The Utilization of Cytologic Fine-Needle Aspirates of Lung Cancer for Molecular Diagnostic Testing
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>279</td>
<td>A Review of Inflammatory Processes of the Breast with a Focus on Diagnosis in Core Biopsy Samples</td>
<td>Timothy M. D’Alfonso, Paula S. Ginter, Sandra J. Shin</td>
</tr>
<tr>
<td>288</td>
<td>Pathology Reporting of Thyroid Core Needle Biopsy: A Proposal of the Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group</td>
<td>Chan Kwon Jung, Hyo Jin Park, Dong Eun Song, Jang Hee Kim, So Yeon Park, Hyunju Yoo, Mi Kyung Shin, Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group</td>
</tr>
<tr>
<td>300</td>
<td>The Utilization of Cytologic Fine-Needle Aspirates of Lung Cancer for Molecular Diagnostic Testing</td>
<td>Michael H. Roh</td>
</tr>
<tr>
<td>310</td>
<td>Analysis of Histologic Features Suspecting Anaplastic Lymphoma Kinase (ALK)-Expressing Pulmonary Adenocarcinoma</td>
<td>In Ho Choi, Dong Won Kim, Sang Yun Ha, Yoon-La Choi, Hee Jeong Lee, Joungho Han</td>
</tr>
<tr>
<td>318</td>
<td>Cancers with Higher Density of Tumor-Associated Macrophages Were Associated with Poor Survival Rates</td>
<td>Kyong Yeun Jung, Sun Wook Cho, Young A Kim, Daerin Kim, Byung-Chul Oh, Do Jong Park, Young Joo Park</td>
</tr>
<tr>
<td>331</td>
<td>A Rare Case of Primary Tubular Adenocarcinoma of the Thymus, Enteric Immunophenotype: A Case Study and Review of the Literature</td>
<td>Hae Yoen Jung, Hyundeuk Cho, Jin-Haeng Chung, Sang Byoung Bae, Ji-Hye Lee, Hyun Ju Lee, Si-Hyang Jung, Mee-Hye Oh</td>
</tr>
<tr>
<td>335</td>
<td>Sclerosing Extramedullary Hematopoietic Tumor Mimicking Intra-abdominal Sarcoma</td>
<td>Serap Karaarslan, Nalan Nese, Guray Oncel, Nazan Ozsan, Taner Akalin, Hasan Kaplan, Filiz Basyurekocu, Mine Hekimgil</td>
</tr>
<tr>
<td>339</td>
<td>Ureteral Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue, Chronic Inflammation, and Renal Artery Atherosclerosis</td>
<td>Hyung Lee, Jong Eun Joo, Young Oh Hong, Won Mi Lee, Eun Kyung Kim, Jeong Joo Woo, Soo Jung Gong, Joonyung Huh</td>
</tr>
</tbody>
</table>
Late Bone Metastasis of Histologically Bland Struma Ovarii: The Unpredictability of Its Biologic Behavior
Sun-Ju Oh, Minjung Jung, Young-Ok Kim

Necrotizing Sarcoid Granulomatosis: Possibly Veiled Disease in Endemic Area of Mycobacterial Infection
Yosep Chong, Eun Jung Lee, Chang Suk Kang, Tae-Jung Kim, Jung Sup Song, Hyosup Shim

Salivary Gland Hyalinizing Clear Cell Carcinoma
Jung-Chia Lin, Ja-Bin Liao, Hsiao-Ting Fu, Ting-Shou Chang, Jyh-Seng Wang

Instructions for Authors for Journal of Pathology and Translational Medicine are available at http://jpatholtm.org/authors/authors.php
A Review of Inflammatory Processes of the Breast with a Focus on Diagnosis in Core Biopsy Samples

Timothy M. D’Alfonso
Paula S. Ginter · Sandra J. Shin

Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, USA

Received: June 2, 2015
Revised: June 10, 2015
Accepted: June 11, 2015

Inflammatory and reactive conditions of the breast are relatively uncommon among benign breast lesions, and may present with clinical and radiologic abnormalities akin to malignant processes. As such, core biopsy may be performed to exclude the possibility of malignancy. In most cases, the diagnosis based on microscopy is clear, but in fragmented core biopsy samples, some conditions may mimic malignancy. Conversely, some malignancies can also simulate benign inflammatory or reactive conditions.

The clinical, radiographic, and histologic features of commonly encountered inflammatory and reactive breast lesions, namely, fat necrosis, mammary duct ectasia, granulomatous lobular mastitis, diabetic mastopathy, and abscess will be reviewed (Table 1). In addition, we will discuss the histologic features on core biopsy that distinguish the items on this differential.

FAT NECROSIS

Fat necrosis is most often seen in traumatized breast tissue, particularly in areas of prior surgery or biopsy. Radiation therapy can also lead to fat necrosis in breast and has been seen in up to 50% of patients following balloon-based brachytherapy. Clinically, fat necrosis can present as a palpable mass with or without skin retraction, or it can be asymptomatic.

On mammography, oil or lipid cysts, which are often calcified, are characteristic of fat necrosis (Fig. 1A). Calcifications in fat necrosis may be clustered, pleomorphic, and linear, simulating ductal carcinoma in situ (DCIS). Fat necrosis can also appear as a stellate mass with irregular margins on mammography and ultrasound.

Microscopically, fat necrosis is characterized by infiltration of foamy histiocytes and foreign body type giant cells around necrotic fat cells and lipid vacuoles (Fig. 1B). Cysts lined by foamy histiocytes are often present (Fig. 1C). Lymphocytic infiltration, often accompanied by plasma cells, can be present to varying degrees. As fat necrosis evolves, fibrosis develops within the lesion, forming a scar and fibrous walled cysts that can calcify (Fig. 1D–F). Adjacent glandular structures in a biopsied sample may show changes indicative of radiation therapy, such as cytologic atypia, squamous metaplasia, and thickened basement membranes.

The differential diagnosis for fat necrosis includes less common types of invasive carcinoma, including the lipid-rich variant and carcinomas with “histiocytoid” morphology, such as apocrine carcinoma and invasive lobular carcinoma. A broad-spectrum cytokeratin can be used to exclude the presence of carcinoma.
Some histiocytic processes, although rare, should also be included in the differential diagnosis of fat necrosis. Rosai-Dorfman disease is a histiocytic proliferation that rarely involves the breast and is characterized by large histiocytes accompanied by a lymphoplasmacytic infiltrate.\textsuperscript{11} Histiocytes show emperipolesis and is characterized by large histiocytes accompanied by a lymphoplasmacytic infiltrate.\textsuperscript{11} Histiocytes show emperipolesis and are suggestive of malignancy or are discordant with the pathologic diagnosis of fat necrosis.\textsuperscript{11}

Duct ectasia

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Etiology and associations</th>
<th>Imaging</th>
<th>Microscopic features</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat necrosis</td>
<td>Mass</td>
<td>Trauma</td>
<td>MMG: oil cyst; calcifications; spiculated mass</td>
<td>Lipid vacuoles and necrotic adipocytes surrounded by foamy histiocytes and foreign body giant cells</td>
</tr>
<tr>
<td></td>
<td>Nipple/skin retraction</td>
<td>Surgery/biopsy</td>
<td>US: oil cyst; solid mass; complex cyst</td>
<td>Chronic inflammation Fibrosis, calcification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiation</td>
<td>Dilated ducts with luminal histiocytes</td>
<td>Periducal fibrosis Periducal chronic inflammation</td>
</tr>
<tr>
<td>Duct ectasia</td>
<td>Unilateral or bilateral non-bloody nipple discharge</td>
<td>MMG: branching calcifications; stellate mass</td>
<td>Periducal fibrosis</td>
<td>Ruptured cyst Ductal dilatation due to intraducal mass</td>
</tr>
<tr>
<td></td>
<td>Nipple/skin retraction</td>
<td>US: irregular architectural distortion</td>
<td>Periducal chronic inflammation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>MMG: ill-defined mass; mass</td>
<td>Intraepithelial histiocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>US: dilated subareolar ducts; mass</td>
<td>Calcifications in duct lumen or duct wall</td>
<td></td>
</tr>
<tr>
<td>Granulomatous lobular mastitis</td>
<td>Mass</td>
<td>Idiopathic Corynebacterim spp.</td>
<td>Often suspicious for malignancy</td>
<td>Non-necrotizing granulomatous inflammation in lobules</td>
</tr>
<tr>
<td></td>
<td>Erythema</td>
<td>MMG: ill-defined mass</td>
<td>Microabscesses</td>
<td>Granulomatous mastitis secondary to:</td>
</tr>
<tr>
<td></td>
<td>Nipple/skin retraction</td>
<td>US: irregular hypoechoic mass</td>
<td>Suppurative granulomas with cystic vacuoles: “cystic neutrophilic granulomatous mastitis”</td>
<td>- Infection (TB, fungal)</td>
</tr>
<tr>
<td></td>
<td>Pain</td>
<td>MMG: ill-defined mass; dilated ducts</td>
<td>Does not form well-defined mass</td>
<td>- Sarcoïdosis</td>
</tr>
<tr>
<td></td>
<td>Draining sinus</td>
<td>US: irregular hypoechoic mass</td>
<td>Stomal fibrosis: dense with keloidal features and epithelioid fibroblasts</td>
<td>- Other systemic granulomatous disease</td>
</tr>
<tr>
<td>Diabetic mastopathy</td>
<td>Palpable mass or nodularity</td>
<td>Diabetes mellitus (types I-ll)</td>
<td>MMG: ill-defined mass; architectural distortion</td>
<td>Invasive carcinoma (apocrine or lobular)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autoimmune dx.</td>
<td>US: irregular hypoechoic solid mass</td>
<td>Granular cell tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endocrine dx.</td>
<td>MRI: non-specific</td>
<td>Multinucleated stromal giant cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(i.e., thyroid dx.)</td>
<td></td>
<td>Lymphoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Breast abscess</td>
<td>Tender mass with redness, warmth, swelling ± Nipple discharge</td>
<td>Lactational/puerperal abscess: (- S. aureus)</td>
<td>Mixed inflammatory infiltrate, mainly neutrophils</td>
<td>Abscess secondary to granulomatous lobular mastitis or duct ectasia</td>
</tr>
<tr>
<td></td>
<td>± Fever</td>
<td>Subareolar/ non-puerperal abscess: (- SMOLD)</td>
<td>Granulation tissue and chronic inflammation with resolution ± Gram-positive cocci in clusters (i.e., S. aureus)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± Draining sinus</td>
<td>- Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± Nipple discharge</td>
<td>- Trauma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Mixed flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MMG, mammography; US, ultrasound; spp., species; TB, tuberculosis; dx, disease; MRI, magnetic resonance imaging; SMOLD, squamous metaplasia of lactiferous ducts.

**Table 1. Pertinent features of inflammatory lesions of the breast**

**MAMMARY DUCT ECTASIA**

Mammary duct ectasia is an inflammatory condition characterized by dilatation of the central ducts with associated fibrosis and chronic inflammation. Perimenopausal and postmenopausal women are most often affected.\textsuperscript{13,14} Duct ectasia presents as unilateral or bilateral non-bloody nipple discharge, nipple retraction, or a palpable mass that is typically subareolar and sometimes associated with pain. On mammography, duct ectasia appears as branching calcifications, ductal dilatation, or a stellate mass.\textsuperscript{15} Ultrasound shows dilated subareolar ducts or a mass-like lesion filled with echogenic material, which may not be evident on mammography.\textsuperscript{17} On magnetic resonance imaging (MRI), duct ectasia can present with a pattern of enhancement that can mimic DCIS.

The microscopic appearance of duct ectasia is variable and depends on the disease stage. In early stages, mild ductal dilatation...
Inflammatory Processes of the Breast

with luminal histiocytes is seen (Fig. 2A, B). Ductal epithelium is not hyperplastic and may be flattened or completely absent. Sloughed epithelium may be seen in ductal lumens (Fig. 2C). Lipid-laden foamy histiocytes can be seen within the duct lumen and in adjacent stroma. Intraepithelial histiocytes can also be seen. “Ochrocytes” refers to histiocytes in periductal stroma that show accumulation of lipofuscin pigment, which imparts a brown color to the cells (Fig. 2D). A periductal chronic inflammatory cell infiltrate composed of lymphocytes and plasma cells is also present, particularly when leakage of duct contents into the surrounding stroma has occurred. In the later stages of duct ectasia, fibrosis of duct walls, sometimes accompanied by elastosis, is the predominant histologic feature. The fibrotic wall of the duct may calcify, and can result in calcifications within duct lumens (Fig. 2E). A small portion of a fibrotic duct, with or without accompanying inflammation, may be the only finding in a limited core biopsy sample. Cholesterol granulomas, or “cholesterolomas,” may form within ducts and rupture, spilling contents into the surrounding stroma (Fig. 2F).

The differential diagnosis for duct ectasia includes cysts associated with fibrocystic change. Cysts are seen in terminal duct lobular units, in contrast to the subareolar duct involvement seen in duct ectasia. Making this distinction is not critical, as both lesions are typically managed conservatively. Juvenile papillomatosis is an uncommon localized lesion showing a constellation of histologic findings, including ductal stasis with luminal histiocytes. Florid ductal hyperplasia, apocrine metaplasia, papillary proliferations, and sclerosis are other characteristic findings in juvenile papillomatosis. Ductal hyperplasia and papillary epithelial hyperplasia are not seen in duct ectasia. Finally, duct ectasia occur because of duct obstruction due to an intraductal mass. If an intraductal mass is suspected and not represented in the core biopsy, excisional biopsy is necessary.

When duct ectasia is diagnosed in a screening core biopsy, excisional biopsy is not necessary, provided there is radiologic-pathologic concordance. Symptomatic cases of duct ectasia are treated by excision of the involved ducts. Duct ectasia is not associated with an elevated risk for breast carcinoma.

GRANULOMATOUS LOBULAR MASTITIS

Granulomatous mastitis have various etiologies, including infection (bacterial, fungal, mycobacterial), sarcoidosis, and other systemic granulomatous disease. After such causes are excluded, idiopathic “granulomatous lobular mastitis” describes a condition causing chronic, destructive non-necrotizing granulomatous inflammation of lobules. The etiology of granulomatous lobular mastitis is unknown, though some cases are associated with Co-
Most cases of granulomatous lobular mastitis occur in women of reproductive age (20s–40s). Most women have been pregnant at least once prior to presentation, though the condition does not usually occur during pregnancy or lactation. Patients present with a unilateral palpable mass that is often accompanied by skin or nipple retraction and pain. Symptoms may be accompanied by axillary lymphadenopathy. On imaging, granulomatous lobular mastitis is often suggestive of malignancy. A spiculated mass or multiple nodular masses may be seen on mammography. Ultrasound often shows an irregular hypoechoic mass, fluid collection, tubular structures, or parenchymal mixed echogenicity.

Microscopically, granulomatous lobular mastitis is characterized by non-necrotizing granulomas concentrated in lobules. The granulomas contain epithelioid histiocytes, Langhans giant cells, and lymphoplasmacytic inflammation (Fig. 3A, B). Neutrophilic microabscesses may also be seen. Granulomatous lobular mastitis may be complicated by frank abscess formation and draining skin sinuses. Cystic vacuoles, representing dissolved lipid, are often present within the granulomas, and can be lined by neutrophils; this has been termed “cystic neutrophilic granulomatous mastitis” (Fig. 3C). In such cases, gram-positive bacilli representing *Corynebacterium* can be seen within the cystic vacuoles (Fig. 3D). The bacteria show “coryneform” features, such as arrangement into palisades and “V” shapes, as well as clubbing of the organisms. These bacteria are not readily identifiable on hematoxylin and eosin examination, and in most cases only rare (<10) bacteria may be present in one or two vacuoles in one core biopsy sample. In fact, gram stains and microbial cultures of these samples are often negative in these cases, in part due to the fastidious nature of these organisms.

We recently reported on a series of twelve patients with histologically identified cystic neutrophilic granulomatous mastitis. All patients presented with a unilateral breast mass that was painful in six of twelve cases. Imaging was either suspicious (BI-RADS 4) or highly suggestive of malignancy (BI-RADS 5) in over half of the studied cases. Gram-positive bacilli were identified in five of twelve cases, and all microbial cultures were negative for bacterial growth. Patients showed a variable response to treatment, with time to resolution of symptoms ranging from two weeks to six months.

Granulomatous lobular mastitis is often initially encountered in a core biopsy sample, with the differential including other
Inflammatory Processes of the Breast

causes of granulomatous lobular inflammation. Special stains should be performed to rule out infection with fungi and acid-fast bacilli as the cause of the granulomatous process. Sarcoidosis should be excluded based on clinical, imaging, and laboratory findings. Sarcoidosis involvement of the breast is uncommon, and rarely the initial site of disease detection. Sarcoid granulomas tend to show less inflammation and are not associated with abscess or microabscess formation, as in granulomatous lobular mastitis. Schaumann bodies and asteroid bodies may be identified within granulomas. In cases where cystic neutrophilic granulomatous mastitis is seen, gram stains should be performed to identify gram-positive bacilli. At our institution, when this pattern is seen on biopsy, we include a note in the pathology report stating its known association with *Corynebacterium* infection, in order to help guide treatment. Microbial cultures should be obtained in all cases of granulomatous lobular mastitis to rule out infection.

Patients with granulomatous lobular mastitis are typically treated using a combination of antibiotics and surgery. Steroids have also been shown to be effective when added to the treatment regimen or used alone.\(^{22,25,36,38,51}\) Despite various treatments options, patients often experience persistent and recurrent disease, with complications including draining sinuses and abscess formation. Patients often have to undergo multiple surgical procedures and courses of antibiotics.

**DIABETIC MASTOPATHY**

Diabetic mastopathy, also known as "lymphocytic mastopathy" or "sclerosing lymphocytic lobulitis" is an uncommon mass-forming lesion seen in patients with insulin-dependent (type 1) diabetes mellitus, particularly in those who have long-standing disease with microvascular complications.\(^{32-34}\) The characteristic histologic findings seen in diabetic mastopathy can also be seen in lesions of patients with type 2 diabetes mellitus, autoimmune diseases such as Hashimoto’s thyroiditis, and even those with no history of diabetes or autoimmune disease.\(^{34,35}\) The cause of diabetic mastopathy is not known. One theory is that hyperglycemia leads to stromal matrix expansion and accumulation of advanced glycosylation end products, leading ultimately to an inflammatory B-cell response.\(^{32}\) It has also been suggested that diabetic mastopathy develops as a result of an immunologic re-

---

Fig. 3. Granulomatous lobular mastitis. (A, B) Non-necrotizing granulomas are centered within lobules. Granulomas contain Langhans giant cells, and are associated with lymphocytes and plasma cells. (C) Cystic neutrophilic granulomatous mastitis showing neutrophil-lined cysts within granulomas. (D) Gram-positive coryneform bacilli are present within the cysts.
response to exogenous insulin; however, this is unlikely the sole cause, as the lesion also develops in patients who have not taken exogenous insulin.36

Diabetic mastopathy most often occurs in premenopausal women, although rare cases have been reported in men.32,34,35,37 The typical presentation is a palpable unilateral mass, though screening mammography may detect the first changes. In some instances, multiple masses or ill-defined nodules are clinically detectable. The mammographic and sonographic features of diabetic mastopathy may be suspicious for malignancy: mammography may reveal an ill-defined mass, distortion, or dense glandular breast tissue;38 ultrasound may show an irregular hypoechoic mass with posterior shadowing.38-40 MRI shows nonspecific enhancement.40-42

Tomaszewski et al.32 were the first to put forth criteria for the microscopic diagnosis of diabetic mastopathy. The characteristic constellation of findings includes lymphocytic lobulitis and ductitis, lymphocytic perivascularitis, and stromal fibrosis with epithelioid fibroblasts.32 Lymphocytic infiltrates, which can be fairly dense, surround ducts, lobules, and small vessels, and may sometimes be associated with plasma cells (Fig. 4A–C). Immunohistochemical characterization of these infiltrates reveals mature B-lymphocytes with a small population of T cells.43 Germinal centers are not typically seen here. Involved lobules may be atrophic or unremarkable. The stroma in diabetic mastopathy is dense and has a keloidal appearance. Intra-stromal epithelioid fibroblasts appear as plump cells with eosinophilic cytoplasm (Fig. 4B–D). Nuclei are oval to round with vesicular nuclei. Neither significant nuclear atypia nor mitotic figures are seen. The distribution of fibroblasts within the stroma can be heterogeneous, and show a whorled or nodular growth pattern.35 These distinctive fibroblasts are not present in all cases of diabetic mastopathy. In a series by Ely et al.,35 epithelioid fibroblasts were absent in five of 19 (26%) cases, including the two cases occurring in men. However, these fibroblasts were present in all three non-diabetic patients in their study.

