiltrate and fibrosis associated with tubular atrophy is the commonest morphologic finding. IgG4 deposits can be demonstrated on the peritubular basement membrane as well as amorphous electron-dense granules by electron microscopy. The glomeruli are usually spared, but concurrent membranous nephropathy with IgG4-containing immune complex deposition has occasionally been reported -- it is uncertain whether the glomerulonephritis represents part of IgG4-related nephropathy or merely a co-incidental occurrence. The tubulointerstitial nephritis responds favorably to steroid, whereas the membranous glomerulonephritis shows variable response.

Breast

Breast involvement manifests as painless mass lesions in one or both breasts. Histologically, there are dense lymphoplasmacytic infiltrates, prominent stromal sclerosis and loss of breast lobules. Reactive lymphoid follicles are often intermingled. The lymphoid infiltrate can be so dense as to mimic malignant lymphoma.

Male genital tract

The prostate and seminal vesicles may show involvement by IgG4-related sclerosing disease, characterized histologically by lymphoplasmacytic infiltration and mild sclerosis.

Central nervous system

Uncommonly, the central nervous system can be involved. Some patients develop hypophysitis presenting as mass lesion with optic nerve compression, hypopituitarism or central diabetes insipidus. Involvement of the epidural tissue and dura can also occur (IgG4-related sclerosing pachymeningitis), and it appears at least a proportion of cases diagnosed as idiopathic hypertrophic pachymeningitis represent this entity.

Skin and soft tissues

Uncommonly, skin involvement can occur. The patients present with plaques or nodules in the head and neck region. Histologically, there is dermal and subcutaneous involvement by a nodular lymphoid infiltration often interspersed with lymphoid follicles and sclerotic stroma. The infiltrate is rich in plasma cells, small lymphocytes, and sometimes plasmablasts. There is invariably accompanying sclerosis. Thus IgG4-related sclerosing disease is also one of the possible etiologies of cutaneous pseudolymphomas.

Soft tissues can also be affected, such as cervical soft tissues. The histologic features are similar to those of retroperitoneal fibrosis or mediastinal fibrosis. Nerves are commonly entrapped by the sclerosing inflammatory process.

DIAGNOSTIC CRITERIA OF IgG4-RELATED SCLEROSING DISEASE

Our proposed histologic diagnostic criteria are listed in Table 1. These criteria are relatively stringent, and might result in exclusion of rare cases of IgG4-related sclerosing disease.

Why not just an absolute count of IgG4+ plasma cells, but require an additional IgG4+/IgG+ cell percentage? Although IgG4+ cells normally account for only ~5% of all IgG+ cells, they can be present in significant numbers in any inflammatory lesion containing numerous plasma cells (most of which express IgG); in such circumstance, the percentage will still be within the normal range.

Then why not just accept a high IgG4+/IgG+ percentage as being diagnostic of IgG4-related sclerosing disease? In lesions with a very low number of plasma cells, a misleadingly high percentage of IgG4+/IgG+ may be sometimes be obtained, e.g. 3 IgG4+ cells/HPF, and 6 IgG+ cells/HPF, giving a high percentage of 50%. Therefore we require both an increase in absolute number of IgG4+ plasma cells and a raised IgG4+/IgG+ cell proportion in the diagnostic criteria.

Table 1. Histologic diagnostic criteria of IgG4-related sclerosing disease in various anatomic sites

All of the following criteria have to be satisfied:

- Compatible morphology:
 - Extranodal sites[#]
 - a) Lymphoplasmacytic infiltration +/- lymphoid follicle formation
 - b) Sclerosis
 - c) +/- Phlebitis
 - d) No significant population of proliferated myofibroblasts
 - Lymph node
 - a) Increased plasma cells
 - b) Usual reactive lymphoid follicles +/- hyaline-vascular follicles or interfollicular expansion with increased activated lymphoid cells
- Absolute number of IgG4+ cells >50/HPF*
- Percentage of IgG4+/IgG+ cells >40%*

[#] Not all features may be present in small biopsies

* For enumeration of IgG4+ or IgG+ cells, select areas with the highest density of positive cells. Three high power fields (HPF) are counted, and an average number of positive cells per HPF is calculated. One HPF covers an area of 0.196 mm² (x 40 objective, x 10 eyepiece, 20 mm field of view).

UNRELATED ENTITIES WITH INCREASED IgG4+ CELLS

Increased IgG4+ plasma cells or proportion of IgG4+/IgG+ plasma cells can occasionally be found in lesions probably unrelated to IgG4-related sclerosing disease, such as splenic angiomatoid nodular transformation, Rosai-Dorfman disease, perforating

collagenosis, cutaneous plasmacytosis and rare cases of granulomatous mastitis. The significance of increased IgG4+ plasma cells in these cases is currently unclear.