The differential diagnosis for diabetic mastopathy as seen in core biopsy sample depends on which components of the lesion are present in the limited sample. If dense keloidal fibrosis is the predominant finding, fibrocystic change should be considered. It would be atypical for fibrosis to occur in diabetic mastopathy without coexisting perilobular or perivascular lymphocytic in-

![Fig. 4. Diabetic mastopathy. (A) Lymphoid infiltrates surround ducts, lobules, and small vessels. The stroma has a hyalinized appearance. (B, C) Plump epithelioid fibroblasts are present in the stroma (C inset, high power). Perilobular (B) and perivascular (C) chronic inflammation is seen. (D) Fibroblasts in diabetic mastopathy compared with granular cell tumor (E) and multinucleated stromal giant cells (F).](http://jpatholtm.org/ "http://jpatholtm.org")
filtrates, unless the sample is quite limited. The most diagnostically relevant caveat would be to avoid misclassifying the epithelioid fibroblasts as neoplastic proliferations. Further, care should be taken to exclude carcinomas with abundant eosinophilic cytoplasm, such as pleomorphic lobular carcinomas that exhibit “histiocytoid” and/or apocrine features. These carcinomas, as in diabetic mastopathy, may not be associated with desmoplastic stroma; a broad-spectrum cytokeratin stain can be performed to rule out carcinoma in such cases. Granular cell tumors are composed of cells with abundant pink granular cytoplasm and bland nuclear features (Fig. 4E). These tumors stain positive for S100 and CD68, which helps to distinguish them from diabetic mastopathy. Multinucleated stromal giant cells, a benign incidental finding in breast tissue, can be distinguished from cells of diabetic mastopathy by their multiple hyperchromatic nuclei and scant, versus abundant, cytoplasm (Fig. 4F). Multinucleated stromal giant cells are incidental microscopic findings, while diabetic mastopathy is a mass-forming proliferation. Other mass-forming lesions with lymphoid infiltrates should be considered when lymphocytic ductitis, lobulitis, and perivasculitis are present in the absence of epithelioid fibroblasts. Lymphoma in the breast tends to diffusely infiltrate the stroma, a pattern of inflammation distinct from that seen in diabetic mastopathy. Additionally, immunostaining and molecular analysis will reveal a clonal proliferation of lymphocytes in lymphoma, but not in diabetic mastopathy.

Diabetic mastopathy is a benign condition, and patients can be managed with routine mammographic surveillance. In patients who have undergone excision of the lesion, recurrences may be ipsilateral or contralateral. In a series reported by Dorokhova et al., five of 34 cases (15%) of diabetic mastopathy recurred (2 bilateral, 3 bilateral). Patients with diabetic mastopathy are not at increased risk for subsequent development of breast carcinoma. Further, the lymphocytes in diabetic mastopathy do not show evidence of clonality by immunoglobulin heavy chain gene rearrangement studies, and patients also do not appear to be at risk for developing lymphoma.

ABSCESS

Breast abscesses occur most commonly during lactation, but may also occur in the subareolar breast tissue of non-lactating breasts. Abscess formation is a consequence of ductal stasis in both lactational and non-lactational breasts. Though most patients with mastitis or breast abscess are treated with empiric antibiotics, core biopsy is indicated in some cases to rule out malignancy.

Lactational abscess can develop as a complication of mastitis, which occurs in up to 10% of lactating women, most commonly during the initiation and weaning phases of breastfeeding. The most common organism isolated from these abscesses is S. aureus, identified approximately 50% of the time. Primitivity, prior lactational mastitis, and improper nursing technique are risk factors for the development of mastitis and abscesses. Cracking of the nipple skin may facilitate the entry of bacteria into the ductal system. In a series reported by Dener and Inan, 22 out of 128 patients (17%) with lactational abscess or mastitis had cracked nipples. Bacteria that enter the ducts are supplied with a lactose-rich environment from milk within the ductal lumen. Patients with lactational mastitis and abscess can present with redness, swelling, tenderness, or a palpable mass. Fever may additionally be present in some cases.

Subareolar, or non-puerperal abscesses, most often develop as a consequence of squamous metaplasia of lactiferous ducts. Squamous metaplasia of ducts leads to keratin accumulation with eventual rupture and spillage of duct contents into the surrounding stroma, which leads to abscess formation. This may be complicated abscess rupture and the formation of a sinus tract. The clinical scenario of recurring subareolar abscesses and sinus formation has been referred to as Zuska’s Disease or periductal mastitis. Patients with subareolar abscesses are typically premenopausal, though older women and men may also be affected. Cigarette smoking is a strong risk factor for the development of subareolar abscesses, with approximately 70%–90% of patients reporting a history of smoking. Diabetes and obesity are other associated risk factors. Patients present with a painful subareolar or periareolar mass, with or without nipple retraction. Nipple discharge, if present, may have a pasty consistency. Most cases are unilateral, but bilateral disease can also occur. In a series of 152 patients reported by Habif et al., 40 (26%) presented with bilateral abscesses. In contrast with lactational mastitis, the bacteria isolated from non-puerperal abscesses are most commonly mixed and predominantly anaerobic. In one study, coagulase-negative staphylococci was the most common aerobic organism isolated.

Mammographic features of abscesses are non-specific and may include a mass, architectural distortion, or skin thickening. Ultrasound shows a hypoechoic mass or a multiloculated fluid collection with a thick, echogenic rim. Microscopically, an abscess shows a mixed inflammatory infiltrate composed mainly of neutrophils in breast tissue, which may additionally show lactational changes in a lactational abscess.
In abscess resolution, neutrophilic inflammation is replaced by granulation tissue and chronic inflammatory changes. In some cases, subareolar abscesses may show dilated ducts that contain squamous metaplasia; a foreign body giant cell reaction to keratin may also be seen within the abscess. The differential diagnosis for breast abscess includes duct ectasia and granulomatous lobular mastitis, both of which can be associated with abscess formation.

Lactational mastitis can be adequately treated with antibiotics, though surgical drainage may be necessary in unresponsive cases. Nursing should be continued throughout treatment, and may in fact help resolve the infection. Treatment of subareolar abscess involves a combination of antibiotics and surgery, involving excision of the abscess and adjacent lactiferous duct. Significantly lower rates of recurrence are seen when the infected duct is also excised, compared excision of abscess alone. Subareolar abscesses are often chronic and recurring, with longer time to resolution and higher rates of recurrence, when compared to lactational abscesses.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**REFERENCES**

25. Al-Khawari HA, Al-Manfouhi HA, Madda JP, Kovacs A, Sheikh M,
Inflammatory Processes of the Breast


Pathology Reporting of Thyroid Core Needle Biopsy: A Proposal of the Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group

Chan Kwon Jung1 · Hye Sook Min2,3 · Hyo Jin Park4 · Dong Eun Song4 · Jang Hee Kim5 · So Yeon Park2 · Hyunju Yoo6 · Mi Kyung Shin7

Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group

1Department of Hospital Pathology, College of Medicine, The Catholic University of Korea, Seoul; 2Department of Pathology, Seoul National University College of Medicine, Seoul; 3Department of Epidemiology and Preventive Medicine, Graduate School of Public Health, Seoul National University, Seoul; 4Department of Pathology, University of Ulsan College of Medicine, Seoul; 5Department of Pathology, Ajou University School of Medicine, Suwon; 6Department of Pathology, Daerim Saint Mary’s Hospital, Seoul; 7Department of Pathology, Hallym University College of Medicine, Seoul, Korea

Received: April 16, 2015
Revised: June 2, 2015
Accepted: June 3, 2015

Corresponding Authors
Chan Kwon Jung, MD, PhD
Department of Hospital Pathology, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Korea
Tel: +82-2-2258-1622
Fax: +82-2-2258-1627
E-mail: cjung@catholic.ac.kr

Mi Kyung Shin, MD, PhD
Department of Pathology, Hallym University Kangnam Sacred Heart Hospital, Hallym University College of Medicine, 1 Singil-ro, Yeongdeungpo-gu, Seoul 150-950, Korea
Tel: +82-2-829-5443
Fax: +82-2-829-5268
E-mail: smk0103@yahoo.co.kr

Key Words: Thyroid nodule; Guideline; Image-guided biopsy; Preoperative period; Diagnosis

Ultrasound-guided fine needle aspiration cytology (FNAC) is the most accurate, effective and safe method for the preoperative assessment of thyroid nodules. Since 2007, pathologists and clinicians have widely adopted the six-level diagnostic scheme of the Bethesda System for Reporting Thyroid Cytopathology (TB-SRTC). The use of standard diagnostic categories has a significant clinical benefit in terms of risk stratification for malignancy, and enhances communication between pathologists and physi-
However, a major limitation of FNAC for the diagnosis of thyroid nodules, is the relatively high incidence of nondiagnostic aspirates (10%–15%) and indeterminate aspirates (10%–30%).4,5 Patients with thyroid nodules of nondiagnostic or indeterminate aspirates typically undergo repeated aspirations for FNAC or diagnostic surgery.1

Ultrasound-guided core needle biopsy (CNB) has been successfully employed for the last decade as a complementary tool for the evaluation of thyroid nodules.6-9 CNB reportedly reduces the rate of nondiagnostic or indeterminate results.6 Therefore, CNB can be used when FNAC results are nondiagnostic or fail to correlate with clinical findings or results of ultrasound imaging studies.8,10,11 Limited data suggest that CNB can be used as a first-line method for the preoperative diagnosis of thyroid nodules with a high risk of malignancy.12 Thyroid CNB also allows ancillary immunohistochemical and molecular tests to be carried out on the tissue sample.

Ultrasound-guided thyroid CNB is a safe procedure in contrast to large CNB, which was employed in the 1990s and requires the use of a larger diameter needle.6 The overall complication

---

**Fig. 1.** Core needle biopsies of fibrotic nodules. The right column images represent the high-power views of the lesional area in the left column images. (A) The specimen consists of an acellular fibrotic lesion and adjacent normal parenchyma. (B) The fibrotic area contains no follicular cells, but contains a few lymphocytes and stromal cells. This lesion is classified in the nondiagnostic category. (C) The specimen shows a paucicellular structure with marked fibrosis and calcification. (D) Scattered atypical cells with suspicious morphological features of papillary carcinoma are embedded in the fibrosis. This lesion contains suspicious follicular cells and should therefore be diagnosed as suspicious for malignancy or as a malignancy, depending on the degree of nuclear atypia. (E) The specimen shows marked fibrosis and calcification. (F) The high-power view of the lesion shows relatively numerous benign-appearing follicular cells. This lesion can be diagnosed as a benign follicular nodule.
rate is similar to what has been reported for FNAC.\textsuperscript{5,9,13} The main goal of histopathologic examination by CNB is to triage patients with thyroid disease who need to be surgically managed. Through the use of CNB, a definitive diagnosis is possible in most, but not necessarily all patients. These concepts are the same as those of FNAC. However, in contrast to FNAC, there is no consensus on the diagnostic criteria for reporting thyroid CNB.

**CONSENSUS CONFERENCE**

Since May 2013, the Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group has held three consensus conferences on thyroid CNB pathology reporting in Seoul, Korea. The purpose of the conferences was to bring together endocrine pathologists who were thyroid specialists (1) to review existing reporting formats and published literature related to thyroid

### Table 1. Diagnostic categories of thyroid core needle biopsy

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Nondiagnostic or unsatisfactory</td>
<td>Normal thyroid tissue only, Extrathyroid tissue only (e.g., skeletal muscle, mature adipose tissue), A virtually acellular specimen, Acellular/paucicellular fibrotic nodule, Blood clot only, Other</td>
</tr>
<tr>
<td>II. Benign lesion</td>
<td>Benign follicular nodule or consistent with a benign follicular nodule, Hashimoto’s thyroiditis, Granulomatous (subacute) thyroiditis, Nonthyroidal lesion (e.g., parathyroid lesions, benign neurogenic tumors, benign lymph node), Other</td>
</tr>
<tr>
<td>III. Indeterminate lesion</td>
<td>IIIA. Indeterminate follicular lesion with nuclear atypia, Follicular proliferative lesions with focal nuclear atypia, Atypical follicular cells embedded in a fibrotic stroma, IIIB. Indeterminate follicular lesion with architectural atypia, Microfollicular proliferative lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen, Solid or trabecular follicular lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen, Macrofollicular proliferative lesion with a fibrous capsule, Hürthle cell proliferative lesion lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen, Other indeterminate lesions</td>
</tr>
<tr>
<td>IV. Follicular neoplasm or suspicious for a follicular neoplasm</td>
<td>Microfollicular proliferative lesion with a fibrous capsule, Mixed microfollicular and normofollicular proliferative lesion with a fibrous capsule, Solid/trabecular follicular proliferative lesion with a fibrous capsule, Hürthle cell proliferative lesion with a fibrous capsule, Follicular neoplasm with focal nuclear atypia</td>
</tr>
<tr>
<td>V. Suspicious for malignancy</td>
<td>Suspicious for papillary carcinoma, medullary carcinoma, poorly differentiated carcinoma, metastatic carcinoma, lymphoma, etc.</td>
</tr>
<tr>
<td>VI. Malignant</td>
<td>Papillary thyroid carcinoma, poorly differentiated carcinoma, undifferentiated (anaplastic carcinoma), medullary thyroid carcinoma, lymphoma, metastatic carcinoma, etc.</td>
</tr>
</tbody>
</table>

**Comments**

1. The core needle biopsy provides an accurate diagnosis in most cases; however, it may miss some cancers or sometimes may be inconclusive.
2. Definitive therapeutic surgery (i.e., a total thyroidectomy) should not be undertaken as a result of a category III, IV, or V core needle biopsy diagnosis.
3. The management of a thyroid lesion must be based on a multidisciplinary approach.
terminology: (1) some clinicians and pathologists may understand the meaning of terms in a CNB pathology report differently from the terms in an FNAC report; (2) the phrase "atypia of undetermined significance" in TBSRTC is inappropriate terminology in the field of histopathology; (3) there is no consensus regarding specimen adequacy; and (4) the sample representativity of the CNB may be different from that of the FNAC. Therefore, we recommend that the limitations of CNB should be explained in the pathology report.

Most participants in the conferences agreed that a sample composed primarily of an acellular/paucicellular fibrotic nodule should be considered nondiagnostic if it did not contain any atypical cells (Fig. 1). However, a categorical diagnosis should be rendered, irrespective of the number of follicular cells, if a sample contains atypical cells (Fig. 1).

Another dilemma is the differential diagnosis of follicular proliferative lesions without nuclear atypia in a CNB sample. Histologically, hyperplastic nodules are usually found in multiples and are partially encapsulated by a fibrous capsule. Follicular adenoma is a completely encapsulated follicular nodule that is mostly solitary and has microscopic features that are different from those of the surrounding normal thyroid tissue. Fibrous

Fig. 2. (A, B) The ultrasound images show well-circumscribed solid, homogeneous, nodules with peripheral hypoechoic rims. (C, D) The core needle biopsies show only microfollicular proliferation. These specimens do not contain a fibrous capsule or adjacent normal tissue that is required to make a diagnosis of follicular neoplasm. (E, F) Images are the high-power views of Fig. 2C and D, respectively. No nuclear atypia is present. The left and right columns show the conventional and Hürthle cell types, respectively. Typical ultrasound features of follicular neoplasms, when present, can lead to the diagnosis of follicular neoplasms, even when specimens are not contained in a fibrous capsule.
capsules of follicular neoplasms vary in thickness. Tumor capsules are usually thin on follicular adenomas and tend to be thicker on follicular carcinomas. It is not possible to differentiate between follicular adenoma and carcinoma using FNAC or CNB samples because the diagnosis of follicular carcinoma is based on the presence of tumor capsular invasion or vascular invasion. Nonetheless, in CNB samples, it may be possible to determine whether a nodule is a follicular neoplasm or a non-neoplastic lesion. Most participants agreed that a diagnosis of “follicular neoplasm or suspicious for a follicular neoplasm” is appropriate when a follicular proliferative lesion is separated from the surrounding thyroid parenchyma by a definite fibrous capsule, has features that are architecturally and cytologically different from the adjacent thyroid glands, and lacks the nuclear features of a papillary carcinoma. However, the CNB specimen of a widely invasive follicular carcinoma may not show a fibrous capsule.

Fig. 3. Core needle biopsy findings of a follicular neoplasm with a macrofollicular growth pattern. The images in the left column and the right column show the core needle biopsy specimen and the resected specimen, respectively. (A) The ultrasound image shows a well-circumscribed, isoechoic, aviod nodule with a peripheral hypoechoic rim. A focal cystic change is present. (B) The surgical specimen exhibits a thick fibrotic capsule surrounding the nodule. (C) The core needle biopsy shows a macrofollicular proliferative lesion with a fibrous capsule (arrows). (D) The high-power view of the biopsy specimen shows benign-appearing follicular cells. The typical ultrasound features and thick fibrous capsule can lead to a diagnosis of follicular neoplasm, even in a macrofollicular lesion. The microscopic examination of the surgical specimen shows that the tumor is well encapsulated (D) and capsular invasion is minimal (F).
capsule because the tumor is not encapsulated although it has invaded into the thyroid parenchyma and extrathyroidal tissue. Some hyperplastic nodules have a cellular microfollicular or solid growth pattern and a fibrous capsule. Therefore, misdiagnosing a widely invasive follicular carcinoma as a benign follicular lesion or a hyperplastic nodule as a follicular neoplasm on the CNB specimens remains a concern.

No study has yet been conducted to define the number of tissue cores that are required for an accurate diagnosis of a thyroid nodule. However, most conference participants agreed that two cores are adequate to diagnose a lesion.

CONSENSUS DIAGNOSTIC CATEGORIES

In the histologic examination of CNB samples, a diagnostic category is selected from the following six standardized options. This proposal was approved by the Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group (Table 1).

I. Nondiagnostic or unsatisfactory

This category includes specimens with an insufficient number of follicular cells to provide an appropriate diagnosis or when the specimen is not representative of the ultrasound image of the lesion. This diagnosis is subjective; and therefore, the report should explain why the sample is nondiagnostic or unsatisfactory. For example, the sample may be normal thyroid tissue only, extrathyroid tissue only (e.g., skeletal muscle and mature adipose tissue), a virtually acellular specimen, an acellular/paucicellular fibrotic nodule, a blood clot only, or it may show other nondiagnostic or unsatisfactory findings. However, any CNB specimen containing atypical cells should not be considered nondiagnostic or unsatisfactory.

Normal thyroid tissue or extrathyroid tissue only implies that the thyroid lesion has not been sampled. For some benign follicular lesions with normofollicular structures, it may be difficult to assess specimen adequacy. It is important to find the transition between the follicular lesion and the surrounding normal parenchyma and compare between the pathologic finding and its ultrasound images.

II. Benign

This category includes all benign thyroidal and nonthyroidal diseases. A CNB specimen can be classified, based on the specific diagnosis for the lesion, as benign. For example, the sample may

Fig. 4. (A) The core needle biopsy shows a microfollicular proliferative lesion and surrounding normal tissue. (B) The high-power view of the boxed area in Fig. 4A shows that the lesion has no nuclear atypia or fibrous capsule. This lesion should be diagnosed as a benign follicular nodule. (C, D) When microfollicular proliferative lesions show a definite fibrous capsule (arrows) in the core needle biopsy, the specimens should be diagnosed as a follicular neoplasm.
be a benign follicular nodule or consistent with a benign follicular nodule, Hashimoto’s thyroiditis, subacute granulomatous thyroiditis, a nonthyroidal lesion (e.g., a parathyroid lesion, benign neurogenic tumors, and benign lymph node), or another benign lesion.

A benign follicular nodule encompasses nodular hyperplasias (adenomatoid nodule), colloid nodules, a nodule in Graves’ disease, nodular Hashimoto’s thyroiditis, and a subset of follicular adenomas. CNB specimens of these lesions show a benign-appearing normofollicular or macrofollicular structure and do not have a well-defined fibrous capsule.

### III. Indeterminate lesion

The cytologic atypia and histologic growth patterns in this category are of uncertain significance and insufficient to be classified under other diagnostic categories. The diagnostic category III “indeterminate lesion” corresponds to “atypia of undetermined significance” or “follicular lesion of undetermined signifi-

![Fig. 5.](image-url)
Thyroid Core Needle Biopsy Diagnosis

The diagnosis of category III is appropriate when a follicular proliferative lesion shows focal nuclear atypia such as nuclear enlargement with pale chromatin, irregular nuclear membranes, and nuclear grooves in a background of predominantly benign-appearing follicles. If a microfollicular proliferative lesion is separated by a fibrous capsule from the surrounding normal parenchyma it is diagnosed as a follicular neoplasm (see category IV). If a fibrous capsule or adjacent nonlesional tissue is not identified in a CNB specimen that shows a predominantly microfollicular or trabecular growth pattern, it is reasonable to classify the lesion as diagnostic category III because it cannot be determined whether the nodule has a fibrous capsule. However, if sono-graphic features suggest a follicular neoplasm in such lesions, the sample can be considered a category IV, “follicular neoplasm” (Fig. 2).13 A multidisciplinary approach to a thyroid nodule can improve the preoperative diagnostic accuracy of FNAC and CNB specimens.16

FNAC specimens of macrofollicular lesions are usually diagnosed as benign. However, when ultrasound images show the typical features of a follicular neoplasm and the CNB specimen microscopically shows a fibrous capsule, then the CNB specimen can be diagnosed as a follicular neoplasm, even in a macrofollicular lesion (Fig. 3). However, in surgical pathologies, most participants agreed that category III is appropriate in a macrofollicular proliferative lesion with a definite fibrous capsule (Fig. 3).

The following are common scenarios that may be encountered in case of follicular proliferative lesions:

IIIA. Indeterminate follicular lesion with nuclear atypia

Examples in this category are follicular proliferative lesions with focal nuclear atypia, follicular proliferative lesions with equivocal or questionable nuclear atypia, and atypical follicular cells embedded in fibrotic stroma.

IIIB. Indeterminate follicular lesion with architectural atypia

Examples in this category are microfollicular proliferative lesions lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen; solid or trabecular follicular lesions lacking a fibrous capsule or the adjacent nonlesional tissue in the specimen; macrofollicular proliferative lesions with a fibrous capsule; Hürthle cell proliferative lesions lacking a fibrous capsule or the

![Image of normal tissue, Galectin 3, and Cytokeratin 19](http://dx.doi.org/10.4132/jptm.2015.06.04)

**Fig. 6.** The core needle biopsy shows a follicular proliferative lesion with nuclear atypia and diffuse strong immunohistochemical staining for galectin 3 and cytokeratin 19 in the tumor cells. Images in the left and right columns show the low magnification and high magnification views, respectively, of the samples.
adjacent nonlesional tissue in the specimen.

III. Other indeterminate lesions

IV. Follicular neoplasm or suspicious for a follicular neoplasm

In CNB and FNAC, the term “follicular neoplasm or suspicious for a follicular neoplasm” is used to encompass neoplastic lesions with follicular proliferative patterns (e.g., follicular adenoma, follicular carcinoma, follicular variant of papillary carcinoma, follicular variant of medullary carcinoma). The histologic diagnosis of “follicular neoplasm or suspicious for a follicular neoplasm” in a CNB specimen is based on the presence of a fibrous capsule and microscopic features that differ from the adjacent thyroid parenchyma (Fig. 4). Follicular cells do not show the typical nuclear features of papillary carcinomas. It is important to identify a well-formed fibrous capsule in the CNB specimen. The FNAC diagnosis for follicular neoplasm is primarily based on the presence of microfollicular or trabecular architecture and the lack of colloid. In a CNB specimen, the growth patterns of a follicular neoplasm can be microfollicular, normofollicular, solid, or trabecular when a fibrous tumor capsule is identified in the sample. Examples in this category include microfollicular proliferative lesions with fibrous capsules, mixed microfollicular and

Fig. 7. Core needle biopsies of malignant thyroid nodules. (A, B) The biopsy specimen maintains the typical morphological features of papillary carcinoma. Poorly differentiated carcinoma shows solid, trabecular, and insular growth patterns (C) and mitosis (arrow) (D) under the high-power view. The medullary carcinoma shows the typical morphological features under the low-power view (E) and the high-power view (F).
normofollicular proliferative lesions with fibrous capsules, solid or trabecular follicular lesions with fibrous capsules, Hürthle cell proliferative lesions with fibrous capsules, and follicular neoplasms or samples suspicious for follicular neoplasms with focal nuclear atypia.

A recent study reported that the neoplasm or malignancy rate was not different among these architectural patterns of follicular lesions. A CNB cannot discriminate between a follicular carcinoma and a follicular adenoma because the diagnosis of these neoplasms requires examination of the entire thick fibrous capsule. However, in a subset of this category, focal nuclear atypia raises the possibility of a follicular variant of papillary carcinoma (Fig. 5). A follicular variant of papillary carcinoma is reportedly the most common malignancy found after surgery of thyroid nodules with a preoperative diagnosis of follicular neoplasm.

Histological findings of a follicular neoplasm on the CNB sample should be correlated with ultrasound imaging features. Typical ultrasound images of a follicular neoplasm show a well-circumscribed, solid, ovoid or round mass with a surrounding halo or a hypoechoic rim around the thyroid nodule (Figs. 3, 5). The neoplasm can be hypoechoic, isoechoic, hyperechoic, or mixed. The sonographic halo corresponds with the fibrous capsule surrounding the follicular lesion (Figs. 3, 5). Focal cystic change can be present, but calcification is rare.

V. Suspicious for malignancy

The “suspicious for malignancy” diagnosis is given when histologic features are strongly suspicious for malignancy, but they are insufficient for a definitive diagnosis of malignancy. In this category, a lesion may be suspicious for papillary thyroid carcinoma, suspicious for medullary thyroid carcinoma, suspicious for poorly differentiated thyroid carcinoma, suspicious for metastatic carcinoma, suspicious for lymphoma, or show other suspicious findings.

Ancillary immunohistochemical or molecular studies can be helpful for the diagnosis of CNB specimens with findings that are suspicious for malignancy. An immunostaining panel consisting of galectin-3, HBME1, cytokeratin 19, or CD56 is helpful in the diagnosis of lesions suspicious for papillary thyroid carcinoma (Fig. 6). A combination of at least two immunostaining markers is recommended to improve the diagnostic ac-

![Fig. 8. Diagnostic pitfalls in thyroid core needle biopsy. Follicular cells are smaller and darker in core needle biopsies in comparison (A) to resected specimens (B). These images have been obtained from the same patient as those pictured in Fig. 6. (C) The core needle biopsy shows the histologic features of a benign follicular nodule. (D) The high-power view of the boxed area in Fig. 8C shows nuclear vacuoles that mimic intranuclear cytoplasmic pseudoinclusions in papillary carcinoma (arrows).](http://jpatholtm.org/10.4132/jptm.2015.06.04)
Accuracy.22 Presence of the BRAF V600E mutation, detected by molecular test or immunostaining, strongly suggests a diagnosis of the papillary carcinoma.21

A diagnosis of medullary thyroid carcinoma can be confirmed with positive immunostaining for calcitonin on a CNB specimen. A diagnosis of lymphoma can be rendered using immunophenotyping studies on a CNB specimen that is suspicious for lymphoma.5

VI. Malignant

Most thyroid malignancies, except for follicular carcinoma, have typical histologic features and are easily diagnosed as a malignancy on a CNB specimen. The following diagnoses are included in this category: papillary thyroid carcinoma (Fig. 7A, B), poorly differentiated carcinoma (Fig. 7C, D), undifferentiated (anaplastic) carcinoma, medullary thyroid carcinoma (Fig. 7E, F), lymphoma, and metastatic carcinoma.

COMMON PITFALLS OF A DIAGNOSIS OF CORE NEEDLE BIOPSY

The follicular cells in a CNB specimen appear smaller and show darker chromatin than in typical surgical specimens (Fig. 8A, B). Nuclear artifacts that mimic intranuclear cytoplasmic pseudoinclusions in papillary carcinoma may also be present in benign follicular cells (Fig. 8C, D). The artifactual vacuoles are irregularly shaped and appear pale on staining, whereas pseudoinclusions in papillary carcinoma are round and sharply delineated by the rim of the nuclear membrane.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

The authors would like to thank the members of the Endocrine Pathology Study Group of the Korean Society of Pathologists for their valuable expertise and suggestions throughout this study. This research was supported by the Korean Society of Pathologists Grant 2013 and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2013R1A2A2A01068570).

REFERENCES

In this era of precision medicine, our understanding and knowledge of the molecular landscape associated with lung cancer pathogenesis continues to evolve. This information is being increasingly exploited to treat advanced stage lung cancer patients with tailored, targeted therapy. During the management of these patients, minimally invasive procedures to obtain samples for tissue diagnoses are desirable. Cytologic fine-needle aspirates are often utilized for this purpose and are important not only for rendering diagnoses to subtype patients' lung cancers, but also for ascertaining molecular diagnostic information for treatment purposes. Thus, cytologic fine-needle aspirates must be utilized and triaged judiciously to achieve both objectives. In this review, strategies in utilizing fine-needle aspirates will be discussed in the context of our current understanding of the clinically actionable molecular aberrations underlying non-small cell lung cancer and the molecular assays applied to these samples in order to obtain treatment-relevant molecular diagnostic information.

Key Words: Lung neoplasms; Cytology; Biopsy, fine-needle; Molecular testing; Precision medicine

During this current era of precision medicine, our current knowledge regarding the molecular drivers underlying cancer continues to evolve. Therefore, anatomic pathology specimens are becoming increasingly utilized for molecular diagnostic assays in order to detect clinically actionable genetic abnormalities in addition to routine diagnoses. This allows for tailored, personalized therapy in a subset of patients afflicted with malignancies harboring actionable genetic mutations for which approved targeted therapeutic agents exist. Molecular testing is therefore most relevant in the context of high-stage, surgically unresectable cancers. In this setting, minimally invasive small biopsy procedures are preferable in that they are associated with low risk of complications for the patient. Cytologic fine-needle aspiration (FNA) specimens are especially suitable, in this regard, as FNA procedures represent rapid, efficient, and minimally invasive means to sample superficial and deep lesions. Technological advances in imaging modalities and instruments such as computed tomography and endobronchial ultrasound (EBUS) have facilitated accurate and precise sampling of the latter. This constellation of circumstances poses unique challenges and opportunities for pathologists as FNA samples must be judiciously utilized, not only for rendering accurate cytologic diagnoses, but also effectively preserved and triaged for anticipated, relevant downstream molecular diagnostic assays.

In the past decade, significant strides have been made in further optimizing the use of small biopsy specimens and FNAs of non-small cell lung carcinomas (NSCLCs), especially adenocarcinomas and mixed histological subtypes with a component of adenocarcinomatous differentiation, for molecular studies. One commonly held misconception is that FNAs are generally insufficient for the performance of molecular assays. However, for instance, we and others have observed and demonstrated that cellularity on the order of 100–500 cells are sufficient for DNA sequencing-based assays. For fluorescence in-situ hybridization (FISH) assays, 100 analyzable tumor cell nuclei are generally sufficient.

Successfully integrating molecular ancillary techniques into routine cytology practice is essential in facilitating the appropriate management of advanced stage cancer patients by their oncologists. The purpose of this review is to describe the approach to processing lung cancer FNA specimens at our institution, within the contexts of our current understanding of clinically actionable genetic abnormalities in NSCLCs and diverse cytopreparatory platforms on which molecular diagnostic tests are performed.
FINE-NEEDLE ASPIRATION SPECIMEN PROCESSING

FNAs represent an advantageous, effective means to obtain diagnostic cellular material as they can often sample a wide area of the target lesion, acquire tumor cells with lower contamination by background stromal connective tissue elements, and allow for immediate assessment during rapid on-site evaluation (ROSE). During the on-site assessment, a member of the cytopathology team is present and prepares direct smears, using the contents expelled from the needle, at the location of the procedure. This is advantageous for three reasons: each needle pass can be examined to determine tumor cell adequacy; there is an opportunity to engage the clinical care provider in a conversation regarding the preliminary diagnosis and relevant molecular diagnostic tests; and the cytopathology team member can help ensure that the specimen is processed in a manner that optimizes judicious triage for ancillary tests, including molecular studies.4,5 FNAs can be performed without ROSE and in this setting, the contents of the FNA needles are typically expelled and rinsed in a cell preservative solution for use in liquid-based cytology (LBC) preparations. Examples of LBC preparations include ThinPrep (Hologic, Bedford, MA, USA), SurePath (Becton Dickinson, Franklin Lakes, NJ, USA), thin-layer advanced cytology assay system (TACAS, MBL, Tokyo, Japan), and Liqui-PREP (LGM International Inc., Ft. Lauderdale, FL, USA). Nonetheless, ROSE is an effective means to maximize the chances of success in acquiring adequate tumor cells for diagnosis and anticipated molecular studies. The ultimate goal is to prevent unnecessary repeat procedures to obtain additional tissue just for molecular studies which can lead to delays in treatment.

For patients afflicted with lung cancer at our institution, we perform ROSE for cytopathologist-performed FNAs and for EBUS-guided FNAs, performed by our clinical colleagues. The contents of each needle pass are expelled onto a slide which is utilized to prepare direct smears. The needle is then rinsed into buffered media; we utilize RPMI media for this purpose. Commonly, a pair of direct smears is prepared per needle pass; one is air-dried and the other is immediately alcohol-fixed. The air-dried smear is stained on-site with the Diff-Quik (Romanowsky) stain and the stained slide can be examined under the microscope immediately thereafter. The alcohol-fixed smear is stained later in the cytopathology laboratory with the Papanicolaou stain. Alternatively, the needle contents can be distributed over multiple smears allowing for flexibility in the utilization of direct smears for cytomorphologic evaluation and ancillary studies (Fig. 1).4,5 Based on the findings in the Diff-Quik stained smears, the determination can be made to perform additional needle passes to ob-

![Example of fine-needle aspiration (FNA) processing workflow. The contents of a needle pass, obtained during an FNA procedure, are expelled onto a slide to prepare smears. Typically, a pair of smears (one Diff-Quik stained and one Papanicolaou stained) is prepared per needle pass. However, additional smears can be prepared from a single needle pass by distributing the cellular material across more than two slides. The additional smears can be directly triaged for ancillary studies. For instance, an unstained smear can be sent to the Immunohistochemistry Laboratory for immunostaining (e.g., thyroid transcription factor-1 [TTF-1] immunocytochemistry). Also, a stained smear can be directly triaged to the Molecular Diagnostics Laboratory for tumor cell macro- or microdissection, nucleic acid isolation, and subsequent molecular testing (e.g., epidermal growth factor receptor [EGFR] mutation analysis).](image-url)
tain more tumor cells for diagnosis and/or anticipated ancillary studies while the patient is still accessible. For instance, additional direct smears can be prepared and/or dedicated needle passes for the rinse solution (i.e., the needle contents are rinsed in the RPMI solution without the preparation of smears for those needle passes) obtained for the cell block preparation. This overall approach is flexible, forgiving, and engages the cytopathology team in maximizing the chance of success in obtaining adequate cellular material for diagnosis and ancillary studies. This approach can help minimize the chances of encountering the scenario in which diagnostic cellular material is present on only one smear; microdissection of the cells for molecular studies can result in sacrificing this only diagnostic slide in this context, which can have medico-legal consequences. If this scenario is encountered, however, this risk can be mitigated by digital archiving prior to microdissection either via digital slide scanning and/or obtaining photomicrographs. After immediate assessment of the cytomorphologic findings on the Diff-Quik stained smears, a preliminary diagnosis can be rendered by the cytopathologist and communicated to the clinical care providers.

After the conclusion of the procedure, the needle rinse solution, containing a suspension of aspirated cells, is centrifuged in the cytopathology laboratory to pellet the cells. Once the supernatant is removed, the cell pellet is congealed in a matrix; an agar-like substance such as HistoGel (Thermo Scientific, Waltham, MA, USA) can be used or the cells can be mixed with plasma and thrombin to create a clot. At our institution, we employ HistoGel for this purpose. The resulting cell button is then placed in a cassette. Commonly, this cassette is fixed in formalin and processed to ultimately create a formalin-fixed, paraffin-embedded (FFPE) cell block. This is analogous to a FFPE block of small biopsy tissue, which can be sectioned for evaluation via the hematoxylin and eosin stain and ancillary tests such as immunocytochemistry or molecular diagnostic assays. It should be noted that heavy metal fixatives such as Zenker’s fixative and acid-zinc-formalin, acid fixatives such as Bouin’s solution, or decalcification solutions should generally be avoided as these render specimens unusable for molecular testing.

Cell blocks are traditionally utilized for ancillary immunocytochemistry and molecular diagnostic assays. The main advantage of using cell blocks is that the majority of ancillary tests are validated for FFPE sections; FFPE cell blocks are treated similarly to traditional surgical pathology FFPE blocks. Furthermore, multiple serial sections from cell blocks can be utilized to perform a battery of ancillary studies. There are two main disadvantages associated with cell blocks, however. First, adequate cellularity in cell blocks is not guaranteed at the time of the procedure. Performing dedicated needle passes, for the needle rinse solution during the FNA procedure, may improve the chances of obtaining a sufficiently cellular cell block but still does not guarantee success. In addition, for hypocellular cell blocks, there is a risk of depleting the cells of interest upon deeper sectioning for molecular tests. Second, the cell block is derived from a needle rinse solution which is a pooled specimen that contains contents of all the needle passes. Thus, if one or more needle passes contain tumor cells but other needle passes contain abundant benign background cellular elements, the tumor cells will be diluted by these benign cells in the cell block.

Recently, given the shortcomings of cell blocks, there has been an increasing appreciation of alternative cytopreparatory platforms for molecular testing such as direct smears and LBC samples. In the subsequent sections below, the utilization of these cytopreparatory platforms will be discussed in the context of the clinically relevant mutations and translocations analyzed during molecular diagnostic testing of NSCLCs.

**EPIDERMAL GROWTH FACTOR RECEPTOR**

The epidermal growth factor receptor (EGFR) gene encodes a transmembrane growth factor receptor that exhibits tyrosine kinase activity. Upon activation, intracellular signaling is mediated by cytoplasmic effectors in the RAS-RAF-MEK-ERK, PI3K-AKT, and STAT pathways. Approximately 10%–15% of NSCLC harbor clinically relevant, sensitizing mutations in EGFR. EGFR mutations are predominantly observed in lung adenocarcinomas; the L858R substitution and small in-frame deletions in exon 19 are the most commonly observed mutations and account for up to 90% of all EGFR mutations in this setting. These mutations are more commonly associated with East Asian ethnicity, female gender, and non-smoking history. However, these are not absolute rules and clinical characteristics should not be used to exclude lung cancer patients from mutation testing. Lung adenocarcinomas harboring these sensitizing mutations in EGFR have been shown to respond to EGFR tyrosine kinase inhibitors (TKIs). The first-line TKIs include erlotinib and gefitinib. In clinical trials, TKI therapy has been demonstrated to result in improved progression-free survival, compared to standard chemotherapy, for patients with lung adenocarcinoma har-
boring EGFR mutations.\textsuperscript{11-16}

EGFR mutation analysis is commonly performed via polymerase chain reaction (PCR) and sequencing-based approaches; advances in the development of testing modalities have afforded a multitude of methodologies.\textsuperscript{1,17,18} Sanger sequencing is considered the gold standard as this involves direct DNA sequence acquisition and can provide information regarding the presence of all potential mutations including common, known mutations and novel mutations. Nonetheless, this test requires a relatively higher enrichment of tumor cell DNA content in the sample. The typical analytic sensitivity for Sanger sequencing is 15\%–20\% mutant allele, which equates to 30\%–40\% tumor cells assuming that the genetic mutation is a heterozygous event without amplification.\textsuperscript{17} This can be problematic in both small biopsy and cytology specimens, especially cell blocks, in which the tumor cell population can be diluted by background benign cellular elements such as inflammatory cells, bronchial epithelial cells, and/or stromal mesenchymal cells. Especially in this setting, a negative mutation result can be either due to the true absence of the mutation in the tumor cells or insufficient percent tumor cellularity that falls below the analytic sensitivity threshold thereby resulting in the failure to detect the mutation even despite the presence of the mutation.\textsuperscript{4,9,17,19} Therefore, often times, there is more reliance on tumor cell enrichment by either macrodissection or microdissection to obtain a reliable result.\textsuperscript{7,9,17,20} Sanger sequencing is also relatively more labor intensive and time consuming than targeted methods and can lead to longer turnaround times.\textsuperscript{18} In contrast to the general Sanger sequencing approach, targeted mutation detection methods such as PCR-restriction fragment length polymorphism, real-time PCR, pyrosequencing, high resolution melting analysis (HRMA), and PCR fragment analysis can be utilized.\textsuperscript{17} The advantages of these approaches include their improved analytic sensitivity and less time-consuming nature leading to reduced turnaround times. At our institution, we utilize a multiplex PCR fragment analysis assay for EGFR mutation testing; this allows for the simultaneous assessment of the two most commonly observed EGFR mutations (Fig. 2). The analytic sensitivity of this method is better than that of Sanger sequencing; a minimum of only 10\% tumor cells is required. In the past decade, myriad studies have been reported demonstrating that a variety of cytologic samples and cytopreparatory platforms can be effectively utilized for EGFR mutational analysis. These have been reviewed elsewhere\textsuperscript{18,21} but salient examples will be discussed below.

As mentioned previously, FFPE cell blocks represent the traditional cytopreparatory platform on which ancillary molecular diagnostic tests are performed. Much of the reasoning behind this lies in that FFPE cell blocks best approximate FFPE blocks containing tissue specimens and the majority of the molecular assays are validated using FFPE material. Nonetheless, in light of the inherent disadvantages of cell blocks mentioned previously, investigation into other cytopreparatory platforms for molecular diagnostic assays have been performed by our group and many others. Cytopathology specimen preparation is diverse and ver-

![Fig. 2. Examples of epidermal growth factor receptor (EGFR) mutations detected by the polymerase chain reaction based fragment analysis assay utilized at our institution. Our assay is a multiplex assay designed to detect the two most common mutations in EGFR: the L858R substitution (case 1) and small deletions within exon 19 (case 2).](http://dx.doi.org/10.4132/jptm.2015.06.16)
satiable; the consequence of this is that each type of cytologic
analyte platform needs to be carefully validated for any given mo-
lecular test. Cytologic direct smears may ultimately prove to be the
cytologic platform best suited for PCR-based analysis due to the
high quality of nucleic acids and immediate nature of specimen
assessment for tumor cell adequacy. Cells on direct smears are
not exposed to formalin but rather undergo an alcohol-based fixa-
tion process prior to staining; thus, the negative effects of formalin
fixation on nucleic acid quality is non-contributory for direct
smears. Furthermore, the tumor cells of interest can be directly
visualized on the direct smear and isolated for nucleic acid extrac-
tion and molecular analysis; in essence, “what you see is what you
get.” We have previously demonstrated that uncoverslipped and
previously coverslipped/decoverslipped Diff-Quik stained smears
are robust sources of cellular material for PCR-based mutational
analysis such as EGFR mutation testing of lung adenocarcino-
ma and BRAF mutation testing of metastatic melanoma FNA
specimens. We and others have observed that destaining the
smears is not necessary for successful DNA extraction and PCR.