Training Program for Residents: Diagnostic Pathology and Laboratory Medicine combined

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Introduction

Medical education in Japan is similar to that in Germany. Medical school training consists of a six-year undergraduate medical education. After graduation from medical schools, students work as residents according to the curriculum for two years.

Pathologists in Japan are much fewer than those in the United States. As of October 2008, there were 2,053 pathologists in Japan. Doctors specializing in laboratory medicine are only about 740. In many hospitals in Japan, one pathologist also works as a laboratory medicine doctor, so it is often necessary that a pathologist have a wide range of knowledge of clinical pathology.

Juntendo University Nerima Hospital

Juntendo University Nerima Hospital was founded in July 2005. It is a medium size hospital with 400 beds. Two pathologists diagnose 6,000 cases and perform 40 autopsies a year. It has been contributing to the local community as a center of healthcare in Nerima prefecture, Tokyo. In the hospital, various kinds of medical staffs take part in conferences using electronic records. They discuss the diagnoses and treatments of each case lively. At the same time residents make presentation of clinical courses, biopsies or radiological images. Pathologists and radiologists add comments on their findings.

In the hospital, 25 doctors have been working as residents since April 2008 and many doctors of each department have enthusiasm for education of residents. In the first year of residency, they have to work in internal medicine, surgery, anesthesiology and emergency room. Then, in the second year, they are able to choose any course from the elective courses.

How the residents think of “pathology or pathologists”

Why is the number of pathologists small in Japan? I conducted a questionnaire survey on the image of pathology or pathologist to 19 residents in Juntendo University Nerima Hospital. 13 residents think of “pathology or pathologists” as “a basic medicine”, 13 residents, “high degree of professionalism” and 11 residents, “interesting”. Five residents answered “one of clinical medicines”. Those who think of pathology as “clinical medicine” are fewer than those who think of it as “basic medicine”.

According to the results, I realized the necessity to emphasize that pathology is “a clinical medicine” and *a pathologist is “a clinical doctor”*. So, I have devised a training system for the elective course in my hospital including diagnostic pathology and laboratory medicine combined for the residents.

Training program for residents: diagnostic pathology and laboratory medicine combined

I proposed three training programs to meet the requirement of the residents. Residents study both diagnostic pathology and laboratory medicine in all three programs. In diagnostic pathology, residents can study surgical pathology including intra-operative pathological diagnoses and autopsies. In laboratory medicine, they can study microbiology and the way of physiological examinations, such as ultrasonography. In program A, residents can mainly study diagnostic pathology. In program B, residents mainly study in laboratory medicine. In program C, residents evenly study both.

20 of the 25 residents chose one of these training programs and these three programs became the most popular in the elective courses. Now, two or three residents work at the laboratory every month and they can develop a broad range of knowledge and skills of diagnostic pathology and laboratory medicine.

Conclusion

To increase the number of pathologists in Japan, we should work hard to propose more attractive training programs for residents. I would like to emphasize that a pathologist is not only a basic researcher but also “a clinical doctor”.

Cytology of Pulmonary Neuroendocrine Carcinoma

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Case 1

Clinical History:

A 50 year old female presents with a lung mass. Her past medical history was significant for invasive mammary ductal carcinoma, poorly differentiated, for which she received mastectomy. On follow up, CT Scan of the chest revealed a right middle lobe lung mass, measuring 2 cm. A CT scan guided fine needle aspiration was obtained with immediate assessment. Subsequently a tissue core biopsy was also taken.

Cytologic findings:

The smears show a cellular aspirate, composed of large sheaths of epithelial cells, as well as numerous single cells. The tumor cells demonstrate moderate nuclear pleomorphism with salt and pepper chromatin and occasional cells with prominent nucleoli. Occasional mitotic figures and apoptotic bodies were identified.

Histologic findings:

The core biopsy shows an epithelial neoplasm with intervening hyalinized areas. No necrosis or significant mitotic activity (0-1/10hpf) was identified.

Immunohistochemical studies:

Immunohistochemical studies were performed on the core biopsy. The tumor was positive for synaptophysin, chromogranin and TTF-1. The tumor was negative for estrogen receptors (ER), progesterone receptors (PR), GCDFP-15 and mammoglobin.