Our preference in utilizing Diff-Quik stained smears over Pana-
nicolaou stained smears is rooted in the following observations:
(1) cellular material on a Diff-Quik stained smear can be easily vi-

dualized without a coverslip and immediately triaged for molec-

ular diagnostic testing; and (2) the report by Killian et al. that
nucleic acids extracted from Diff-Quik stained smears show bet-


reduction of background blood as the Cytolyt solution exhibits
hemolytic properties. The cell suspension can be divided and
an aliquot is used to prepare a ThinPrep slide that is stained via
the Papanicolaou method, similar to alcohol-fixed Papanicolaou
stained direct smears. Tumor cells can be isolated from the Thin-


between ALK rearrangements involve fusions be-
Other less common
genes.50,51 The fusion gene product displays oncogenic ac-
Pechinoderm microtubule-associated protein-like 4 (EML4) and
of chromosome 2. This results in the fusion of portions of the
interstitial deletions and small inversions within the short arm
Most
exclude lung cancer patients for
tions to this and clinical characteristics should not be used to
patients who are never-smokers. However, there can be excep-
tions to this and clinical characteristics should not be used to
exclude lung cancer patients for ALK rearrangement testing.10
Most ALK rearrangements in lung adenocarcinoma result from
interstitial deletions and small inversions within the short arm
of chromosome 2. This results in the fusion of portions of the
echinoderm microtubule-associated protein-like 4 (EML4) and
ALK genes.50,51 The fusion gene product displays oncogenic ac-
tivity that drives these ALK rearranged lung adenocarcinomas.50
Other less common ALK rearrangements involve fusions be-
tween ALK and other genes, such as KIF5B and TFG.50 ALK
rearranged lung adenocarcinoma has been recognized as a legiti-
mate target for small molecular inhibitor therapy; crizotinib
was shown to be efficacious in treating patients with these can-
cers. In a phase 1 study evaluating 143 patients, an overall re-
sponse rate of 61% and estimated overall survival rates of 74.8%
at 12 months were observed.32 Therefore, the evaluation of lung
adenocarcinoma FNA samples for ALK rearrangements, in ad-
dition to EGFR mutations, has become increasingly incorporat-
ed into patient management algorithms.

FISH is currently the preferred approach to assaying lung ad-
enocarcinomas for ALK rearrangements according to expert re-
commendations based on review of the literature.10 In the United
States of America, there is only one test approved by the Food
and Drug Administration (FDA) for this purpose. This assay uti-
izes a dual ALK breakapart probe strategy in which orange and
green labeled probes hybridize to the highly conserved translo-
cation breakpoint region in the ALK gene. ALK gene loci that
have not undergone rearrangement typically display fused orange
and green signals (yellow) or juxtaposed touching orange and
green signals. When an ALK rearrangement occurs, the orange
and green signals become separated. However, as the majority of
the ALK rearrangements involve a small inversion within chro-
mosome 2p, rather than a rearrangement involving another chro-
mosome, the extent to which the two signals are split is finite.
In order to score a nucleus as positive for the ALK rearrangement,
the orange and green signals must be separated by a distance of
> 2 signal diameters (Fig. 3); a nucleus can also be scored positive
if a single orange signal without a corresponding green signal is
observed.33 Typically, up to 100 tumor cell nuclei are scored in this
assay and a lung adenocarcinoma is considered positive for the
ALK rearrangement if at least 15% of the nuclei are scored as
positive for the rearrangement.33,54

**ANAPLASTIC LYMPHOMA KINASE**

The anaplastic lymphoma kinase (ALK) protein is a receptor
tyrosine kinase and rearrangements involving the ALK gene lo-
cus is observed in approximately 5% of lung adenocarcinomas.9,50
These mutations are more commonly observed in younger age
patients who are never-smokers. However, there can be excep-
tions to this and clinical characteristics should not be used to
exclude lung cancer patients for ALK rearrangement testing.10
Most ALK rearrangements in lung adenocarcinoma result from
interstitial deletions and small inversions within the short arm
of chromosome 2. This results in the fusion of portions of the
echinoderm microtubule-associated protein-like 4 (EML4) and
ALK genes.50,51 The fusion gene product displays oncogenic ac-
tivity that drives these ALK rearranged lung adenocarcinomas.50
Other less common ALK rearrangements involve fusions be-
tween ALK and other genes, such as KIF5B and TFG.50 ALK
rearranged lung adenocarcinoma has been recognized as a legiti-
mate target for small molecular inhibitor therapy; crizotinib
was shown to be efficacious in treating patients with these can-
cers. In a phase 1 study evaluating 143 patients, an overall re-
sponse rate of 61% and estimated overall survival rates of 74.8%
at 12 months were observed.32 Therefore, the evaluation of lung
adenocarcinoma FNA samples for ALK rearrangements, in ad-
dition to EGFR mutations, has become increasingly incorporat-
ed into patient management algorithms.

FISH is currently the preferred approach to assaying lung ad-
enocarcinomas for ALK rearrangements according to expert re-
commendations based on review of the literature.10 In the United
States of America, there is only one test approved by the Food
and Drug Administration (FDA) for this purpose. This assay uti-
izes a dual ALK breakapart probe strategy in which orange and
green labeled probes hybridize to the highly conserved translo-
cation breakpoint region in the ALK gene. ALK gene loci that
have not undergone rearrangement typically display fused orange
and green signals (yellow) or juxtaposed touching orange and
green signals. When an ALK rearrangement occurs, the orange
and green signals become separated. However, as the majority of
the ALK rearrangements involve a small inversion within chro-
mosome 2p, rather than a rearrangement involving another chro-
mosome, the extent to which the two signals are split is finite.
In order to score a nucleus as positive for the ALK rearrangement,
the orange and green signals must be separated by a distance of
> 2 signal diameters (Fig. 3); a nucleus can also be scored positive
if a single orange signal without a corresponding green signal is
observed.33 Typically, up to 100 tumor cell nuclei are scored in this
assay and a lung adenocarcinoma is considered positive for the
ALK rearrangement if at least 15% of the nuclei are scored as
positive for the rearrangement.33,54

![Fig. 3. Example case of anaplastic lymphoma kinase (ALK) rearrangement fluorescence in-situ hybridization assay performed on a direct smear containing lung adenocarcinoma cells. The arrows point to the split orange and green signals that are separated by a distance of >2 signal diameters. These nuclei would be scored as positive for the ALK rearrangement.](http://dx.doi.org/10.4132/jptm.2015.06.16)
The FDA has approved this dual breakapart probe set for use on paraffin embedded tissue sections. As mentioned previously, paraffin embedded cell blocks are processed similarly to paraffin embedded tissue blocks; thus, cell blocks are traditionally used for the performance of ALK rearrangement FISH assays. Nonetheless, there is an observed noticeable failure rate when using and relying on cell blocks for these assays due to insufficient tumor cell material in a significant proportion of cases.\(^5\)\(^5\)\(^4\) Therefore, we and others have investigated alternative cytopreparatory platforms for ALK FISH. In our study, we demonstrated the effective use of Diff-Quik stained direct smears for this purpose and observed that the performance of this assay using direct smears was better than the performance using cell blocks.\(^5\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\)\(^6\) Proietti et al.\(^5\)\(^6\) also examined the use of Papanicolaou stained direct smears and observed that the failure rate due to insufficient cellularity was significantly higher for cell blocks than for direct smears. Therefore, the utilization of both Diff-Quik and Papanicolaou stained smears are feasible analytes for ALK FISH. Recently, ThinPrep slides have also been shown to be a feasible platform for ALK FISH.\(^5\)\(^4\) The advantage of utilizing cytologic preparation platforms such as direct smears and ThinPrep slides for this purpose is that entire tumor cell nuclei are being analyzed. FISH evaluation on paraffin sections derived cell blocks and other FFPE blocks are prone to signal loss in some of the tumor cells due to section truncation artifacts.\(^5\)\(^3\)\(^4\)

In addition, alternative methods to FISH have been the subject of recent investigation. Immunohistochemistry utilizing antibodies directed against ALK is an attractive alternative as it is simpler, quicker, and less expensive.\(^1\)\(^0\) The challenge associated with this approach is that the ALK protein is expressed at much lower levels in ALK rearranged lung tumors than in anaplastic large cell lymphoma, the prototypical ALK rearranged tumor.\(^1\)\(^0\) Fortunately, monoclonal anti-ALK antibodies (clones D5F3, D9E4, and 5A4) have been shown to exhibit high sensitivity and specificity.\(^1\)\(^0\)\(^5\)\(^7\) Immunohistochemistry using the D5F3 and 5A4 monoclonal antibodies on cytologic specimens have been recently described and shown to exhibit a high degree of concordance with ALK FISH testing.\(^5\)\(^5\)\(^8\) This supports the feasibility of utilizing an immunohistochemical approach as a first-line screening methodology to select specimens for ALK FISH testing. Of note, the mouse monoclonal anti-ALK1 antibody (CD246) typically used for the diagnosis of anaplastic large cell lymphoma is less reliable for identifying ALK rearrangements in lung adenocarcinoma; this is most likely attributable to the limited sensitivity of this particular antibody in detecting the lower expression levels of the ALK fusion proteins in lung adenocarci-

While EGFR mutations and ALK rearrangements represent the two best characterized, clinically actionable molecular alterations in NSCLC, other molecular markers are becoming increasingly appreciated and investigated. \(\text{ROS1}\) encodes a receptor tyrosine kinase and is rearranged in approximately 2% of lung adenocarcinomas.\(^1\)\(^9\) Early data seem to indicate that these tumors are responsive to crizotinib. \(\text{MET}\) encodes another receptor tyrosine kinase, hepatocyte growth factor receptor, and is amplified in a subset of NSCLC; a significant proportion of these cases are seen in context of acquired resistance to EGFR TKIs.\(^1\) As crizotinib also targets this receptor, studies are under way to determine whether this agent will be effective in \(\text{MET}\) amplified lung cancers. Next, mutations in \(\text{BRAF}\) are seen in approximately 3% of lung adenocarcinomas.\(^1\)\(^0\) Therapeutic agents targeting \(\text{BRAF}\), such as dabrafenib, are currently being investigated in clinical trials.\(^1\)\(^6\) In addition, gene rearrangements involving the \(\text{RET}\) tyrosine kinase gene (e.g., \(\text{KIF5B-RET}\)) have been observed in approximately 2% of lung adenocarcinomas; RET specific TKIs such as sunitinib, sorafenib, and vandetanib may be useful in treating patients with these lung cancers.\(^1\) Finally, amplification of \(\text{FGFR1}\) and mutations in \(\text{PIK3CA}\) have been observed in some lung squamous cell carcinomas and agents targeting these gene products are also under investigation.\(^1\) The above mentioned genetic markers highlight the distinct molecular profiles that are becoming increasingly appreciated for different subtypes of NSCLC, especially adenocarcinomas and squamous cell carcinomas. Therefore, efforts to correctly subtype cases of NSCLC are important, including challenging cases of poorly differentiated NSCLC.\(^1\)\(^0\) At our institution, if the subtyping of NSCLC is deemed to be challenging based on cytomorphologic evaluation alone, immunohistochemistry is utilized; we have de-

\text{http://jpatholtm.org/}\n
\text{http://dx.doi.org/10.4132/jptm.2015.06.16}
monstrated that this can be accomplished using smears as well as cell blocks. For example, Napsin-A and thyroid transcription factor-1 are often positive in lung adenocarcinomas and can be utilized for confirming this subtype of NSCLC. In contrast, p63 and p40 are useful markers for confirming a diagnosis of squamous cell carcinoma. Ultimately, utilizing FNA samples of NSCLC for immunocytochemistry must be judiciously leveraged to ensure that sufficient material still exists for molecular ancillary testing.

CONCLUDING THOUGHTS

Given the continuously evolving landscape in our understanding of the genetic events responsible for lung cancer pathogenesis, the integration between anatomic pathology, particularly cytopathology, and molecular diagnostics will become even more essential. Emerging molecular technological advances, such as next generation sequencing (NGS), that allow for high-throughput, high-sensitivity molecular analyses will likely play an important role in the management of patients with NSCLC. Cytopathic specimens, based on recent reports, represent a robust source of cellular material for NGS.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

I wish to thank my colleague in the Molecular Diagnostics Laboratory at University of Michigan Health System, Dr. Bryan Betz, for providing the images pertaining to molecular diagnostic assays displayed in this review.

REFERENCES


Analysis of Histologic Features Suspecting Anaplastic Lymphoma Kinase (ALK)-Expressing Pulmonary Adenocarcinoma

In Ho Choi · Dong Won Kim
Sang Yun Ha1 · Yoon-La Choi1
Hee Jeong Lee2 · Joungho Han1

Department of Pathology, Soonchunhyang University Seoul Hospital, Seoul; 1Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 2Department of Pathology, Gwangmyeong Sungei Hospital, Gwangmyeong, Korea

Background: Since 2007 when anaplastic lymphoma kinase (ALK) rearrangements were discovered in non-small cell lung cancer, the ALK gene has received attention due to ALK-targeted therapy, and a notable treatment advantage has been observed in patients harboring the EML4/ALK translocation. However, using ALK-fluorescence in situ hybridization (FISH) as the standard method has demerits such as high cost, a time-consuming process, dependency on interpretation skill, and tissue preparation. We analyzed the histologic findings which could complement the limitation of ALK-FISH test for pulmonary adenocarcinoma. Methods: Two hundred five cases of ALK-positive and 101 of ALK-negative pulmonary adenocarcinoma from January 2007 to May 2013 were enrolled in this study. The histologic findings and ALK immunohistochemistry results were reviewed and compared with the results of ALK-FISH and EGFR/KRAS mutation status. Results: Acinar, cribriform, and solid growth patterns, extracellular and intracellular mucin production, and presence of signet-ring-cell element, and psammoma body were significantly more often present in ALK-positive cancer. In addition, the presence of goblet cell-like cells and presence of nuclear inclusion and groove resembling papillary thyroid carcinoma were common in the ALK-positive group. Conclusions: The above histologic parameters can be helpful in predicting ALK rearranged pulmonary adenocarcinoma, leading to rapid FISH analysis and timely treatment.

Key Words: Lung; Adenocarcinoma; Anaplastic large cell lymphoma kinase; Anaplastic lymphoma kinase; in situ hybridization, fluorescence

Two major genetic mutations in non-small cell lung carcinomas (NSCLCs), epidermal growth factor receptor (EGFR) and KRAS, have been well studied. Since 2007 when Soda et al.1 discovered rearrangements of anaplastic lymphoma kinase (ALK) in NSCLC, the ALK gene has received attention as being responsible for another molecular subtype of lung cancer, accounting for about 5% to 6% of NSCLCs.2,3 It is important that the presence of ALK gene rearrangement can be an indication for targeted therapy, like EGFR-tyrosine kinase inhibitor (ex. gefitinib) as first-line therapy in patients with advanced pulmonary adenocarcinoma. Moreover, studies have shown that the ALK-targeted inhibitor ‘crizotinib’ produces a notable positive effect in patients harboring the EML4/ALK translocation.2

For the most effective use of ALK inhibitor, an accurate method to detect ALK gene rearrangement should be performed. To date, fluorescence in situ hybridization (FISH) is the universally accepted standard method for detecting ALK rearrangement, with immunohistochemistry (IHC) used as a screening method for identifying ALK rearrangement.4,5 However, there is a need for a more rapid method to detect ALK rearrangement due to the highly time-consuming nature of FISH. We encountered a patient who showed a dramatic response to crizotinib treatment before confirming ALK rearrangement by FISH based only IHC-positive results for ALK and suspicious histological findings from a biopsy. Therefore, we were curious whether the combination of IHC and suspicious histological findings can improve upon the shortcomings of FISH. Additionally, limited specimen obtained by biopsy is common and often results in lack of remaining tissue for ALK study after the two major mutation tests (EGFR and KRAS) for lung cancer. If the histologic finding is more suggestive of ALK mutation,6 priority can be given to the ALK mutation test because EGFR, KRAS, and ALK are known to be virtually mutually exclusive.

Our study aimed to investigate the characteristic histologic
features of ALK-positive pulmonary adenocarcinoma, in order to identify important histologic findings in small biopsied specimens whose architecture is difficult to assess and to evaluate whether the combination of these morphologic features and IHC results can overcome the shortcomings of FISH.

**MATERIALS AND METHODS**

**Case selection**

Two hundred five ALK-positive cases identified using IHC or FISH on biopsied and resected specimens from Samsung Medical Center were enrolled from January 2007 to May 2013. The basic characteristics of these cases (age, sex, and tumor stage), method of sampling (biopsy or operation), and results of IHC and FISH were investigated.

As a control group for comparing the histologic findings, all consecutive cases of pulmonary resection performed during 2012 were collected. The ALK-IHC had been performed in all cases of the control group, regardless of **EGFR** and **KRAS** analyses or microscopic findings.

The Institutional Review Board of Samsung Medical Center approved this study (IRB No. 2014-01-146).

**Review of the histological findings**

All resected tumors were classified according to histologic subtype based on the new International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) classification; the predominant pattern was determined as lepidic, acinar, papillary, solid, and invasive mucinous. All other components (>5% of tumors) were also noted, including the cribriform growth pattern. Presence of extracellular and intracellular mucin, presence of signet-ring-cells, abrupt presence of goblet cell-like cells that contain amphophilic mucin like those in the intestinal mucosa, and presence of psammoma body, nuclear inclusion and groove, bizarre nuclei and multilobated nuclei were evaluated. As one of the other cytomorphological parameters, presence of prominent large eosinophilic nucleoli was investigated using a cut-off value of >30% for tumor cells. In the biopsied specimens, we did not determine the predominant subtype, but we recorded all identifiable growth patterns, nuclear features and presence of psammoma body, bizarre nuclei, and nuclear inclusion and groove. We applied the parameter of ‘nuclear inclusion and groove’ to cells showing cytomorphologic features of thyroid papillary carcinoma. All cytomorphological features in all presented growth components were evaluated in three fields of each growth pattern using high magnification (×400).

**IHC and scoring for ALK**

IHC for **ALK** fusion was performed using formalin-fixed paraffin-embedded (FFPE) tumor tissue (4-µm thickness) and an antibody to NCL-**ALK** (1:30, clone 5A4, Novocastra, Newcastle upon Tyne, UK). The interpretation of IHC results was based on the a four-tier scoring system: 0 (none), 1+ (faint cytoplasmic staining, ≥10% of tumor cells), 2+ (moderate, smooth cytoplasmic staining), and 3+ (intense, granular cytoplasmic staining). IHC scores of 2+ or 3+ were regarded as ALK-positive results.

**FISH of ALK**

The FISH analysis for **ALK** rearrangement on FFPE tissue was examined using a probe specific to the **ALK** locus (Vysis LSI **ALK** dual-color, Break-Apart Rearrangement Probe, Abbott Molecular, Abbott Park, IL, USA). At least 50 nonoverlapping tumor cells were examined, and the cut-off value for positive **ALK** rearrangement was defined as >15% of tumor cells showing split signals or lone 3′ (orange color) signals (Fig. 1).

**EGFR and KRAS mutation status**

**EGFR** and **KRAS** mutation data of **ALK**-negative and **ALK**-positive groups were investigated. The mutation analyses of **EGFR** (exon 18, 19, 20, and 21) and **KRAS** (exons 2 and 3) were examined using direct sequencing-polymerase chain reaction...
Genomic DNA was extracted from the FFPE tissue and purified with a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Bidirectional sequencing was performed using the BigDye Terminator v 1.1 kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3130xl genetic analyzer (Applied Biosystems).

Statistical analysis

Fisher exact test was used to compare the features of ALK-positive pulmonary adenocarcinoma according to sex, and linear by linear association was used for age and tumor stage. Normal distribution according to age in each group was tested by Kolmogorove-Smirnove and Shapiro-Wilk test. Chi-square test/Fisher exact test, independent samples t test, and logistic regression analysis were applied where appropriate in order to determine the relationship between morphological features and ALK positivity. A p-value of < .05 was considered to be statistically significant in all statistical tests. All analyses were performed using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Comparison of ALK-IHC and ALK-FISH

The status of ALK-FISH and ALK-IHC was compared in 78 cases (Table 1). Among 69 ALK-FISH positive cases, 63 cases showed 2+ or 3+ positivity in ALK-IHC staining; therefore, the sensitivity of ALK-IHC (2+ and 3+) was determined to be 91.3% (63/69).

Characteristics of patients with ALK-positive pulmonary adenocarcinoma

We settled ALK-positive group (n = 205) as ALK-IHC or ALK-FISH positive cases, including 127 cases of ALK-IHC positive (2+ or 3+) without ALK-FISH examination, 63 cases of positive ALK-IHC and ALK-FISH, 9 cases of positive ALK-IHC and not detected or failed ALK-FISH, and 6 cases of nega-

Table 1. Arrangement of ALK-FISH results according to ALK-IHC and sampling method

<table>
<thead>
<tr>
<th>ALK-IHC</th>
<th>Rearranged (n = 69)</th>
<th>Not detected (n = 6)</th>
<th>Failed (n = 3)</th>
<th>No. of cases (n=78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1+</td>
<td>3 (4.3)</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2+</td>
<td>42 (60.9)</td>
<td>3 (50.0)</td>
<td>3 (100)</td>
<td>48</td>
</tr>
<tr>
<td>3+</td>
<td>21 (30.4)</td>
<td>3 (50.0)</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Failed</td>
<td>2 (2.9)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

| Sampling method | | | | |
|-----------------|-----------------|-----------------|-----------------|
| Biopsy | 56 (81.2) | 2 (33.3) | 0 | 58 |
| Resection | 13 (18.8) | 4 (66.7) | 3 (100) | 20 |

Values are presented as number (%).
ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

Table 2. Characteristics of patients with ALK-positive and -negative pulmonary adenocarcinoma

<table>
<thead>
<tr>
<th>Total cases (n=306)</th>
<th>ALK-positive</th>
<th>ALK-negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biopsy (n = 129)</td>
<td>Resection (n = 76)</td>
<td>Total (n = 205)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>47:82</td>
<td>36:40</td>
<td>83:122</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>53.9 ± 11.5</td>
<td>55.1 ± 11.7</td>
<td>54.4 ± 11.5</td>
</tr>
<tr>
<td>TNM stage, n (%)</td>
<td>a</td>
<td>b</td>
<td>1b</td>
</tr>
<tr>
<td>1</td>
<td>1 (0.8)</td>
<td>29 (38.2)</td>
<td>30 (14.8)</td>
</tr>
<tr>
<td>2</td>
<td>4 (3.1)</td>
<td>8 (10.5)</td>
<td>12 (5.9)</td>
</tr>
<tr>
<td>3</td>
<td>1 (0.8)</td>
<td>0</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>4</td>
<td>106 (82.2)</td>
<td>9 (11.8)</td>
<td>115 (56.1)</td>
</tr>
</tbody>
</table>

ALK, anaplastic lymphoma kinase; M, male; F, female.
*p-value between the ALK-positive and -negative group; **p-value between the ALK-positive and -negative resected group; *p-value by Student’s t test; *Linear by linear association by chi-square test.
The characteristics of patients in the ALK-positive group and control group are presented in Table 2. The ALK-positive group consisted of 129 biopsy cases and 76 resection cases. The sex ratio in the ALK-positive group was male:female = 83:122 in contrast to 53:48 in the ALK-negative group. This finding was statistically significant (p = .047) and suggested that ALK-positive pulmonary adenocarcinoma occurs more frequently in women, with odds ratio of 1.623. ALK-positive cancer occurred in patients 7.3 years younger than the ALK-negative group, and this difference was statistically significant (p < .001). The distribution of tumor stage in the ALK-positive biopsy group was one-sided in stage 4 (82.2%). However, a statistically significant difference was not noted between the ALK-positive and -negative resection groups (p = .254).

**Table 3.** Correlation of histologic findings in resection groups according to ALK positivity

<table>
<thead>
<tr>
<th></th>
<th>ALK-positive resection (n = 76)</th>
<th>ALK-negative resection (n = 101)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic subtype (predominant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td>31 (40.8)</td>
<td>35 (34.7)</td>
<td>.403</td>
</tr>
<tr>
<td>Papillary</td>
<td>24 (31.6)</td>
<td>36 (35.6)</td>
<td>.572</td>
</tr>
<tr>
<td>Solid</td>
<td>19 (25.0)</td>
<td>23 (22.8)</td>
<td>.730</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>0</td>
<td>3 (3.0)</td>
<td>.130</td>
</tr>
<tr>
<td>Lepidic</td>
<td>0</td>
<td>3 (3.0)</td>
<td>.130</td>
</tr>
<tr>
<td>Invasive mucinous</td>
<td>2 (2.3)</td>
<td>1 (1.0)</td>
<td>.402</td>
</tr>
<tr>
<td>Growth pattern (&gt;5% of entire tumor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td>65 (85.5)</td>
<td>73 (72.3)</td>
<td>.035</td>
</tr>
<tr>
<td>Papillary</td>
<td>52 (68.4)</td>
<td>66 (65.3)</td>
<td>.668</td>
</tr>
<tr>
<td>Solid</td>
<td>45 (59.2)</td>
<td>36 (35.6)</td>
<td>.002</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>31 (40.8)</td>
<td>21 (20.8)</td>
<td>.004</td>
</tr>
<tr>
<td>Lepidic</td>
<td>7 (9.2)</td>
<td>20 (19.8)</td>
<td>.052</td>
</tr>
<tr>
<td>Cribriform</td>
<td>51 (67.1)</td>
<td>29 (28.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of growth patterna</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (3.9)</td>
<td>14 (13.9)</td>
<td>&lt;.001b</td>
</tr>
<tr>
<td>2</td>
<td>14 (18.4)</td>
<td>44 (43.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>26 (34.2)</td>
<td>32 (31.7)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23 (30.3)</td>
<td>9 (8.9)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10 (13.2)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Microscopic findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of signet-ring-cell element</td>
<td>20 (26.3)</td>
<td>4 (4.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Extracellular mucin production</td>
<td>34 (44.7)</td>
<td>18 (17.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intracellular mucin content</td>
<td>58 (76.3)</td>
<td>57 (56.4)</td>
<td>.006</td>
</tr>
<tr>
<td>Hobnail cells with abundant cytoplasm</td>
<td>4 (5.3)</td>
<td>35 (34.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Abrupt presence of goblet cell-like cells</td>
<td>49 (64.5)</td>
<td>33 (32.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Psammoma body</td>
<td>33 (43.4)</td>
<td>5 (5.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Presence of bizarre cells</td>
<td>19 (25.0)</td>
<td>43 (42.6)</td>
<td>.015</td>
</tr>
<tr>
<td>Presence of multilobated cells</td>
<td>5 (6.6)</td>
<td>6 (5.9)</td>
<td>.862</td>
</tr>
<tr>
<td>Nuclear inclusion and groove</td>
<td>16 (21.1)</td>
<td>10 (9.9)</td>
<td>.038</td>
</tr>
<tr>
<td>Prominent macronucleoli</td>
<td>58 (46.6)</td>
<td>78 (53.4)</td>
<td>.034</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

ALK, anaplastic lymphoma kinase.

aNumber of all presented growth patterns presented in ‘Growth pattern (>5% of entire tumor)’; b p-value by Student’s t-test.
Among the examined cytomorphologic parameters, extracellular and intracellular mucin production (Fig. 2C), presence of signet-ring-cell element (Fig. 2D), presence of psammoma body (Fig. 2E), abrupt presence of goblet cell-like cells (Fig. 2F), and presence of nuclear inclusion and groove (Fig. 2G) were significantly more frequent in the ALK-positive resection group. Hobnail cells with abundant cytoplasm, bizarre cells, and presence of macronucleoli were more frequently observed in the ALK-neg-

![Fig. 2. Histologic features of anaplastic lymphoma kinase (ALK)-rearranged pulmonary adenocarcinoma. (A) ALK-rearranged tumors show variable growth patterns. At low magnification, the tumor shows solid, acinar, and papillary growth patterns. (B) Cribriform and micropapillary patterns are more frequent in ALK-positive pulmonary adenocarcinoma. (C) Tumors have frequent extracellular and intracellular mucin production. (D) Solid growth is frequently found to contain signet-ring-cells. (E) A few psammoma bodies are noted in the background of mixed tumor and extracellular mucin. (F) Some tumors contain goblet cell-like cells. (G) Intranuclear inclusion and nuclear groove resembling papillary thyroid carcinoma are frequently found. (H) On the biopsied specimen from the patient showing dramatic response to ALK-targeted therapy before ALK-fluorescence in situ hybridization, many signet-ring-cells are found. (I) Tumor cells in Fig. 2H show 3+ ALK-immunohistochemistry score.](http://jpatholm.org/)
According to logistic regression analysis (Table 4), acinar, solid, micropapillary, and cribriform growth patterns were more frequent in ALK-positive group, and the significant cytomorphologic features included presence of signet-ring-cell elements, extracellular mucin production, hobnail cells with abundant cytoplasm, and psammoma body.

Morphological features of small biopsy specimens compared with resection specimens

To evaluate whether ALK-specific morphologic features in the resection group can be applied to small biopsy specimens, cytomorphologic findings were compared between the ALK-positive biopsy and resection groups (Table 5). The parameters except for extracellular mucin production, psammoma body, and nuclear inclusion and groove showed no differences between the biopsy and resection groups (p > .05).

Table 4. Multivariate analysis: histologic findings in ALK-positive or -negative resection groups

<table>
<thead>
<tr>
<th>Growth pattern (&gt;5% of entire tumor)</th>
<th>ALK-positive resection (n = 76)</th>
<th>ALK-negative resection (n = 101)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinar</td>
<td>65 (85.5)</td>
<td>73 (72.3)</td>
<td>3.178</td>
<td>1.298–7.779</td>
</tr>
<tr>
<td>Solid</td>
<td>45 (59.2)</td>
<td>36 (35.6)</td>
<td>2.694</td>
<td>1.301–5.577</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>31 (40.8)</td>
<td>21 (20.8)</td>
<td>3.414</td>
<td>1.581–7.372</td>
</tr>
<tr>
<td>Cribriform</td>
<td>51 (67.1)</td>
<td>29 (28.7)</td>
<td>3.771</td>
<td>1.877–7.575</td>
</tr>
</tbody>
</table>

| Microscopic findings                |                               |                                  |            |       |
|-------------------------------------|                               |                                  |            |       |
| Presence of signet-ring-cell element| 20 (26.3)                     | 4 (4.0)                          | 5.466      | 1.488–20.090 |
| Extracellular mucin production      | 34 (44.7)                     | 18 (17.8)                        | 3.372      | 1.411–8.057 |
| Hobnail cells with abundant cytoplasm| 4 (5.3)                     | 35 (34.7)                        | 0.096      | 0.026–0.356 |
| Psammoma body                       | 33 (43.4)                     | 5 (5.0)                          | 16.548     | 5.303–51.641 |

Values are presented as number (%).

ALK, anaplastic lymphoma kinase; CI, confidence interval.

Table 5. Correlation of morphologic features between ALK-positive biopsy and resection groups

<table>
<thead>
<tr>
<th></th>
<th>ALK-positive biopsy (n=129)</th>
<th>ALK-positive resection (n=76)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of signet-ring-cell element</td>
<td>40 (31.0)</td>
<td>20 (26.3)</td>
<td>.476</td>
</tr>
<tr>
<td>Extracellular mucin production</td>
<td>15 (11.6)</td>
<td>34 (44.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intracellular mucin content</td>
<td>101 (78.3)</td>
<td>58 (76.3)</td>
<td>.743</td>
</tr>
<tr>
<td>Hobnail cells with abundant cytoplasm</td>
<td>5 (3.9)</td>
<td>4 (5.3)</td>
<td>.640</td>
</tr>
<tr>
<td>Abrupt presence of goblet cell-like cells</td>
<td>69 (53.5)</td>
<td>49 (64.5)</td>
<td>.124</td>
</tr>
<tr>
<td>Psammoma body</td>
<td>20 (15.5)</td>
<td>33 (43.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nuclear inclusion and groove</td>
<td>7 (5.4)</td>
<td>16 (21.1)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

ALK, anaplastic lymphoma kinase.

DISCUSSION

Since the presence of ALK rearrangement has become an important predictive biomarker for the use of the ALK-targeted inhibitor ‘crizotinib’ for NSCLC, the detection of ALK rearrangement is also becoming increasingly important. However, ALK-FISH as a standard method for detecting ALK rearrangement is expensive, time-consuming, and influenced by many factors (skill and material). On the other hand, ALK-IHC has been used as a screening method due to its lower specificity compared to ALK-FISH. We evaluated the histologic findings that can be helpful in predicting ALK rearrangement and in saving time until ALK-targeted inhibitor treatment.

Although there might have been a selection bias due to the excessive number of biopsied cases in stage IV, the ALK-positive group showed more frequent occurrence of lung adenocarcinoma in an advanced tumor stage than did the ALK-negative group (stage IV: 56.1% vs 13.9%). Although the ALK-positive resection group showed no statistically significant difference in the distribution of tumor stage compared with the ALK-negative group (p = .234), ALK-positive lung cancer is suspected to show more aggressive behavior, considering that ALK-positive lung cancer presents in an advanced stage with low resectability, leading to the need for chemotherapy or palliative therapy instead of surgical treatment.

There have been several studies on the morphologic findings of ALK-rearranged NSCLCs. Some authors7-9 have suggested that acinar growth pattern and extracellular mucin production
are typical of ALK-rearranged pulmonary adenocarcinoma. Another author recommended signet-ring-cell elements associated with solid growth as a feature of ALK-rearranged pulmonary adenocarcinoma.\textsuperscript{10} In the present study, histologic subtype based on ‘predominant’ growth pattern was not effective in discriminating between the ALK-positive and -negative groups. However, ALK-positive pulmonary adenocarcinoma showed more variable growth patterns (Fig. 2A) with significantly more frequent presence of acinar, solid, micropapillary, and cribriform growth patterns (Fig. 2A, B, D) compared with non-ALK rearranged adenocarcinoma (Table 3).

As reported by Jokoji \textit{et al.},\textsuperscript{7} and Inamura \textit{et al.},\textsuperscript{9} who observed that mucin production was one of the important findings of EML4-ALK-positive lung adenocarcinoma, our study also showed that extracellular mucin production was statistically significant in the ALK-positive resection group (p < .001).

Rodig \textit{et al.}\textsuperscript{10} showed that tumor cells forming a solid or sheet-like pattern were easily distinguishable from other growth patterns in ALK-rearranged adenocarcinoma, and that the majority (56%) of ALK-rearranged tumors showed a solid growth pattern with > 10% signet-ring cells. Our study also showed frequent solid growth (45/76 cases, 59.2%) in the ALK-positive resection group, and signet-ring-cell elements were extremely frequent in the ALK-positive resection group (20/76 cases, 26.3% vs 4/101 cases, 4.0%) (Table 3, Fig. 2D). Indeed, the signet-ring-cell element was significantly more frequent in the ALK-positive resection group showing solid growth than in the ALK-negative group (odds ratio, 21.9 vs 1.9).

A recent study about histomorphologic features of ALK-rearranged lung adenocarcinoma by Kim \textit{et al.}\textsuperscript{11} showed several significant microscopic features, including cribriform formation, presence of extracellular mucin, presence of mucin-containing cells, close relation to adjacent bronchioles, presence of psammoma body, presence of cholesterol cleft, and solid predominant pattern. Their data are similar to those of the present study except for two parameters that we did not evaluate close relation to adjacent bronchioles and presence of cholesterol cleft. However, we are more interested in the abrupt presence of goblet cell-like cells and presence of nuclear inclusion and groove resembling papillary thyroid carcinoma.

Abrupt presence of goblet cell-like cells (Fig. 2F) was frequent in the ALK-positive resection group (64.5% vs 32.7%, p < .001). We also found that many cases with a signet-ring-cell element in the ALK-positive resection group showed abrupt presence of goblet cell-like cells (16/20 cases, 80.0%). Considering the cytomorphologic similarity between signet-ring cells and goblet cell-like cells, we supposed that presence of goblet cell-like cells is part of a process to form signet-ring-cell and is predictive parameter for ALK-positive pulmonary adenocarcinoma. However, this relation was not statistically significant (p = .091).

One of the notable findings of our study was that the presence of nuclear inclusion and groove resembling papillary thyroid carcinoma (Fig. 2G) was noted more often observed in the ALK-positive lung cancer cases compared to the negative cases. We hypothesized that this feature is valuable in predicting ALK-positive lung cancer. However, there is no available data on why papillary thyroid carcinoma-like features are more frequent in ALK-positive lung cancer, suggesting additional study cases are needed.

On the correlation between biopsy and resection cases in ALK-positive tumors (Table 5), we found that some parameters, such as presence of signet-ring-cell elements, intracellular mucin content, and presence of goblet cell-like cells, showed no statistically significant differences between the biopsy and resection groups. However, these features are believed to be helpful for predicting ALK positivity in small biopsy specimens. Furthermore, presence of signet-ring-cell, psammoma body, nuclear inclusion and groove, and goblet cell-like cells are meaningful in small biopsy sections, if present (Fig. 2H).

Paik \textit{et al.}\textsuperscript{4} reported that the sensitivity and specificity of ALK-IHC was 100% and 95.8%, respectively, with a well-organized interpretation flow and concluded that ALK-IHC might be useful as a screening method to identify ALK rearrangements; a 91.3% sensitivity of ALK-IHC was noted in our study. However, ALK-IHC alone is not yet adequate to replace ALK-FISH and is applicable only as a screening method. For example, we excluded 4 cases showing discordance between the result of ALK and that of \textit{EGFR} and \textit{KRAS} before the present study. These cases included three \textit{EGFR}-mutated cases (two ALK-IHC- and ALK-FISH-positive cases with no examination by ALK-FISH and one concurrent \textit{EGFR}-mutated and ALK-FISH-positive case) and one concurrent ALK-IHC- and \textit{KRAS}-positive case without examination by ALK-FISH; \textit{KRAS} missense mutation in the 12th codon: c.34G>T (p.G12C). Upon reviewing a case showing \textit{EGFR} missense mutation in exon 18 (G719X), ALK-IHC positivity (2+), and ALK-FISH-positivity, there was a suspicion of over-interpretation of ALK-FISH analysis; about 20% of tumor cells showed only ‘lone 3’ (orange color) signals without ‘split signals.’ The reasons for the other three false positive cases by ALK-IHC could not be determined, although they showed definite 2+ and 3+ ALK-IHC scoring.

However, ALK-FISH has some demerits, such as high cost,
time-consuming process, dependency on many conditions, including skill of interpretation, and preparation of material such as quantity and quality of tumor cells. In our study, 9 cases showed no detection or failed results on ALK-FISH, and they were all negative for EGFR and KRAS, despite having 2+ or 3+ ALK-IHC scores (Table 1). After review of ALK-IHC and ALK-FISH results, the 6 cases with no detection were also confirmed as failures due to interpretation difficulty of ALK-FISH caused by excessive mucin content and low cellularity. In the practice, ALK-FISH is usually the second choice after analyses of both major mutations (EGFR and KRAS), and it leads to scarcity of tissue availability for testing from small biopsied specimens. Therefore, we believe that ALK-FISH could be complemented for rapid treatment, and the microscopic findings might be predictors of ALK rearrangement. If the morphologic findings are suggestive of ALK-rearranged pulmonary adenocarcinoma and are supported by a positive result in ALK-IHC, the patients can benefit from timely treatment. For example, we encountered a patient who showed dramatic response to ‘crizotinib’ before confirmation by ALK-FISH using only positive ALK-IHC and suspicious histological findings via biopsy (Fig. 2H, I).

Conclusively, we found that acinar, micropapillary, cribriform, and solid growth patterns with intracellular mucin content, signet-ring-cell elements, and psammoma body were useful in predicting ALK rearrangement. Indeed, the notable findings of our study were that presence of goblet cell-like cells or nuclear inclusion or groove resembling papillary thyroid carcinoma was more common in ALK-positive adenocarcinoma of the lung, although these findings should be evaluated further. In the biopsied specimens, presence of signet-ring-cell elements, goblet cell-like cells, intracellular mucin content, and psammoma body can be helpful in predicting ALK rearrangement. Additionally, there is a limitation of our study as the histologic comparison between FISH-positive cases and IHC-positive cases could not be made due to weighted biopsied specimens.

We hope to clarify the histologic findings of ALK rearrangement in a prospective cohort study and to evaluate the histologic findings with newly appearing genes in ALK rearrangement.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

REFERENCES


Cancers with Higher Density of Tumor-Associated Macrophages Were Associated with Poor Survival Rates

Kyong Yeun Jung1,2 · Sun Wook Cho1,3 · Young A Kim4 · Daein Kim3 · Byung-Chul Oh4 · Do Joon Park1 · Young Joo Park1

1Department of Internal Medicine, Seoul National University College of Medicine, Seoul; 2Department of Internal Medicine, Eulji University School of Medicine, Seoul; 3Department of Internal Medicine, National Medical Center, Seoul; 4Department of Pathology, SMG-SNU Boramae Medical Center, Seoul; 5Lee Gil Ya Cancer and Diabetes Institute, Gachon University Graduate School of Medicine, Incheon, Korea

Background: Macrophages are a component of a tumor’s microenvironment and have various roles in tumor progression and metastasis. This study evaluated the relationships between tumor-associated macrophage (TAM) density and clinical outcomes in 14 different types of human cancers.

Methods: We investigated TAM density in human tissue microarray sections from 14 different types of human cancers (n = 266) and normal thyroid, lung, and breast tissues (n = 22). The five-year survival rates of each cancer were obtained from the 2011 Korea Central Cancer Registry.

Results: Among 13 human cancers, excluding thyroid cancer, pancreas, lung, and gallbladder cancers had the highest density of CD163-positive macrophages (7.0 ± 3.5%, 6.9 ± 7.4%, and 6.9 ± 5.5%, respectively). The five-year relative survival rates of these cancers (pancreas, 8.7%; lung, 20.7%; gallbladder, 27.5%) were lower than those of other cancers. The histological subtypes in thyroid cancer exhibited significantly different CD163-positive macrophages densities (papillary, 1.8 ± 1.6% vs anaplastic, 22.9 ± 17.1%; p < .001), but no significant difference between histological subtypes was detected in lung and breast cancers. Moreover, there was no significant difference in CD163-positive macrophages densities among the TNM stages in lung, breast, and thyroid cancers.

Conclusions: Cancers with higher TAM densities (pancreas, lung, anaplastic thyroid, and gallbladder) were associated with poor survival rate.

Key Words: Tumor-associated macrophage; Prognosis; Neoplasms

The tumor microenvironment includes cancer cells and various stromal cells, including immune cells, fibroblasts, and vascular endothelial cells.1 Tumor-associated macrophages (TAMs) are also present in the tumor microenvironment and are important in tumor progression and metastasis.2 TAMs can induce neoplastic cell transformation, elicit tumor destructive reactions, and have either negative or positive effects on tumor growth.3 Several reports have suggested that TAMs are associated with tumor growth, disease progression, and poor prognosis in some human cancers.4 Moreover, high densities of TAMs are present in the more advanced stages of cancers that have poor prognoses, such as breast,4 lung,4 thyroid,3 and bladder3 cancers. In contrast, several reports, including those on colorectal,5 stomach,6 lung,7,8 and endometrial9 cancers, have shown that a high density of TAMs is associated with a high survival rate. Collectively, these results suggest that TAMs can have either positive or negative effects depending on the specific tissue type, tumor location, and tumor stage. The aim of this study was to evaluate the relationship between TAM density and clinical outcome in various human cancers.