FNA Diagnosis:

NEUROENDOCRINE CARCINOMA

Diagnosis:

NEUROENDOCRINE CARCINOMA, FAVOR WELL-DIFFERENTIATED
(CARCINOID TUMOR)

Diagnosis after excision:

MODERATELY DIFFERENTIATED NEUROENDOCRINE CARINOMA
(ATYPICAL CARCINOID)

Case 2

Clinical History:

A 43 year old male presents with a lung nodule. His past medical history is significant for medullary thyroid carcinoma, for which he underwent total thyroidectomy and cervical lymph node dissection.

Cytologic findings:

The smears show a cellular aspirate, composed loosely cohesive clusters and single cells. The cells are round to oval to plasmacytoid with mild to moderate nuclear pleomorphism and granular chromatin.

Histologic findings:

The core biopsy shows an epithelial neoplasm with intervening hyalinized areas. No necrosis or significant mitotic activity (0-1/10hpf) was identified.

Immunohistochemical studies:

Immunohistochemical studies were performed on the core biopsy. The tumor was positive for synaptophysin, chromogranin and calcitonin. The tumor was negative for thyroglobulin.

FNA Diagnosis:

NEUROENDOCRINE CARCINOMA, CONSISTENT WITH METASTATIC
MEDULLARY THYROID CARCINOMA

CASE 3

Clinical History:

A 64 year old female presents with a lung mass. Her past medical history is unremarkable.

Cytologic findings:

The smears show a cellular aspirate, composed loosely cohesive large groups of loosely cohesive tumor cells as well as single cells and numerous naked nuclei. The cells show moderate nuclear pleomorphism with occasional nuclear molding. The chromatin is granular with occasional tumor cells with inconspicuous nucleoli. There are abundant mitotic figures and apoptotic bodies. The background is remarkable for tumor necrosis.

Immunohistochemical studies:

Immunohistochemical studies were performed on the core biopsy. The tumor was positive for synaptophysin, chromogranin, CD56 and TTF-1.

FNA Diagnosis:

SMALL CELL CARCINOMA

Diagnosis after excision:

LARGE CELL NEUROENDOCRINE CARCINOMA

CASE 4

Clinical History:

A 47 year old female presents with right sided chest pain. Her past medical history is unremarkable. Imaging of the chest showed a 12 cm lung mass with enlarged mediastinal lymph nodes and pleural effusion. Patient underwent transthoracic CT scan guided FNA and core biopsy.

Cytologic findings:

The smears show a cellular aspirate, composed of loosely cohesive tumor cells as well as numerous single cells. The cells show mild nuclear pleomorphism. The chromatin is granular with occasional tumor cells with inconspicuous nucleoli. There are abundant mitotic figures and apoptotic bodies.

Immunohistochemical studies:

Immunohistochemical studies were performed on the core biopsy. The tumor was positive for CD99 and bcl-2 with focal immunoreactivity for pan-keratin. The tumor was negative for LCA, S100 and desmin.

FNA Diagnosis:

SMALL ROUND CELL MALIGNANT TUMOR

Diagnosis after excision:

EWING'S SARCOMA/PNET

CASE 5**Clinical History:**

A 58 year old male presents with weight loss and cough. Imaging of the chest showed a 3 cm lung mass with enlarged mediastinal lymph nodes. Patient underwent endobronchial guided FNA and core biopsy.

Cytologic findings:

The smears show a cellular aspirate, composed of loosely cohesive tumor cells as well as numerous single cells. The cells show moderate nuclear pleomorphism. The chromatin is granular with occasional tumor cells with inconspicuous nucleoli. There are numerous apoptotic cell, single cell necrosis and mitotic figures. Nuclear molding is present.

Immunohistochemical studies:

Immunohistochemical studies were performed on the core biopsy. The tumor was negative for keratin with focal immunoreactivity for synaptophysin and CD56. The tumor was negative for LCA.

FNA Diagnosis:

SMALL CELL CARCINOMA

Diagnosis on core biopsy:

SMALL CELL CARCINOMA

DISCUSSION

Neuroendocrine tumors represent a group of neoplasms that share certain ultrastructural, cytologic and molecular phenotype. Neuroendocrine tumors can be classified to two major categories; epithelial and neural tumors. The epithelial tumors include carcinoid tumor, small cell carcinoma, Merkel cell carcinoma, medullary thyroid carcinoma and pancreatic endocrine tumors amongst others. The neural tumors include pheochromocytoma/paraganglioma, pituitary adenoma, neuroblastoma, medulloblastoma and more.

Regardless of the anatomic origin, neuroendocrine tumors share one important feature; they are at least potentially malignant tumors. Therefore, current recommended terminology is different from those used in the past. The term “neuroendocrine carcinoma” is the recommended term for previously known “carcinoid tumor”. For neural tumors however, the nomenclature has not changed and the traditional terminology has been retained.