MATERIALS AND METHODS

Study subjects and tissue microarrays

We purchased 14 different type of human cancer human tissue microarray sections (lung, 49; breast, 49; thyroid, 10; pancreas, 10; gallbladder, 9; larynx, 9; esophagus, 10; liver, 10; cervix, 10; ovary, 10; stomach, 10; prostate, 9; kidney, 9; and endometrium, 9 sections) and normal tissues (lung, 9; breast, 8 sections) from SuperBioChips Laboratories (Seoul, Korea). The supplier pro-
vided the clinical information associated with each section, including age at surgery; gender; pathologic diagnosis; TNM staging for cancers; survival/death follow-up result for lung and breast cancers; and estrogen receptor (ER), progesterone receptor (PR), and C-erbB2 expression for breast cancers. Thyroid microarrays contained normal thyroid (n = 5), papillary thyroid cancer (PTC; n = 35), and anaplastic thyroid cancer (ATC; n = 18), and one of the thyroid microarray slides was used in a previous study. Clinical and pathologic data for the thyroid samples were obtained from medical records at Seoul National University Hospital and SMG-SNU Boramae Medical Center. The five-year cancer relative survival rates were obtained from the 2011 annual report of cancer statistics in Korea.

Immunohistochemistry on tissue array blocks
CD68 and CD163 were used as TAM markers. Immunohistochemical (IHC) staining for CD68 and CD163 was performed using the BenchMark XT Slide Preparation System (Ventana Medical Systems, Tucson, AZ, USA) and CD68 (ready-to-use, 514H12, Novocastra, Newcastle upon Tyne, UK) and CD163 (1:200, 10D6, ER2, Novocastra). The proportion of CD163-positive area in each tumor was evaluated after IHC staining (Fig. 1). We divided the area of each tissue core into quarters and a central area and randomly chose all five fragments to determine the positive stain proportion (Fig. 1A). Areas of fibrosis or tumor necrosis among the randomly chosen fragments were excluded. We used a color deconvolution plug-in for Image J software to identify the positive stains and to calculate the percent CD163-positive area (Fig. 1B, C). TAM density was determined by calculating the average CD163-positive area (%) at a minimum of four different sites in each tissue.

Statistical analysis
The CD163-positive macrophages density (%) is presented as mean ± standard deviation. Differences in CD163-positive macrophages densities among TNM cancer stages, histological subtypes, and pathological characteristics were determined using one-way analysis of variance (ANOVA) or Student’s t test. Statistical significance is indicated by a p-value less than .05. All data were analyzed with IBM SPSS Statistics ver. 20.0 (SPSS Inc., Chicago, IL, USA).

Ethics statement
This study was approved by the Institutional Review Boards of Seoul National University Hospital (1107-060-369) and SMG-SNU Boramae Medical Center (06-2010-176). The need for informed consent was waived by those boards.

RESULTS
CD163-positive macrophage densities and prognoses in human cancers
The average CD163-positive area was significantly correlated with the average CD68-positive area (%) in thyroid (r = 0.775, p < .001) (Appendix 1A), breast (r = 0.806, p < .001) (Appendix 1B), and lung (r = 0.780, p < .001) (Appendix 1C) cancers. Therefore, our remaining IHC assessments were performed with CD163 data.

The CD163-positive macrophages densities and the five-year relative survival rates in the 14 different types of human cancers are summarized in Fig. 2. Excluding thyroid cancer, of the 13 other human cancers, pancreas, lung, and gallbladder cancers had the highest density of CD163-positive macrophages (7.0 ±
3.5%, 6.9 ± 7.4%, and 6.9 ± 5.5%, respectively). In contrast, endometrium, prostate, and kidney cancers had the lowest densities of CD163-positive macrophages (3.6 ± 4.6%, 2.8 ± 2.5%, and 2.8 ± 1.8%, respectively). Interestingly, among the tested cancers, five-year relative survival rate (%) was inversely correlated with CD163-positive macrophages density. The five-year overall survival rates of the cancers with highest TAM densities (pancreas, 8.7%; lung, 20.7%; gallbladder and biliary tract, 27.5%) were lower than those of the cancers with the lowest TAM densities (endometrial, 86.5%; kidney, 78.8%; prostate, 92%).

Of the 14 cancers assessed, thyroid cancer exhibited an extremely wide range of TAM densities that varied according to pathologic subtype (Fig. 3). The ATC cases had the highest density of CD163-positive macrophages (22.9 ± 17.1%), whereas the PTC cases had the lowest CD163-positive macrophage density (1.8 ± 1.3%).

**Clinicopathological correlations with TAM density in thyroid, lung, and breast cancers**

To investigate the role of TAMs in human cancers, we evaluated the correlations between TAM density and clinicopathological features in lung, breast, and PTC cancers. The CD163-positive macrophage density was significantly higher in cancer tissues than normal tissues in breast (4.7 ± 4.5% vs 0.6 ± 0.2%; p < .001), lung (6.9 ± 7.4% vs 1.6 ± 1.6%; p < .001), and thyroid (PTC, 1.8 ± 1.3% vs normal, 0.2 ± 0.2%; p < .001) tissues (Fig. 3). Among the histological subtypes of lung (adenocarcinoma, 5.8 ± 4.4% vs squamous cell carcinoma, 5.9 ± 4.5% vs large cell carcinoma, 8.7 ± 7.3%; p = .428) and breast cancers (infiltrating ductal carcinoma, 4.0 ± 3.4% vs medullary carcinoma, 9.3 ± 3.4%; p = .055), there were no significant differences in CD163-positive macrophages density (Appendix 2). Differences among TNM stages were ev-

**Fig. 2.** CD163-positive macrophage densities and five-year survival rates in 14 different types of human cancers. Left axis and bar graphs represent the average CD163-positive area (%) as mean ± standard error. Right axis and star-shaped markers represent the five-year overall survival (OS, %) obtained from the 2011 Annual Report of Cancer Statistics in Korea. PTC, papillary thyroid cancer; GB, gallbladder; ATC, anaplastic thyroid cancer; NA, not acquired.

<table>
<thead>
<tr>
<th>CD163 (%)</th>
<th>1.8</th>
<th>2.8</th>
<th>2.8</th>
<th>3.6</th>
<th>4.4</th>
<th>4.7</th>
<th>5.0</th>
<th>5.6</th>
<th>5.9</th>
<th>6.1</th>
<th>6.3</th>
<th>6.9</th>
<th>6.9</th>
<th>7.0</th>
<th>22.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Yr OS</td>
<td>100</td>
<td>86.5</td>
<td>78.8</td>
<td>92</td>
<td>69.4</td>
<td>91.3</td>
<td>61.6</td>
<td>80.1</td>
<td>28.6</td>
<td>30.2</td>
<td>71.7</td>
<td>27.5</td>
<td>20.7</td>
<td>8.7</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Fig. 3.** The CD163-positive macrophage densities of normal tissue, papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC).
valuated by assessing adenocarcinoma and squamous cell carcinoma of lung cancer, infiltrating ductal carcinoma of breast cancer, and PTC. There were no significant differences in CD163-positive macrophage density associated with TNM stage in lung (p = .821), breast (p = .060), or thyroid (PTC, p = .943) cancer (Appendix 2). Within the breast cancer samples, several prognostic markers including ER, PR, p53, and C-erbB2 were analyzed. The ER-positive (3.4 ± 4.5% vs 5.6 ± 4.8%; p = .182) and PR-positive (3.7 ± 4.4% vs 5.4 ± 4.9%; p = .319) breast cancers showed non-significant trends toward lower CD163-positive macrophages density than those in the ER- or PR-negative breast cancers. In contrast, p53-positive (5.9 ± 5.9% vs 4.2 ± 3.6%; p = .269) and C-erbB2-positive (6.4 ± 6.8% vs 4.4 ± 3.8%; p = .233) breast cancer showed nonsignificant trends toward higher CD163-positive macrophage density than those in p53- or C-erbB2-negative breast cancers.

**DISCUSSION**

Our results demonstrate that CD163-positive macrophage density is inversely correlated with five-year cancer survival rate in a variety of human cancers. This finding suggests the presence of a potential pro-tumorigenic role of TAMs in some human cancers. Among the 14 different types of human cancers assessed, ATC, the most aggressive cancer in humans,16,17 had the highest CD163-positive macrophages density (23%), which was markedly higher than the second highest TAM density (7%) detected in the pancreas cancer samples. ATC accounts for up to 2% of thyroid cancers and is characterized by an extremely poor survival rate with a one-year average survival rate of 20% and a median survival duration of five months.18,19 In contrast, the most common well-differentiated thyroid cancer, PTC, had the lowest CD163-positive macrophages density (1.8%). PTC is associated with lower recurrence and mortality rates20 than other solid and relatively indolent tumors and has a five-year survival rate higher than 90%.21 Since the differentiation of pathologic subtypes is distinctive and survival rates markedly differ among subtypes, our observation of a striking difference in CD163-positive macrophages density between two thyroid cancer subtypes led us to hypothesize that TAM density is a potential prognostic factor in human cancers.

In contrast, histological subtypes of lung and breast cancers did not exhibit significant differences in CD163-positive macrophage density. Correlations between TAM density and clinicopathological features have been reported in ductal and lobular carcinomas in breast cancer22 and in adenocarcinoma23,24 and non-small cell25,26 lung cancers. Nonetheless, only a few reports have compared TAM density based on histological subtype. Although our data did not detect a significant difference related to histological subtype in lung or breast cancers, the number of each subtype in our sample population was limited. Therefore, we recommend a larger-scale study of TAM density in a variety of histological subtypes.

In this study, there were no significant differences in CD163-positive macrophage density among TNM stages in lung, breast, or thyroid cancers. In addition, cancer tissues showed higher CD163-positive macrophage densities than those in normal tissues in lung, breast, and thyroid samples. In breast cancer, there were trends toward negative correlations of TAM density with ER and PR expression, whereas there were trends toward positive correlations of TAM density with p53 and C-erbB2 expression. Previous reports indicate that TAM infiltration is inversely correlated with ER expression in breast cancer.7,27 However, this correlation might vary with TAM location.23 Medrek et al.23 reported that a dense infiltration of CD163-positive macrophages in tumor stroma was associated with ER- and PR-negativity, but there was no such association in tumor nest. Our study results show a non-significant trend toward negative correlations between ER and PR expressions and density of CD163-positive macrophages. Due to data limitations, we could not assess such correlations according to infiltration location.

Selecting the most appropriate marker for TAM assessment can be challenging. Originally, a TAM was seen as a residual macrophage within the tumor microenvironment, and the biological characteristics of TAMs have been shown to be anti-tumorigenic, cytotoxic, or pro-tumorigenic.1,28 Furthermore, macrophages can undergo polarization and change from an M1-macrophage (classically activated, pro-inflammatory) to an M2-macrophage (alternatively activated, anti-inflammatory or regenerative).29 Collectively, these findings indicate the difficulty in choosing a cancer-specific TAM marker. In the beginning of this study, we performed IHC investigations using both CD68, a pan macrophage marker,29 and CD163, a more specific marker for M2-macrophages.30 Those investigations revealed that the CD163-positive areas were significantly positively correlated with the CD68-positive areas in lung, breast, and thyroid cancers; thus, our remaining IHC assessments were performed with CD163 data.

Previous clinical studies have shown a correlation between TAM density and cancer prognosis. Most studies have shown a significant correlation between TAM density and poor prognosis, especially among breast, thyroid, and lung cancers.1,7,8,22
However, several studies have reported that TAM density is associated with good prognosis. Such contradictory results might be due to differences in the number, grade, stage, or size of tumors among the studies. In addition, previous clinical studies have used various methods to assess TAM infiltration. The use of different approaches could have also contributed to the inconsistent results. Although the sample size for each cancer subtype in our study population was small, the results in this study comparing TAM density in various human cancers are significant; therefore, further studies with larger sample sizes are warranted.

In summary, we detected a trend toward an inverse correlation between CD163-positive macrophage density and five-year survival rate in 14 different types of human cancers. In particular, PTC and ATC clearly showed an inverse correlation between TAM density and prognosis. Our results suggest that TAM density is a potential biomarker of poor prognosis in human cancers.

Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

Acknowledgments
This work was supported by a grant from the Next-Generation BioGreen 21 Program (No.PJ00954003), Rural Development Administration, Republic of Korea.

REFERENCES


Appendix 1. The correlation between average CD163-positive area (%) and average CD68-positive area (%). (A) Thyroid cancer. (B) Breast cancer. (C) Lung cancer.

Appendix 2. Average CD163-positive area (%) according to clinicopathologic characteristics in lung, breast, and thyroid cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>CD163 (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>19</td>
<td>5.8±4.4</td>
<td>.428</td>
</tr>
<tr>
<td>SQCC</td>
<td>20</td>
<td>5.9±4.5</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>4</td>
<td>8.7±7.3</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>2</td>
<td>1.0±0.5</td>
<td></td>
</tr>
<tr>
<td>Mixed carcinoma</td>
<td>4</td>
<td>5.0±2.3</td>
<td></td>
</tr>
<tr>
<td>Stage*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>16</td>
<td>5.7±4.4</td>
<td>.821</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>6.6±5.2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>5.5±3.8</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>15</td>
<td>3.6±3.4</td>
<td>.104</td>
</tr>
<tr>
<td>Death</td>
<td>15</td>
<td>5.9±3.9</td>
<td></td>
</tr>
<tr>
<td><strong>Breast</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal</td>
<td>45</td>
<td>4.0±3.4</td>
<td>.055</td>
</tr>
<tr>
<td>Medullary</td>
<td>2</td>
<td>9.3±3.4</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>1</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Metaplastic</td>
<td>1</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>Stage*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>13</td>
<td>5.3±4.9</td>
<td>.060</td>
</tr>
<tr>
<td>IIB</td>
<td>10</td>
<td>4.2±2.4</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>12</td>
<td>1.8±1.4</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>10</td>
<td>4.5±2.3</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>30</td>
<td>4.2±3.7</td>
<td>.726</td>
</tr>
<tr>
<td>Death</td>
<td>6</td>
<td>3.7±2.5</td>
<td></td>
</tr>
<tr>
<td><strong>Thyroid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTC</td>
<td>35</td>
<td>1.8±1.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ATC</td>
<td>18</td>
<td>22.9±17.1</td>
<td></td>
</tr>
<tr>
<td>Stage*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>1.4±0.6</td>
<td>.943</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>1.7±1.1</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>21</td>
<td>1.8±1.2</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>1.8±0.8</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>15</td>
<td>1.8±1.5</td>
<td>.241</td>
</tr>
<tr>
<td>Death</td>
<td>2</td>
<td>3.3±2.8</td>
<td></td>
</tr>
</tbody>
</table>

SQCC, squamous cell carcinoma; SCC, small cell carcinoma; PTC, papillary thyroid carcinoma; ATC, anaplastic thyroid carcinoma.

*p-value by ANOVA or student t test; *TNM stage and survival was classified in adenocarcinoma and squamous cell carcinoma (lung), infiltrating ductal carcinoma (breast) and papillary thyroid carcinoma (thyroid).
WHO Grade IV Gliofibroma: A Grading Label Denoting Malignancy for an Otherwise Commonly Misinterpreted Neoplasm

We report a 50-year-old woman with no relevant clinical history who presented with headache and loss of memory. Magnetic resonance imaging showed a left parieto-temporal mass with annular enhancement after contrast media administration, rendering a radiological diagnosis of high-grade astrocytic neoplasm. Tumour sampling was performed but the patient ultimately died as a result of disease. Microscopically, the lesion had areas of glioblastoma mixed with a benign mesenchymal constituent; the former showed hypercellularity, endothelial proliferation, high mitotic activity and necrosis, while the latter showed fascicles of long spindle cells surrounded by collagen and reticulin fibers. With approximately 40 previously reported cases, gliofibroma is a rare neoplasm defined as either glio-desmoplastic or glial/benign mesenchymal. As shown in our case, its prognosis is apparently determined by the degree of anaplasia of the glial component.

Key Words: Gliofibroma; Bimorphic neoplasm; Desmoplastic glioma; Adult population; Tumour suppressor protein 53

CASE REPORT

A 50-year-old female presented with headache and loss of memory over the previous six months. Magnetic resonance imaging scans showed a left parieto-temporal mass (70.82 × 48.56 × 43.97 mm) with significant perilesional oedema and postcontrast annular enhancement (Fig. 1A, B). Despite prompt tumour sampling and analysis, the patient ultimately expired one month after the surgery. Necropsy was not authorized.

Histologically, the lesion showed areas unequivocally consistent with glioblastoma due to their microvascular proliferation, palisading necrosis, hypercellularity, pleomorphism, and high mitotic activity (Fig. 1C, D). These areas intermingled with cytologically bland, long spindle-cell fascicles (Fig. 1E, F) enveloped by sturdy collagen and fine reticulin fibers (Fig. 2A–D). As expected, immunoperoxidase-coupled reactions showed strong and diffuse expression of glial fibrillary acidic protein in the glioblastoma areas (Fig. 3A), while the spindled part of the neoplasm was exclusively positive for vimentin (Fig. 3B). Ki-67 expression was variable, with positivity as high as 50% in the astroglial regions (Fig. 3C) but with immunolabeling of less than 1% in the mesenchymal portion. Likewise, expression of p53 was largely diffuse in the gliomatous parts, albeit focally in some fusiform cells (Fig. 3D, E). No reaction was detected for epidermal growth
factor receptor in either element.

The lesion was diagnosed as gliofibroma (mixed astrocytic neoplasm with a low-grade mesenchymal constituent), with special note of its poor prognosis based on the degree of anaplasia of the glial component, suggestive of glioblastoma. This note was written prior to the patient’s demise.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient’s legal guardian, and the anonymity of the patient was preserved.

Fig. 1. Magnetic resonance imaging scans and biphasic histological features. (A) Post-contrast, T1-weighted sagittal section. A heterogeneous tumour with an enhancing peripheral rim is shown. (B) Fluid-attenuated inversion recovery sequence. Considerable outlying oedema can be seen. (C) Glioblastomatous component with palisading necrosis and microvascular proliferation (upper right inset). (D) Boundary zone with neoplastic cells of gemistocytic appearance (left) next to apparently atypical spindle cells (right). Mitoses are noticeable exclusively in neoplastic glial cells (arrow). (E) Mesenchymal component with solid fascicular tissue intermingled with loose astrocytic areas. (F) High-magnification photomicrograph of the cytologically bland mesenchymal constituent.
Fig. 2. Histochemical stains of whole-mount sections. (A, B) Collagen-rich tissue seen with Masson’s trichrome. (C, D) Reticulin pattern showed with reticular fiber stain.

Fig. 3. Immunohistochemistry panel. (A) Glial fibrillary acidic protein. (B) Vimentin. (C) Ki-67. (D) p53 (glial portion) with diffuse immunolabeling. (E) p53 (mesenchymal portion) with very focally positive immunoreactivity.
DISCUSSION

The term gliofibroma was first introduced by Friede in 1978\(^1\) to describe an unusual neoplasm featuring astroglial cells within collagen bundles. Since then, nearly 40 cases of gliofibroma have been reported in the worldwide literature, the vast majority occurring in the paediatric population.\(^2\) Only 11 cases have been recorded in adults, with age ranging from 19 to 54 years.\(^3,4\) More importantly, some cases described in both, children and adults, bear morphological traits indicative of biologically aggressive lesions (Table 1). The prognostic value of these features in gliofibromas has been widely debated, as some non-lethal tumours have a high-grade glial component while other are recurrent lesions despite their low-grade glial associate.\(^5,6\) Moreover, as accurately demonstrated by Suarez et al.,\(^7\) the overall mortality for these lesions is approximately 23%. However, when considering the lethality of the high-grade-only subset, this value increases to 70% or even 100% in cases with an accompanying glioblastoma component (Table 1).\(^8-13\)

In their work, Schober et al.\(^9\) were the first to tentatively assign a World Health Organization (WHO) grade to their diagnosis, while Cerda-Nicolas and Kepes\(^10\) preferred to add a descriptive modifier (anaplastic). In a similar way, Sharma et al.\(^4\) proposed to dichotomize these lesions into low-grade (benign) or high-grade (malignant/anaplastic) based on the Ki-67 proliferation index. In spite of these grading attempts, Snipes et al.\(^8\) seminally pointed out that prognosis seems to be more related to location rather than to any morphological or immunohistochemical feature, though the degree of anaplasia and the mitotic index might be worthy of consideration. On the other hand, the case documented by Altamirano et al.\(^14\) highlights the issue of whether atypia can exist in the fibroma portion of the neoplasm. This is exceedingly difficult to assert, as the boundaries of either a gliosarcoma or sarcoglioma are unavoidably blurred.

In this case, increased immunolabeling for Ki-67 was noted in the nuclei of the mesenchymal part, though it was rated

Table 1. High-grade gliofibroma cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Reference</th>
<th>Age/Sex</th>
<th>Location</th>
<th>Grade according to the described features of the glial component</th>
<th>Outcome (follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Friede (1978)(^1)</td>
<td>3.9 yr/F</td>
<td>Lower medulla oblongata</td>
<td>Dedifferentiation of the glial component</td>
<td>Died (3 mo)</td>
</tr>
<tr>
<td>2</td>
<td>Snipes et al. (1991)(^6)</td>
<td>2 mo/F</td>
<td>Right thalamus and floor of the fourth ventricle</td>
<td>Anaplastic astrocytoma, WHO grade III (mitoses up to 5 per 10-HPF)</td>
<td>Died (16 mo)</td>
</tr>
<tr>
<td>3</td>
<td>Vazquez et al. (1991)(^6)</td>
<td>11 mo/F</td>
<td>Right temporal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (pleomorphism and numerous MFs)</td>
<td>Alive (2 yr)</td>
</tr>
<tr>
<td>4</td>
<td>Schober et al. (1992)(^9)</td>
<td>18 yr/M</td>
<td>Right frontal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (foci of pleomorphism, sparse and abnormal MFs)</td>
<td>Alive (7 days)</td>
</tr>
<tr>
<td>5</td>
<td>Cerda-Nicolas and Kepes (1993)(^11)</td>
<td>4 yr/F</td>
<td>Fourth ventricle, extending to the preoptic cistern and left temporal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III</td>
<td>No data</td>
</tr>
<tr>
<td>6</td>
<td>Cerda-Nicolas and Kepes (1993)(^11)</td>
<td>9 yr/F</td>
<td>Left parietal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (pleomorphism and occasional MFs)</td>
<td>Alive (5.5 mo)</td>
</tr>
<tr>
<td>7</td>
<td>Caldemeyer et al. (1995)(^11)</td>
<td>8 yr/M</td>
<td>Right temporal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (numerous MFs)</td>
<td>No data</td>
</tr>
<tr>
<td>8</td>
<td>Sharma et al. (1998)(^4)</td>
<td>54 yr/F</td>
<td>Right parietal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (pleomorphism, MFs and 10.5% of PI)</td>
<td>Died (6 mo)</td>
</tr>
<tr>
<td>9</td>
<td>Kim et al. (2003)(^12)</td>
<td>25 yr/M</td>
<td>Left parietal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (up to 35.8% of PI)</td>
<td>Alive (2 mo)</td>
</tr>
<tr>
<td>10</td>
<td>Gargano et al. (2014)(^12)</td>
<td>10.7 yr/F</td>
<td>Left fronto-parietal lobe</td>
<td>Anaplastic astrocytoma, WHO grade III (mild to moderate pleomorphism with up to 10% of PI)</td>
<td>Alive (2 yr)</td>
</tr>
<tr>
<td>Subtotal (10 cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mortality rate (cases with available data) (5/8) = 62.5%</td>
</tr>
<tr>
<td>11</td>
<td>Bastos Leite et al. (2002)(^13)</td>
<td>47 yr/F</td>
<td>Left parietal lobe</td>
<td>Glioblastoma, WHO grade IV (nuclear atypia, endothelial proliferation, necrosis and mitoses)</td>
<td>Died (1 yr)</td>
</tr>
<tr>
<td>12</td>
<td>Present case</td>
<td>50 yr/F</td>
<td>Left parieto-temporal lobe</td>
<td>Glioblastoma, WHO grade IV (microvascular proliferation, palisading necrosis and up to 50% of PI)</td>
<td>Died (1 mo)</td>
</tr>
<tr>
<td>Subtotal (2 cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mortality rate (2/2) = 100.0%</td>
</tr>
<tr>
<td>Total (12 cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall mortality rate (cases with available data) (7/10) = 70.0%</td>
</tr>
</tbody>
</table>

F, female; WHO, World Health Organization; HPF, high-power fields; M, male; MFs, mitotic figures; PI, proliferation index (MIB-1, Ki-67).
in less than 5% of the overall tumour. The authors interpreted the mass as a proliferative activity of uncertain relevance. Though our case displays focal nuclear enlargement and vacuolization (Fig. 1D), we did not observe an increment in mesenchymal Ki-67 expression and did not find any areas suggestive of a true sarcomatous component. Nonetheless, this presumed “atypia” might not reliably worsen the prognosis, as in our case it was based on histology (glioblastoma) while in Altamirano’s group was determined by location (thalamus/mesencephalon). p53 immunostaining in our case was also of conjectural significance, indicating not only a possible secondary glioblastoma, but also a common origin of both the glial and mesenchymal elements, similar to the demonstration of Biernat regarding gliosarcomas, and thus possibly representing a true benign mesodermal differentiation as formerly hypothesised by Snipes et al. Furthermore, p53 immunolabeling has been previously tested in nine gliofibroma cases with no positive result. Interestingly, Goyal et al. also applied this immunohistochemical marker in three other cases but never mentioned the final outcome. This lack of p53 expression is shared by the so-called desmoplastic infantile astrocytoma and may hint, at least in these aforementioned cases, at a different developmental pathway from that of diffuse or anaplastic astrocytomas and glioblastomas.

Three more issues briefly deserve our attention. (1) As previously mentioned, the first report of gliofibroma clearly detailed a desmoplastic astrocytoma (Friede-type gliofibroma); nonetheless, this designation was subsequently applied to similar lesions consisting of both glial and mesenchymal derivatives (authentic glioma/fibroma), as in the present case. It is worth mentioning that the glial constituent can range from innocuous to evidently malignant, while the latter is a consistently benign fusocellular component. (2) In the bimorphic subset, it has not been fully corroborated whether the two populations are distinct cell lineages or if the fusiform cells are glia capable of differentiating into fibroblasts, myofibroblasts, Schwann cells or histiocytes. (3) Recently, some desmoplastic lesions displaying a non-astrocytic glial phenotype with immunohistochemical properties more akin to ependymoma (as in the case described by Gargano et al.) have also been diagnosed as gliofibromas. Thus, as pointed out by Suárez et al., a controversial dilemma of “splitting versus lumping” currently prevails in the conceptual and classification schemes regarding these kinds of tumours. Nevertheless, it seems to be more of an academic and histopathological predicament rather than one of clinical concern or prognostic relevance.

As shown in our case and previously advised by other authors, gliofibroma prognosis is greatly influenced by the degree of anaplasia of the glial component. Hence, we suggest adding a grade (WHO I–IV) or descriptive (low-grade, high-grade, anaplastic, malignant/glioblastomatoid) modifier to the diagnosis of gliofibroma.

It had been previously claimed that necrosis and prominent vascular proliferation are not usual features of gliofibromas. Thus, we document the second case of gliofibroma with a glioblastomatous component (though this is the first case involving a mixture of glioma and fibroma) as well as the third case occurring in Latin American population.

Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

REFERENCES
10. Cerda-Nicolas M, Kepes JJ. Gliofibromas (including malignant forms), and gliosarcomas: a comparative study and review of the
A Rare Case of Primary Tubular Adenocarcinoma of the Thymus, Enteric Immunophenotype: A Case Study and Review of the Literature

Hae Yoen Jung · Hyundeuk Cho · Jin-Haeng Chung · Sang Byoung Bae · Ji-Hye Lee · Hyun Ju Lee · Si-Hyong Jang · Mee-Hye Oh

Department of Pathology, Soonchunhyang University Cheonan Hospital, Cheonan; 1Department of Pathology and Respiratory Center, Seoul National University Bundang Hospital, Seongnam; 2Division of Hematology and Oncology, Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, Korea

Received: March 10, 2015
Revised: April 8, 2015
Accepted: April 16, 2015

Corresponding Author
Mee-Hye Oh, MD, PhD
Department of Pathology, Soonchunhyang University Cheonan Hospital, 31 Suncheonhyang 6-gil, Dongnam-gu, Cheonan 330-930, Korea
Tel: +82-41-570-3580
Fax: +82-41-570-3580
E-mail: mhoh0212@schmc.ac.kr

Key Words: Thymus gland; Adenocarcinoma; Keratin-20; Caudal type homeobox 2

Thymic carcinomas are uncommon malignant tumors. According to the World Health Organization (WHO), thymic carcinomas are mostly squamous cell, lymphoepithelioma-like, or basaloid carcinomas. Thymic adenocarcinomas are extraordinarily rare. We herein report a case of primary thymic adenocarcinoma with tubular morphology and enteric immunophenotype as determined by expression of cytokeratin (CK) 20 and caudal type homeobox 2 (CDX2). The immunophenotype suggests metastasis from the gastrointestinal tract; however, extensive clinical evaluation revealed no extrathymic primary tumor. In addition, we review the literature to facilitate a discussion of the clinicopathologic characteristics of thymic adenocarcinoma.

CASE REPORT

A previously healthy 59-year-old woman presented with abdominal pain in the lower left quadrant that had persisted for 3 months. Endoscopic studies failed to reveal specific changes in the whole gastrointestinal tract mucosa. Imaging studies revealed an anterior mediastinal mass (Fig. 1A) and multiple lung nodules. Additionally, a whole-body positron emission tomography scan showed abnormal hyperuptake lesions in the left 10th rib and 10th vertebral body, suggesting metastasis (Fig. 1B). Laboratory tests indicated elevated levels of several serum tumor markers (carbohydrate antigen [CA] 19-9, 252.2 U/mL; CA125, 62.9 IU/mL; and carcinoembryonic antigen, 8.73 ng/mL).

The surgeon prioritized surgery for this patient not only because of her aggravated symptoms, but also because the tumor had invaded the left brachiocephalic vein. Approached through a median sternotomy, an extended thymectomy and combined resection of the pericardium were performed. Incomplete resection was inevitable because of tumor invasion into the pericar-
Gross examination identified a solid mass measuring 6.8 × 4.7 × 3.9 cm. The tumor was unencapsulated and invaded the pericardium. The mass was firm and rubbery with a homogeneous gray-whitish cut surface. There was no internal fibrous septation (Fig. 2A). Microscopic examination revealed that the tumor was surrounded by normal thymic tissue and was composed of various-sized glandular structures (Fig. 2B). The tumor cells were columnar and oval, forming cystic, tubular, and cribriform structures containing necrotic material. Immunohistochemical staining showed focally positive CK7 staining and diffusely strong positive staining for CK20 and CDX2 (Fig. 2C–G) but negative staining for thyroid transcription factor 1 (TTF-1) and Napsin A. CD5 staining was positive for both tumor cells and T lymphocytes in the normal thymus parenchyma and stroma between tumor cell nests (Fig. 2H).

Clinically, the possibility of metastatic adenocarcinoma from the lung was raised; however, the multiple small lung nodules measured less than 1 cm, and the tumor cells were negative for TTF-1 and Napsin A. Furthermore, the pathologic features suggested metastatic adenocarcinoma of enteric origin; however, physical and radiologic examinations did not reveal primary tumors elsewhere, including in the gastrointestinal tract. These observations collectively led to a pathologic diagnosis of primary thymic adenocarcinoma of enteric immunophenotype with tubular morphology. Adjunctively, extensive sampling revealed no mucin pools or papillary structures and no other histologic components such as thymic cysts, thymomas, or teratomas.

After the operation, palliative chemoradiotherapy and radiotherapy were administered. The patient is alive with aggravated bone and lung metastasis after 11 months of follow-up.

**DISCUSSION**

Primary thymic adenocarcinoma is very rare. It was first reported in 1989 by Moriyama et al. but was not accepted as a valid histologic subtype until 1997. The current WHO classification system subtypes thymic adenocarcinomas as either papillary or nonpapillary. Nonpapillary adenocarcinomas include a heterogeneous group of tumors, such as mucinous adenocarcinoma, adenocarcinoma with glandular differentiation, adenocarcinoma with adenoid cystic carcinoma features, and hepatoid carcinoma. This heterogeneity, coupled with the rarity of the carcinoma, has led to a paucity of information on the specific clinicopathologic features of thymic adenocarcinoma.

According to a series of case reports in which descriptive classifications are commonly used, thymic adenocarcinomas comprise papillary, mucinous, glandular (or tubular), and papillotubular adenocarcinomas. In 2003, Maghbool et al. reviewed 26 previous cases of reported thymic adenocarcinomas and demonstrated that the mucinous type shows a significantly worse prognosis than the papillary type. Moser et al. recently reported two cases of primary thymic adenocarcinoma with CK20 and CDX2 expression, which reflects enteric differentiation, and suggested this is a novel subtype along with 11 reported thymic adenocarcinomas with enteric immunophenotype.

For this study, we also reviewed English literature of thymic adenocarcinomas, except for thymic carcinomas with adenoid cystic carcinoma features. In analysis of clinicopathologic features, including the current case, there are currently 39 reports of primary thymic adenocarcinomas, consisting of 16 mucinous adenocarcinomas (41.0%), 13 papillary adenocarcinomas (33.3%), seven tubular adenocarcinomas (17.9%), and three papillotubular...
lar adenocarcinomas (7.7%). Among these cases, information on CK20 and/or CDX2 expression was available for 20 cases, revealing that 15 were enteric type and five were not.

The morphology of these enteric-type adenocarcinomas was mucinous in 11 cases, tubular in three cases, and papillotubular in one case. Furthermore, the enteric-type adenocarcinomas and mucinous/tubular adenocarcinomas have similar clinicopathologic features. A total of seven of the 14 cases of mucinous/tubular adenocarcinomas were accompanied by thymic cysts. Moreover, mucinous/tubular adenocarcinomas showed CD5 expression.

Fig. 2. Pathologic examination. (A) Gross examination of the tumor reveals an ill-defined mass with pericardial invasion (arrows, pericardium). (B) Microscopically, the tumor is surrounded by normal thymic tissues. At higher magnification, the tumor is composed of glandular or tubular structures with large glands lined by tall columnar cells (C) and oval cells forming small glands (D). The tumor cells show positive staining for CK7 (E), CK20 (F), CDX2 (G), and CD5 (H). CK, cytokeratin; CDX2, caudal type homeobox 2.
more frequently (76.4%) than papillary adenocarcinomas (33.3%). Similarly, 12 of 15 enteric-type adenocarcinomas (80%) showed CD5 expression, and eight (53.3%) were accompanied by thymic cysts.

In general, low-grade histology (well-differentiated squamous cell carcinoma, mucopidermoid carcinoma, and basaloid carcinoma), low Masaoka stage, and complete resection are factors known to be associated with better survival from thymic carcinoma.\textsuperscript{16,17} Kaplan-Meier analysis of thymic adenocarcinomas revealed that modified Masaoka stage I or II disease had better disease-free survival than stage III or IV disease (p = .040). Additionally, we found that mucinous/tubular subtype showed a trend toward poorer overall survival than papillary or papillotubular subtype, in line with previous findings; however, this result failed to reach statistical significance in Kaplan-Meier analysis (p = .610). Interestingly, the nonenteric type seemed to have worse prognosis than the enteric type (mean survival time, nonenteric vs enteric type, 7.3 ± 4.2 months vs 91.9 ± 24.4 months); however, the number of cases was too small to analyze for statistical significance. Other factors of sex, tumor size, associated thymic cyst or thymomomas, serum tumor marker elevation, or c-kit or CD5 immunexpression were not related to prognosis.

In summary, we report a rare case of primary tubular adenocarcinoma of the thymus with enteric immunophenotype. To the best of our knowledge, there have been only two such cases worldwide. The tumor presented at an advanced stage and demonstrated aggressive behavior. We believe that identification of more cases is essential in order to investigate the clinicopathologic characteristics of primary thymic adenocarcinomas.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

REFERENCES


Sclerosing extramedullary hematopoietic tumor (SEMHT) is a rare tumor that occurs in patients with chronic myeloproliferative disorders (CMPDs). The tumor is classified in the chronic idiopathic myelofibrosis (MF) subgroup, and cases have been reported at various localizations since 1980.1-4 Such tumors are usually seen in the abdominal organs, retroperitoneum, and mesenteric region.3 The clinical, radiological, and morphological features may complicate differentiation from sarcoma, carcinoma, and lymphoma. It is sometimes also difficult to differentiate between a SEMHT and extramedullary hematopoiesis (EMH). These two lesions have similar clinical features, but EMH is morphologically more cellular. To aid in the differentiation, SEMHT has a more solid mass appearance with dense fibrosis and atypical megakaryocytes.3

EMH is the presence of hematopoietic tissue in locations other than the bone marrow. The basic mechanism is bone marrow dysfunction and decreased production of hematopoietic cells, followed by production of bone marrow cells in other organs and tissues. EMH is seen in many disorders such as sickle cell anemia, hemoglobinopathies, thalassemia, hereditary spherocytosis, and MF.3 EMH is most commonly observed in the liver and spleen and is rarely found in the peritoneum, lymph nodes, kidneys, thymus, central nervous system, retroperitoneum, myocardium, uterus, pleura, paraspinal region, or intestines.6-9

CASE REPORT

A 57-year-old female patient was examined for the chief complaint of fatigue. She was diagnosed with severe anemia, and a palpable intra-abdominal mass was identified on physical examination. Radiological examinations revealed hepatosplenomegaly and hypodense soft tissue lesions measuring 15×6 cm in size along the medial liver contour at the liver portal hilus level and 9.8×6 cm in size along the right iliac vascular structures in the right lower quadrant (Fig. 1), as well as several enlarged lymph nodes in the paraaortic and paracaval regions (the largest measured 5 cm and was located in the right paracaval region). Intra-abdominal free fluid deposition was noted. Radiologically, the soft tissue masses observed along the medial liver contour (15×6 cm) invaded the portal vein, and the characteristics of those along the right iliac vascular structures in the right lower quadrant (~9.8×6 cm) were reported to be consistent with infiltrative sarcomatous lesion or lymphoma (diffuse infiltrative type), and pathologic evaluation was recommended. Whole blood analysis revealed a mildly increased neutrophil count (14,500/μL). Other blood analysis results were as follows: platelets 299×10³/μL, erythrocytes 3.91×10⁶/μL, hemoglobin 10.6 g/dL (reference range, 10.80 to 14.90 g/dL), and hematocrit 34.4% (reference range, 35.60% to 45.40%).

The patient was referred for general surgery, and the right lower quadrant mass was removed with a preliminary diagnosis of intra-abdominal malignant tumor. Macroscopically, the soft tissue mass was solid, off-white in color, and 9.5×7×3 cm in size.
Microscopically, there were thick fibrotic bands, areas with more prominent collagen bands, mixed inflammatory cell infiltration including eosinophils, and occasional foci with individual or grouped enlarged cells with large cytoplasm and pleomorphic nucleoli in the background. Lymphoid follicles with prominent germinal centers were observed mostly in the periphery of the mass, and some contained the large cells described above (Fig. 2A, B). No mitosis or necrosis was found. Increased collagen was seen in the background on Masson’s trichrome stain (Fig. 2C). Immunohistochemistry revealed suspicious large cells that were negative for CD34, DKA, S-100, CD31, desmin, vimentin, CD117, CD10, CD68, CD30, mast cell tryptase, CD1a, CD45, CD30, CD15, CD3, CD20, CD21, CD23, anaplastic lymphoma kinase (ALK), pancytokeratin, and epithelial membrane antigen. The Ki-67 proliferation index was very low at about 2%. The background lymphoid follicle structures became more evident with CD3 and CD20 staining. Additional stains were then applied, and the large cells were positive for CD61 (Fig. 3A), indicating dysplastic megakaryocytes. Some myeloperoxidase-positive cells with mononuclear or polynuclear morphology were seen, indicating granulocytic series. Glycophorin staining revealed some precursor cells belonging to the erythroid series (Fig. 3B). We concluded that all these characteristics were re-

Fig. 1. A soft tissue lesion approximately 9.8 × 6 cm in size with a lobulated contour along the right iliac vascular structures of the right lower quadrant.

Fig. 2. (A) Thick fibrotic bands and increased collagen tissue with occasional mixed inflammatory cell infiltration including eosinophils and foci of individual or grouped cells showing pleomorphic nuclei and large cytoplasm. (B) Features of the large cells are seen more clearly at larger magnification. (C) Increased collagen is seen in the background (Masson’s trichrome).

Fig. 3. (A) Large cells are CD61-positive on immunohistochemical stain, indicating that they are megakaryocytes. (B) Glycophorin positivity in precursor cells (green arrows) belonging to the erythroid lineage and surrounding erythrocytes (red arrow).
 Detailed investigation of the patient history revealed that she had been diagnosed with MF after a bone marrow biopsy 14 years prior. A liver biopsy had been performed at that time, and EMH was reported. The previous bone marrow biopsy evaluation found hypercellular bone marrow (90%) with a prominent increase in occasional clustering of megakaryocytes. The bone marrow showed thick reticulin fibers with reticulin staining and collagen formation according to Masson's trichrome staining. The diagnosis of MF was made, and the patient had been followed for 14 years without treatment.

We diagnosed the retroperitoneal soft tissue mass that was highly suspicious for malignancy as SEMHT given the morphological and immunophenotypical results and the medical history. The patient was started on Hydrea (DEVIA, Istanbul, Turkey) (500 g tablet, twice daily) and Urikoliz (SANDOZ, Istanbul, Turkey) (300 mg, half tablet, three times daily) to suppress the features related to SEMHT. Hydrea treatment was terminated six months later due to development of leukopenia and anemia. No other problems have developed during follow-up thus far.

**DISCUSSION**

SEMHT is a rare tumor more commonly seen with chronic idiopathic MF rather than CMPDs. It is usually located in the abdomen, retroperitoneum, and mesenteric region. Our patient had multiple masses located in the abdomen. EMH generally indicates the presence of bone marrow elements in an area other than the bone marrow. It can develop as a result of bone marrow failure due to various causes in a wide range of disorders including MF. EMH is most commonly observed in the spleen and liver and is rarely seen in the peritoneum, lymph nodes, kidneys, thymus, central nervous system, retroperitoneum, myocardium, uterus, pleura, or paraspinous region. Differentiation of SEMHT from EMH is made based on several characteristics such as cellularity, tendency to form a mass, and presence of fibrosis and atypical megakaryocytes. Marked cellularity indicates EMH, while the presence of fibrosis and atypical megakaryocytes and a tendency to form a mass favors a diagnosis of SEMHT. This case had background fibrosis, atypical megakaryocytes, and mass formation and was therefore diagnosed as SEMHT.