CLASSIFICATION. Tumors of the lung with neuroendocrine morphology by light microscopy encompass a three-grade spectrum of low-grade typical carcinoid, intermediate-grade atypical carcinoid, and the high-grade LCNEC and SCLC. All of these tumors share varying degrees of neuroendocrine features by light microscopy including organoid nesting, palisading, a trabecular pattern, and rosette-like structures. Neuroendocrine differentiation can be demonstrated by immunocytochemistry or electron microscopy in majority of the neuroendocrine tumors.

The widely variable published terminology and criteria for neuroendocrine lung tumors hinder understanding of this complicated subject. Many terms have been used for neuroendocrine lung tumors, including well-, moderately- and poorly differentiated neuroendocrine carcinoma, and neuroendocrine carcinoma (grade 1-3). The last revision of the diagnostic criteria for neuroendocrine tumors by the World Health Organization and the International Association for Study of Lung Cancer (WHO/IASLC) was in 2004. The current WHO schema³ classifies the pulmonary neuroendocrine tumors into four categories; small cell carcinoma, large cell neuroendocrine carcinoma, atypical carcinoid and carcinoid tumor.

CYTOHISTOLOGIC CRITERIA. The most important criterion for separating well differentiated NEC (typical carcinoid) from moderately differentiated NEC (atypical carcinoid) is mitotic activity. Arrigoni, et al, originally proposed that atypical carcinoids had between 5-10 mitoses per 10 high power fields. Other criteria that were described by Arrigoni

et al were nuclear pleomorphism, necrosis, cellularity, architectural distortion and abnormal nuclear cytoplasmic ratio.¹ While, the mitotic range for pulmonary atypical carcinoid was modified to 2 to 10 mitoses per 2 mm² by the World Health Organization and the International Association for Study of Lung Cancer (WHO/IASLC),²⁻³ other investigators have proposed higher mitotic counts for atypical carcinoid in lung and mediastinum (>3/10 hpf).⁴ A second criterion for atypical carcinoid is necrosis. Cytologic atypia has been shown to be less reliable as a diagnostic feature.

A mitotic count of 11 or more mitoses per 2 mm² is the main criterion for separating LCNEC and SCLC from atypical carcinoid.⁵ LCNEC and SCLC usually have very high mitotic rates, with an average of 70-80 per 2 mm². LCNEC and SCLC also generally have more extensive necrosis than atypical carcinoid. LCNEC are separated from SCLC using a constellation of criteria, which include larger cell size, abundant cytoplasm, prominent nucleoli, vesicular or coarse chromatin, polygonal rather than round or fusiform shape and less prominent nuclear molding. LCNEC cells more closely resemble those of a large cell carcinoma than a carcinoid tumor.

Whether mitoses should be counted in the areas of highest mitotic activity or randomly, has recently been investigated. The current data show that mean mitotic activity, counting several fields including areas with lower and higher mitotic activity, more accurately correlates with the clinical outcome.⁶

With increased number of neuroendocrine tumors of the lung first encountered as aspiration cytology specimens, accurate cytologic identification of these neuroendocrine neoplasms and awareness of the pitfalls becomes more critical.

IMMUNOCYTOCHEMISTRY. The most commonly used immunocytochemical markers include synaptophysin, chromogranin and CD56 (NCAM). Synaptophysin, a molecule associated with synaptic vesicles of neurons, is a sensitive marker that is useful in this context. However, it is not specific and can be expressed in a variety of neural, neuroendocrine and neuroectodermal cells. Chromogranin is specific for neuroendocrine differentiation; however, it lacks optimal sensitivity to detect neuroendocrine differentiation. CD56 or neural cell adhesion molecule (NCAM), although sensitive, is not specific for neuroendocrine cells and can be expressed in a variety of neural, neuroectodermal and hematopoietic cells. Due to lack of optimal sensitivity and specificity, application of a panel of these markers is recommended for the work up of neuroendocrine tumors. Neuroendocrine differentiation can be demonstrated by electron microscopy or immunohistochemistry in virtually all typical and atypical carcinoid tumors. However, this is not always true of the high-grade neuroendocrine tumors such as SCLC. Neuroendocrine granules may be absent by electron microscopy in as many as one third of SCLC and up to 25 percent of SCLC fail to stain with an immunohistochemical panel of neuroendocrine markers.

STAGING. Clinically, all neuroendocrine carcinomas regardless of grade of differentiation are malignant and are capable of lymph node metastasis, distant metastasis and causing death. The current proposed staging system for pulmonary neuroendocrine carcinomas is the TNM staging.⁷ The tendency for widespread metastasis in small cell carcinoma has led to applying a different staging system (limited vs. extensive) for this tumor rather than using the TNM staging.

DIFFERENTIAL DIAGNOSIS

Well differentiated neuroendocrine carcinoma WD-NEC (carcinoid tumor) The differential diagnosis of WD-NEC in FNA biopsies includes benign bronchial epithelial cells, moderately differentiated NEC (atypical carcinoid), small cell carcinoma, metastatic neuroendocrine carcinoma of extrapulmonary origin and adenocarcinoma (primary or metastatic). Spindle cell carcinoid may be confused with mesenchymal tumors. The bland appearance and monotony of the WD-NEC may be mistaken for bronchial cells. However, carcinoid tumor cells are less cohesive and lack cilia.

While diagnosis of WD-NEC (carcinoid) can be favored over MD-NEC (atypical carcinoid) based on cytomorphological features such as cellular monotony, low N/C ratio, granular chromatin, absence of nucleoli, rare mitotic figures and absence of necrosis, a definitive classification may be impossible in FNA sample or surgical lung biopsies, simply due to limited sampling and heterogeneity of the tumor.

A major pitfall is misdiagnosis of WD-NEC as small cell carcinoma in limited FNA sample. While absence of necrosis and significant mitotic activity are helpful features in distinguishing carcinoid tumor from small cell carcinoma, the distinction can be difficult in hypocellular and suboptimal samples. Ki67 immunostaining can be helpful, demonstrating immunoreactivity in more than 50% of the tumor cells in small cell carcinoma.

Carcinoid tumor may be mistaken for adenocarcinoma of primary lung or metastasis from extrapulmonary origin. The monotony of the cells, abundance of single cells, granular chromatin and rare mitotic activity in WD-NEC, as opposed to greater cellular variation, large cohesive cell groups, prominent nucleoli and more frequent mitoses in adenocarcinoma can be helpful in the differential diagnosis. Immunocytochemical studies would commonly show diffuse expression of neuroendocrine markers in the WD-NEC.

Although spindle cell carcinoid may have some resemblance to mesenchymal neoplasms, the abundance of single cells as well as large cell groups and finely granular chromatin are helpful features that raise the possibility of spindle cell carcinoid. Immunocytochemical studies with expression of keratin and neuroendocrine markers are helpful in confirming the diagnosis.

Moderately differentiated neuroendocrine carcinoma MD-NEC (atypical carcinoid tumor) The differential diagnosis is similar to those of WD-NEC (typical carcinoid). A major pitfall is distinction from small cell carcinoma and large cell neuroendocrine carcinoma. In limited FNA or biopsy samples, the distinction of a high grade NEC from the MD-NEC (atypical carcinoid) can be very difficult. In such cases, if a repeat biopsy is not feasible, Ki67 immunostaining can be helpful. Although no uniformly accepted criteria for Ki67 immunostain in pulmonary neuroendocrine carcinomas has been established, a >50% immunoreactivity is highly suggestive of high grade NEC, since majority of WD-NEC and MD-NEC show <20% Ki67 expression.

Large cell neuroendocrine carcinoma (LCNEC) The most commonly encountered differential diagnosis is small cell carcinoma. According to the WHO schema, LCNEC is classified under the group of “large cell carcinoma”, a variant of non-small cell carcinoma. The distinction of the LCNEC from SCLC however can be very difficult, and in some cases impossible. It is controversial whether the distinction between SCLC and LCNEC has clinical value, as patients with both neoplasms are being treated with similar therapeutic protocols.

The commonly used cytological criteria are nuclear size, nuclear pleomorphism, nuclear molding, chromatin, nucleoli and amount of cytoplasm. The key cytologic features are pleomorphic cells in large groups as well as single cells and bare nuclei with occasional nuclear molding. The tumor cells show abundant mitotic figures and necrosis. The cells may occasionally show rosette like structures that can be confused with glands. The chromatin is often coarsely granular with prominent nucleoli in some cases.

Studies have shown that there is considerable overlap in cytomorphologic features between SCLC and LCNEC which contributes to considerable difficulty in separating these variants of high grade neuroendocrine lung tumors. Improved diagnostic criteria and future prospective clinicopathologic studies are needed to validate the concept that patients with LCNEC have a different clinical course from that of those with SCLC.

Small cell carcinoma (SCLC) The most commonly encountered entities in the differential diagnosis are non-small cell carcinoma and small blue cell tumors such as lymphoma, melanoma and sarcoma, namely Ewing’s/PNET and rhabdomyosarcoma. Non-small cell carcinoma, particularly large cell neuroendocrine carcinoma and squamous cell carcinoma, basaloid type; are frequently encountered in the differential diagnosis. Cytological features such as nuclear molding and abundant single cell necrosis, although not specific, are characteristic. Immunocytochemical study and molecular studies, namely RT-PCR and FISH (fluorescent in situ hybridization) are valuable techniques in establishing the correct diagnosis.

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Histological Diagnosis of Autoimmune Liver Diseases and Sclerosing Cholangitis

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Autoimmune liver diseases consist of autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Precise diagnosis of autoimmune liver diseases is important for the differences of the treatment of the patients; immunosuppressive therapy in AIH and ursodeoxycholic acid therapy in PBC and PSC. So, in the present lecture, I talk with practical diagnostic procedures for AIH and PBC based on my own experiences, thereafter I describe the new information and diagnostic points of sclerosing cholangitis including PSC.

(1) Diagnostic procedures of AIH

AIH is a liver disease with immunological reactions targeting to hepatocytes. The diagnostic criteria of AIH using scoring system was proposed in International AIH group in 1999,¹⁾ and more recently a simplified criteria by the group was reported in 2008.²⁾ The new criteria consisted of presence of autoantibodies (ANA, SMA, LKM, SLA), increase of serum IgG, absence of viral hepatitis and liver histology.²⁾ So, liver histology is very important for the diagnosis of AIH. Histological findings for diagnosis of AIH are lymphocytic infiltrate with interface hepatitis of moderate or severe degree, intense infiltrate of plasma cells and confluent necrosis. Especially, intense infiltrate of plasma cells is a very important finding for the diagnosis of AIH. The case of AIH with intense infiltrate of plasma cells has a good response to immunosuppressive therapy.

(2) Diagnostic procedures of PBC

PBC is an immunological disorder that characterized by the positivity of antimitochondrial antibodies (AMA), and the presence of non-suppurative destructive cholangitis in intrahepatic bile ducts, especially in the interlobular bile duct. About 10 % frequency, the cases with PBC showed AMA-negative PBC. For the histological diagnosis of PBC, the detection and evaluation of non-suppurative destructive cholangitis are important. Non-suppurative destructive cholangitis occurs segmentally, thus serial and deep cut section examinations are useful for the detection. Among the classification of non-suppurative cholangitis, the classification of Ludwig³⁾ is useful for the routine diagnosis of PBC. The classification consisted of granulomatous cholangitis, lymphoid cholangitis, fibrous cholangitis (fibrous-obliterative cholangitis as for the subtype), and pleomorphic cholangitis, and granulomatous cholangitis occurs PBC only.³⁾

(3) New information and diagnostic points of sclerosing cholangitis

PSC is a main disorder among sclerosing cholangitis. However, recently, Zen et al. reported IgG4-related sclerosing cholangitis (IgG4-SC).⁴⁾ Histological features in intrahepatic large bile duct and hepatic hilar/or extrahepatic bile ducts in IgG4-SC have been highlighted. The histological findings in IgG4-SC consist of extensive and dense fibrosis with marked lymphoplasmacytic infiltrate, many eosinophils and obliterative phlebitis.⁴⁾ Immunohistochemically, many IgG4-positive plasma cells are

found in the bile duct lesion.⁴⁾ By contrast, in PSC, IgG4-positive plasma cells are scarce or hardly found, and fibrous thickening by inflammatory cell infiltrate and fibrosis occurs in the affected bile duct, but inflammatory cell infiltrate is more intense on luminal side.⁴⁾ The precise evaluation of these differences between IgG4-SC and PSC is important for the histological diagnosis of sclerosing cholangitis. Moreover, in PSC, intrahepatic bile ducts are frequently affected, and non-suppurative cholangitis occurs in the injured duct, and fibrous-obliterative cholangitis is present PSC only. In this lecture, I present cases of IgG4-SC and PSC using clinical and pathological pictures that were offered from the members of Liver Disease Working Group – Kanto (The group consisted of clinicians and pathologists who specialize liver disease in Kanto region)

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Histological features of early hepatocellular carcinomas and their developmental process; For daily practical clinical application

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Recently, the following two papers concerning early hepatocellular carcinomas (early HCCs; hepatocellular carcinomas in the early stage) have been published.

- 1) International Consensus Group for Hepatocellular Neoplasia: Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 49: 658-664, 2009
- 2) Kondo, F: Histological features of early hepatocellular carcinomas and their developmental process: for their daily practical clinical application. *Hepatol Int* 3: 283-293, 2009

The former paper reported that the members of the International Consensus Group for Hepatocellular Neoplasia (including myself) reached a consensus on the concept and histological criteria of early HCCs. The latter paper described the concept and the histological features of typical early HCCs precisely. Their developmental process was also presented. Intended especially for daily clinical practice, the total process of the histological diagnosis of early HCCs, various difficult cases, and the points of differentiation were described.

I will present a lecture based on the above two articles. The following abstract is a quotation from the latter paper.

Based on clinical and pathological experience, indistinct margin-type hepatocellular carcinomas (HCCs) were considered to be typical early-stage HCCs with good prognosis. For histological diagnosis, the assessment of stromal invasion (tumor invasion into portal tracts and fibrous septa) is very important. In differentiating stromal invasion from pseudo-invasion (benign hepatic tissue in the fibrous stroma), the following 5 items are useful: 1) macroscopic or panoramic views of the histological specimen, 2) amount of fibrous components of the stroma, 3) destruction of the structure of portal tracts, 4) loss of reticulin fibers around cancer cells, and 5) cytokeratin 7 immunostaining for ductular proliferation. Parenchymal features of early HCCs are summarized as 1) thin trabecular structure, 2) hypercellularity, 3) hyperstainability of cytoplasm, and 4) microacinar formation. Detailed understanding of the total biopsy procedure and various difficult lesions is necessary for a correct biopsy diagnosis. Evaluation of non-cancerous tissue is also required to attain a better understanding of the developmental process and clinical stages of HCC.

BRONCHIOLOALVEOLAR CARCINOMA

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DEFINITION

The definition of BAC has somewhat changed over the years. In 1960, Liebow not only coined the term Bronchiolo-alveolar carcinoma but also provided a definition to diagnose this particular neoplasm. Liebow's definition of BAC was that of a well-differentiated adenocarcinoma, and added that the distinction between this type of tumor and the "ordinary" adenocarcinoma as far as cell of origin was not clear. In addition, Liebow reinforced the concept that there are three main forms of this neoplasm, single nodular, disseminated nodular, and diffuse. Also, stated that this particular neoplasm is capable of invading lymph nodes, pleura, and extra-thoracic organs. This latter feature may be seen in over 50% of the cases at death. In Liebow's opinion, the tumor may have a long dormancy or slow growth, particularly those presenting as isolated nodules. He also alluded to the fact that 50% of patients present with bilateral disease. In 1980, in the fascicle of tumors of the lower respiratory tract of the Armed Forces Institute of Pathology, the definition offered was that of "a lesion with relatively bland cytologic features that arises in the periphery of the lung and spreads on the walls of the distal air spaces." In the 1995 fascicle of tumors of the lower respiratory tract, second series, of the Armed forces Institute of Pathology, the definition is that of a subset of adenocarcinoma common and distinctive enough to warrant separation from the other subtypes. In the two most recent publications of the World Health Organization (WHO) the tumor is defined as an adenocarcinoma with bronchioloalveolar pattern and no evidence of stromal, vascular, or pleural invasion.

Based on those publications, one can gather that the diagnosis of BAC has changed to the point of making that diagnosis possible only by complete examination of the tumor in question and not on biopsy material.

GROSS FEATURES

Essentially, there are three types of presentations for BAC. 1) the localized form of the disease in which there is a peripheral mass in the lung parenchyma, which may be indistinguishable from any other non-small cell carcinoma in the lung. Usually these tumors are under 3 cm in greatest diameter and do not show areas of necrosis and/or hemorrhage. They are well-defined tumors without encapsulation. The cut surface appears homogenous and tan in color; 2) the multinodular pattern in which the tumor involves extensive areas of the lung parenchyma in a milliary fashion almost mimicking a metastatic disease, the nodules are of varying sizes but they are usually under 1 cm in greatest diameter, this type of presentation may involved a lobe of the entire lung parenchyma; 3) the diffuse form in which the appearance is that of a pneumonic process, in this pattern, the tumor also involves extensive areas of lung parenchyma that may take one lobe or the entire lung parenchyma, no tumor masses or nodules are identified in this presentation and the appearance is that of a non-neoplastic process.

HISTOLOGICAL FEATURES

The histopathologic features of BAC recapitulate those of its gross appearance. The nodular form of the tumor shows at light microscopy the presence of an almost intact normal lung parenchyma; however, closer inspection shows areas in which the alveolar walls are being replaced by either a low cuboidal or cylindrical type of epithelium that lines entirely or partially the alveolar wall and that follows very much the outline of the normal alveolar wall. The tumor does not show increase mitotic activity and/or cellular pleomorphism with nuclear atypia. The proliferation is rather bland but distinct from the normal alveolar lining. The multinodular pattern resembles a metastatic tumor in term of the extensive areas of skip normal lung parenchyma. The tumor nodules appear to be discretely affecting extensive areas of the lung parenchyma but not in a continuous form but rather in a nodular pattern. Either low cuboidal and/or columnar type of mucinous epithelium lines the alveoli; in some areas, it is possible to identify normal alveoli that are filled with an acellular mucinous material. Mitotic figures and cellular pleomorphism with nuclear atypia are not common. Necrosis and/or hemorrhage are not common in this pattern. The diffuse pattern of BAC is almost invariably of the mucinous type, in this pattern two important features may be easily identifiable, one is the presence of extensive areas in which the alveoli are filled with mucinous material containing mucinophages, and two, the presence of alveoli that is being replaced by a columnar mucinous type of epithelium. The low power magnification of this type can be easily misread as some type of pneumonic process. Thus, this type of pattern has been called pneumonic type.

DISCUSSION

The literature is filled with controversy on the topic of this particular tumor so-called Bronchioloalveolar Carcinoma. Although there have been several studies addressing specific issues regarding this tumor, unfortunately, many of those studies have generated more controversy. More recent literature on BAC using the latest definition by the WHO has also provided conflicting information regarding bronchioloalveolar carcinomas. For instance, Breathnach et al in a study focusing on the survival and recurrences of BAC stage I disease found that when compared stage I BAC and adenocarcinoma of the conventional type, the 5-year survival rate for BAC is 83% and 63% for other types of adenocarcinoma. Rena et al also reported similar findings in a study of 28 patients of stage I pure bronchioloalveolar carcinoma in which the authors found a 5-year disease free of 81% and a long term survival of 86% against 51 and 71% of adenocarcinoma of the conventional type respectively. Interestingly, even as the authors state that the WHO criteria was followed in these cases, 20 patients were diagnosed with fine needle aspiration biopsy, which very much goes against the current existing criteria by the WHO. Gaeta et al in a study of 20 cases of bronchioloalveolar carcinoma focusing on pattern of recurrence after surgical resection concluded that diffuse BAC may develop from prior focal carcinoma and that the mucinous type is the one most likely to become diffuse. Interestingly in this study the authors included three different types of BAC – mucinous, non-mucinous, and mixed adenocarcinomas with prominent bronchioloalveolar pattern. However, a study by Ebright et al concluded that the most important predictors of survival in BAC are clinical pattern and pathologic stage rather than degree of invasion on histological examination. More recently, Garfield et al raised concern about the current definition of BAC, mainly in cases with multifocal involvement, and stated that the current definition is inapplicable for patients with stage IIIB and IV. Similar concerns were also raised by Damhuis et al, who found an unfavorable prognosis of stage I BAC with a 5-year survival rate of 24%.

The dissatisfaction with the WHO criteria for the diagnosis of BAC has been made clear by different authors. Sidhu et al (54) in an ultrastructural analysis of 155 cases of BAC stated that the unique characteristic of BAC is its cell type, and that the extent of lepidic growth, degree of differentiation, and degree of stromal desmoplasia cannot be used as definitional requirements. The authors further characterized the current definition of the WHO as a form of *in situ adenocarcinoma*, in which BAC is defined as a pattern and not as an entity and when it is accepted as an entity is only when the lesion is not invasive. Thus, the current criteria negates that BAC has the potential to spread and makes the staging of tumor pathology impractical. Therefore, the authors concluded that with the current definition, BAC may become an extremely rare entity if existing at all. Hajdu went even farther in his opinion about the current WHO definition of BAC by stating “a group of pathologists changed the definition of BAC, it was de facto implied that BAC is carcinoma in situ and that invasive BAC does not exist.”

Based on past and current literature, we consider BAC should be reworded as a pathological entity in which it should be categorically stated that it represents a growth pattern of adenocarcinoma with the potential to spread within lung parenchyma and/or outside of the thoracic cavity. The current criteria of implicitly coding BAC as an In Situ adenocarcinoma is problematic not only from the conceptual point of view but also from the practical aspects of it. It is clear that by contrasting past and current studies, there is no important and/or meaningful gain with the current “new and improved” criteria of the WHO, which essentially, without conducting a meaningful and serious study on BAC, decided to make a change in the histological criteria for diagnosis. Far from its possible intended message, it has created new communication problems between clinicians and pathologists.

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