The differential diagnosis of large intra-abdominal or retroperitoneal soft tissue masses varies from case to case, and a clinicopathological correlation is important. A detailed immunohistochemical (IHC) panel is used together with morphological indicators of the degree of differentiation, number of mitoses, and the presence/absence of necrosis in the pathologic evaluation of soft tissue tumors. In this case, there was no necrosis in the background and no mitosis in the large cells, which did not invade the surrounding tissues, vessels, or nerves. We performed IHC stains for all entities that should be considered in the differential diagnosis of a retroperitoneal mass such as liposarcoma, malignant peripheral nerve sheath tumor, leiomyosarcoma, gastrointestinal stromal tumor, solitary fibrous tumor, inflammatory myofibroblastic tumor, and fibrosarcoma. All were negative (large cells were negative for CD34, DKA, S-100, desmin, vimentin, CD117, CD10, ALK), and the diagnosis of SEMHT was made with the help of a detailed medical history and the positivity for CD61 and glycophorin. It is difficult to differentiate some sarcomas (especially sclerosing liposarcoma) from SEMHT without using IHC markers.

Differentiating benign proliferations of bone marrow cells from those that are malignant is important. Myeloid sarcoma, which should also be considered in the differential diagnosis, is a tumor that consists of myeloid blastic cells and can develop in various anatomical sites such as the skin, lymph nodes, small intestine, and mediastinum. IHC analysis is usually used for the differential diagnosis with hematological malignancies (large cells are negative for CD34 and CD117). We also eliminated other lymphomas including Hodgkin lymphoma (lymphocyte-poor type, appearance similar to Reed-Steinberg cells in some of the large cells) and histiocytic lymphoma through IHC investigation (large cells were negative for CD68, CD30, mast cell tryptase, CD1a, CD45, CD30, CD15, CD3, CD20, CD21, CD23, ALK, and S100).

We were able to make a correct diagnosis with detailed clinicopathological correlation although the initial histopathological and radiological findings supported a possible sarcomatous neoplasm. We emphasize the importance of a thorough history and highlight the fact that radiologic and pathologic data may be difficult to interpret when the medical history is inadequate. We presented this case to remind physicians to consider this pathology in the differential diagnosis of soft tissue masses, especially when hematological disorder is also present.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**REFERENCES**

1. Remstein ED, Kurtin PJ, Nascimento AG. Sclerosing extramedul-


Ureteral Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue, Chronic Inflammation, and Renal Artery Atherosclerosis

Hojung Lee · Jong Eun Joo · Young Ok Hong · Won Mi Lee Eun Kyung Kim · Jeong Joo Woo · Soo Jung Gong · Jooryung Huh
Departments of Pathology, Radiology, and Internal Medicine, Eulji General Hospital, Eulji University College of Medicine, Seoul; Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Malignant lymphoma of the upper urinary tract including the renal pelvis and ureter is extremely rare. Less than ten cases have been reported in the literature, of which extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is the most frequent histologic type.\(^1\) MALT lymphoma arising in the stomach, skin, thyroid, and salivary gland is associated with chronic inflammation caused by pathogenic microorganisms or autoimmune disorder;\(^2\) however, the pathogenesis of MALT lymphoma in the upper urinary tract is unknown. Herein, we report a case of upper ureteral MALT lymphoma with extensive peri-ureteropelvic inflammation and atherosclerosis of the renal artery, and discuss the possible correlation between these lesions.

CASE REPORT

A 73-year-old male patient was admitted to the emergency room with a chief complaint of right flank pain for one week. Laboratory tests showed an elevated white blood cell count of \(13.21 \times 10^3/\mu\text{L}\) (neutrophilic leukocytes, 85%; lymphocytes, 5%) and high C-reactive protein (CRP) level of 23.71 mg/dL. Urine analysis was unremarkable, and no microbial growth was observed in blood or urine cultures. Abdominal computed tomography (CT) showed diffuse thickening of the right ureteropelvic wall with hydroureteronephrosis (Fig. 1A). Urine cytology and washing cytology of the right ureter revealed no atypical cells. Right nephroureterectomy was performed for histologic diagnosis, and the intraoperative diagnosis of lymphoid malignancy was made. Positron emission tomography/CT revealed multiple hypermetabolic lesions throughout the neck, chest, and abdomen, suggesting lymphoma seeding (Fig. 1B). The resected specimen showed an elongated, concentric, whitish yellow solid lesion along the walls of the renal pelvis and proximal ureter (Fig. 2). This lesion also surrounded the atherosclerotic renal artery (Fig. 2, upper inset).

Histologic examination revealed peri-ureteropelvic nodular lymphoid infiltrates encroaching upon the ureter wall (Fig. 3A). The perimuscular layer of the ureter was heavily infiltrated with tumor cells. There were centrocyte-like cells with slightly irregular nuclear contours and clear cytoplasm, occasional larger activated cells, monocytoid cells, and plasma cells forming numerous lymphoid follicles. Immunohistochemically, the neoplastic cells were positive for CD20 (Fig. 3B) but negative for CD3, CD5, CD10, Bcl-2, Bcl-6, and cyclin D1. Tumor-infiltrating T cells were mostly CD4+ cells (Fig. 3C). Monotypic expression of \(\lambda\) light chain was demonstrated in the plasma cells. This case was diagnosed as a MALT lymphoma. Adjacent peripelvic adipose tissue showed a dense inflammatory infiltrate composed of lymphoplasmacytic cells, eosinophils, and histiocytes with fibroblastic proliferation (Fig. 3D). Infiltrating lymphocytes were mainly CD4+ T cells, and CD20+ B cells were rare. Neither IgG4-positive plasma cells nor anaplastic lymphoma kinase–stained cells were noted, excluding the possibility of IgG4-associated disease or an inflammatory myofibroblastic tumor.
The patient received rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapya. Complete remission was achieved with no recurrence at the 14-month follow-up.

**DISCUSSION**

Lymphomas constitute approximately 5% of non-urothelial tumors of the urinary tract, affecting urinary bladder in more than 90% of cases. In contrast, lymphoma of the upper urinary tract is extremely rare. Of the reported cases, MALT lymphoma is the most frequent, with eight case reports; seven in the renal pelvis and one in the upper ureter. While MALT lymphoma of the urinary bladder is frequently associated with chronic cystitis and female predominance, MALT lymphoma of the upper urinary tract typically affects middle-aged or elderly males with unknown preceding conditions. MALT lymphoma of the urinary tract presents with incidental mass or thickening on radiographs, although some may complain of pain. It usually presents at a localized stage and has excellent prognosis, although dissemination can occur, as in the present case. Despite its widespread extent, the primary origin in the urinary system of this case was supported by the growth pattern within the renal pelvis and ureter walls. However, the possibility of secondary ureteral involvement of MALT lymphoma in other organs still remains since histologic confirmation at other sites was not performed. Unlike its gastric or even bladder counterpart, ureteropelvic MALT lymphoma rarely shows subepithelial lymphoid infiltrates or lymphoepithelial lesions, mainly involving periureteral fat. Thus, ureteral MALT lymphoma is probably not associated with ‘mucosa’ or luminal insults but rather with periureteral inflammation. Therefore, in the case of lymphoma accompanied by inflammatory lesion, it may be better to be called ‘inflammation-associated lymphoid tissue lymphoma’ rather than MALT lymphoma, if MALT is not present, as in this case.

In MALT lymphoma, a chronic inflammatory microenvironment is important for the development of the tumor. The intratumoral T-cell component is a crucial factor, and CD4+ T cells activate B cells in a CD40-dependent manner in combination with Th2 cytokines (interleukin (IL) 4 and/or IL-10). Thus, the chronic inflammatory infiltrates seen in the peripelvic area in the present case may have provided the appropriate conditions for growth of a malignant clone.

On the other hand, atherosclerosis is defined as a chronic inflammatory response of the arterial wall to endothelial injury triggered by a variety of insults including lipid accumulation. Many inflammatory cells, mostly monocytes and CD4+ T cells, are recruited into the arterial intima, forming atheromatous plaques and accompanied by high blood levels of acute phase reactants such as CRP, as in the present case. Renal artery atherosclerosis in our patient may be a coincidental lesion considering his old age. However, the patient’s laboratory and pathologic findings indicated that the inflammatory process was concurrently ongoing within the renal artery and periureteral adipose tissue. Therefore, in the present case, the final outcome may
manifest as ureteral MALT lymphoma and renal artery atherosclerosis.

Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

REFERENCES

7. Hara M, Satake M, Ogino H, et al. Primary ureteral mucosa-associated lymphoid tissue (MALT) lymphoma: pathological and radio-
Late Bone Metastasis of Histologically Bland Struma Ovarii: The Unpredictability of Its Biologic Behavior

Sun-Ju Oh · Minjung Jung · Young-Ok Kim
Department of Pathology, Kosin University Gospel Hospital, Busan, Korea

Struma ovarii (SO) is a subtype of ovarian teratoma that contains mostly thyroid tissue comprising more than 50% of the tumor volume.1 It is the most common type of monodermal teratoma of the ovary, accounting for approximately 2.7% of all teratomas.1 Most cases of typical SO are benign with exceptional cases showing malignant features of papillary carcinoma, vascular invasion, or invasion into surrounding tissue. However, there are rare cases of histologically benign SO with malignant biological behavior, variously designated as peritoneal strumosis, malignant SO, metastatic SO, or highly differentiated follicular carcinomas.2,3 Here, we report on a patient who developed a vertebral metastasis ten years after oophorectomy for SO that finally proved to be malignant.

CASE REPORT

A 60-year-old woman presented with back pain. Magnetic resonance imaging revealed a destructive bone mass at the thoracic 12 level of the spine (Fig. 1A). Overall image analysis suggested a metastasis, but the origin could not be detected on a positron emission tomography–computed tomography (PET-CT) scan. Needle biopsy was performed on the spinal lesion, and microscopically it revealed thyroid follicles with colloids resembling normal thyroid tissue (Fig. 1B, C). Immunohistochemically, these cells were diffusely positive for thyroid transcription factor-1 and thyroglobulin (Fig. 1D), suggesting thyroid as origin of the tumor. Any evidence of nuclear atypia suspicious for papillary carcinoma was not detected throughout the lesion, which was consequently diagnosed as metastatic follicular carcinoma of thyroid.

Subsequent clinical evaluation revealed a high serum thyroglobulin level of 1,277 ng/mL (normal range, 1.4 to 78.0 ng/mL). However, ultrasonogram and computed tomography showed no distinct mass in the thyroid gland. The patient’s past medical history revealed that she underwent total hysterectomy and bilateral salpingo-oophorectomy for a left ovarian cyst 12 years ago that was initially diagnosed as SO. Follow-up of the patient included periodic ultrasonograms and measurement of serum cancer antigen 125 levels, all of which had been normal since the surgery. A suspicion for metastasis originating from the ovarian tumor was raised, and the initial slides were reevaluated.

Upon review of the pathology report and slides, the ovarian cyst was a 12-cm-sized multilocular mass with a smooth outer surface. Microscopic examination showed benign looking thyroid tissue characterized by round follicles, most of which were dilated, but some follicles were small and others were medium sized. Many follicles contained intraluminal colloids, and they were lined by cuboidal epithelial cells with moderate amounts of cytoplasm (Fig. 2A). However, a complete examination revealed a focus of 0.5-cm-sized closely packed microfollicles with nuclear irregularity, overlapping, and vague clearing (Fig. 2B, C). These features resembled a follicular variant of papillary carcinoma (FVPC), but were insufficient to establish a definite diagnosis of FVPC. Other characteristics suspicious for malignancy such as vascular or ovarian capsular invasion were not noted. No other teratomatous components were identified. A possibility of unusual metastasis from SO was suggested for the spinal lesion.

Subsequent total thyroidectomy and spondylectomy for the spinal lesion were conducted. The resected thyroid gland showed no evidence of neoplasm, as expected, and the spinal lesion ex-
Oh S-J, et al.

hibited the same histological features as the needle biopsy. The patient subsequently received radioactive iodine (RAI) treatment, but her PET-CT scan on the last follow-up showed multiple bone metastases in the scapula, rib, sternum, and pelvic bones.

**DISCUSSION**

The most common thyroid-type carcinomas originating in SO are papillary and follicular carcinomas. The diagnostic criteria for cases of papillary carcinoma are similar to those described for the cervical thyroid gland and are based primarily on nuclear and architectural features. These features are almost invariable and of no clinical importance. With respect to follicular carcinoma, invasion into the surrounding ovarian tissue, vascular invasion, or metastasis is evidence of malignancy.

However, SO can show a wide spectrum of histological features that should not prompt a diagnosis of malignancy. These include the presence of areas resembling follicular adenoma, foci with papillary architecture, and nuclear clearing. SO with these features have a benign clinical course and these tumors are designated “proliferative SO.” These types of tumors generally do not behave in a malignant fashion. However, this claim was denied later when adenomatous proliferative SO also can show the propensity for recurrence or metastasis.

It is difficult to predict the metastatic potential of SO at initial diagnosis. Even SO that histologically resembles non-neo-

---

**Fig. 1.** (A) Sagittal magnetic resonance imaging showing an ill-defined lytic mass involving the thoracic 12 level. The mass destructs the vertebral body and spinal canal extending to the pedicle. (B, C) Needle biopsy of the spinal lesion reveals benign-looking thyroid follicles. (D) These cells are positive for thyroglobulin on immunohistochemical stain, supporting thyroid origin.

**Fig. 2.** Histological features of struma ovarii. Most of the tumor consist of dilated follicles reminiscent of nodular goiter (A) while a small part of the tumor shows densely packed microfollicles (B) with mild nuclear irregularity, overlapping, and vague clearing (C).
plastic thyroid tissue is sometimes associated with recurrence or extra-ovarian metastasis, which have previously been designated as peritoneal strumosis, malignant SO, metastatic SO, or related terms.\(^5\) Roth and Karseladze\(^\text{2}\) described three cases of SO with extra-ovarian dissemination having an innocuous appearance resembling that of a colloid or nodular goiter. They designated this entity as “highly differentiated follicular carcinoma of ovarian origin (HDFCO).” The diagnosis of HDFCO characteristically cannot be made until extra-ovarian dissemination is detected because of its non-neoplastic appearance. They also compared cases of HDFCO with those of typical thyroid-type carcinomas of ovarian origin with peritoneal involvement. They found that biological behavior is not different in the point that the prognosis seems to be favorable.

In a study of 27 cases of biologically malignant SO with extra-ovarian dissemination or metastasis, Shaco-Levy \(\text{et al.}\)\(^6\) found that histologically malignant, adenomatous, or even normal primary tumors can show biologically malignant behavior. The authors found no independent factor to predict its biological behavior. However, severe fibrous adhesions, larger amounts of peritoneal fluid (≥ 1 L), and larger size of the stromal component (≥ 12 cm) have some predictive value. In the following year, the authors analyzed in more detail the natural history of biologically aggressive tumors in a study of patients with more rapid disease progression.\(^7\) Factors predictive of a potentially more aggressive clinical course are large tumor size (> 10 cm), more than 80% stromal tissue, extensive papillary carcinoma histology, more than five mitoses per 10 high-power field, and marked cytological atypia. Our case showed no adverse histological or clinical factors other than the tumor size of 12 cm according to their criteria. However, others have contradicted that the size of the stromal component is not relevant to the metastatic or recurrence potential and no histologic features seem to correlate with a propensity for an adverse clinical outcome.\(^8\)

When no independent factor predicts biologically malignant potential of SO, controversy regarding the extent of pelvic resection and the management of the thyroid gland can arise with respect to the treatment plan. Marti \(\text{et al.}\)\(^9\) suggested that pelvic surgery alone may be sufficient initial therapy for thyroid-type carcinoma confined to the ovary, whereas prophylactic total thyroidectomy with RAI may be reserved for patients with extra-ovarian spread or distant metastasis. However, there are no present series and data from the literature allowing the determination of risk stratification for patients with SO confined to the ovary at presentation. Because recurrence may occur after more than a decade following diagnosis, as in the present case, long-term follow-up is indicated with regular thyroglobulin measurements to detect metastasis.

In summary, we report a patient with a vertebral metastasis 10 years after diagnosis of histologically benign SO. On the basis of our experience with this patient and others in the literature, no determinants can predict its biologically malignant potential. Pathologists are advised to report in detail on tumor size, cytologic atypia, mitosis, and other histological factors affecting the adverse outcome when encountering large SO consisting solely of thyroid tissue as in our case. Long-term follow-up with regular thyroglobulin measurements should be warranted for any evidence of metastasis or recurrence.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**REFERENCES**

Necrotizing sarcoid granulomatosis (NSG) is a rare granulomatous disease that primarily affects the lung and presents as nodular masses of confluent sarcoid-like granulomas with extensive necrosis and vasculitis. Proper diagnosis and treatment are challenging for clinicians, radiologists, and pathologists because of the rarity and diagnostic difficulty of this disease. Since it was first described by Liebow, only 135 cases have been reported. In addition to its rarity, the similarity of the clinical, radiological, and pathological features of NSG to other granulomatous diseases, such as granulomatous infection, nodular sarcoidosis, and Wegener’s granulomatosis (WG), is the largest obstacle in its proper diagnosis. The initial symptoms are non-specific or frequently do not present at all. Radiologically, NSG can present as cavitary lesions, ill-defined pneumonic consolidations, or even as a solitary nodule or a mass. Pathologically, NSG shares features of sarcoidosis and WG.

Thus, it is not surprising that NSG is often easily mistaken for granulomatous infections, such as tuberculosis, especially in endemic areas. Although the general level of hygiene in Korea has dramatically improved, the reported incidence of pulmonary tuberculosis remains the highest among the Organization for Economic Cooperation and Development member countries. Identification of causal microorganisms is essential for proper treatment of tuberculosis; however, empirical treatment with anti-tuberculosis medication is often performed in endemic countries like Korea, in spite of tests failing to show any causative microorganisms.

Here, we report the first two cases of NSG in Korean patients, one of whom showed a dramatic response to immediate application of systemic corticosteroids, and the other of whom showed no response to empirical treatment with anti-tuberculosis medication.

**CASE REPORT**

The publication of the case information and materials was approved by the institutional review board of The Catholic University of Korea, College of Medicine (SC11ZISE0221).

**Case 1**

A 70-year-old man with a history of smoking presented with a three-week history of cough, fever, and chill. He reported no change in body weight, no cyanosis, and no clubbing. Vital signs were stable, and inflammatory markers were slightly increased (erythrocyte sedimentation rate, 90 mm/hr; C-reactive protein [CRP], 3.52 mg/L).

Radiological examination revealed multifocal ill-defined, nodular consolidations in both lungs, with moderate enlargement of paratracheal and hilar lymph nodes, suggesting granulomatous infection (Fig. 1A, B).

On microscopic examination of the wedge-resected tissue, confluent small granulomas with necrosis of variable sizes were seen in the lung parenchyma. The granulomas were accompanied by central necrosis that was suppurative rather than caseous, palisaded by Langerhans-type giant cells and mononuclear
lymphohistiocytes. At first glance, granulomatous vasculitis did not seem evident, but on closer observation, mild transmural vasculitis with fibrosis distant from the necrotic area was clearly noted in a significant portion of the lesion (Fig. 2A–D). The differential diagnosis based on pathologic findings included granulomatous diseases of various causes. Special stains for fungus and acid-fast bacilli on the resected tissue and sputum were negative. Tuberculosis–polymerase chain reaction and culture on bronchial washing fluid and peripheral blood were both negative. Serum cryptococcal antigen and IgG for parasites were all negative.

An extensive battery of autoimmune markers was all negative (antineutrophil cytoplasmic antibody, antinuclear, anti-ds-DNA, anti-sm, anti-SS-A/Ro, anti-Scl72, anti-Jo1, etc.). Serum angiotensin-converting enzyme was unremarkable. With these results, a diagnosis of NSG was highly suspected and treated with oral prednisolone.

After one month of therapy, the patient showed a dramatic improvement of symptoms and chest radiography (Fig. 1C). He has had no symptoms through two years of follow-up.

Case 2

A 41-year-old non-smoking woman presented with a two-week history of cough and sputum. Laboratory findings showed only a mild increase in acute inflammatory marker (CRP, 37.4 mg/L).

Radiologically, multiple nodules with ill-defined margins scattered mainly in the left lower lobe were found with minimal pleural effusion, suggesting hematogenous spread of metastatic cancer, pulmonary lymphoma, or septic pneumonia (Fig. 1D). Hilar lymph nodes were unremarkable.

Histological examination of resected tissue revealed variably-
sized granulomas with varying amounts of suppurative necrosis and frequent granulomatous vasculitis, raising suspicion for sarcoidosis or other kinds of granulomatous vasculitis (Fig. 2E, F). However, autoimmune markers were all negative, and no causative microorganisms were found on various tests.

An anti-tuberculosis treatment regimen was started based on the clinicoradiographic findings in order to exclude the possibility of potential pulmonary tuberculosis. During the first three weeks of anti-tuberculosis treatment, the patient reported no improvement in symptoms. She was treated for another month and slowly improved, irrespective of treatment, and even after its cessation. Bacterial cultures of sputum, blood, and bronchial washing remained negative.

DISCUSSION

In 1973, Liebow described three chief features of NSG that differentiate it from sarcoidosis, WG, and other granulomatous infections. First, histologically, NSG appears as sarcoid-like granuloma with vasculitis and necrosis. Second, radiological
findings include multiple lung nodules without hilar lymphadenopathy. Third, it has a very benign clinical course. However, subsequent reports have since shown that NSG can present with more variable radiologic findings and is commonly accompanied by lymphadenopathy and pleural effusion.\(^4\)

Because the symptoms of NSG are often non-specific and the radiologic findings vary widely, the pathological findings are important for accurate diagnosis. If one finds a mixture of pathologic features of pulmonary sarcoidosis and mild to moderate granulomatous vasculitis, combined with negative tests for causative microorganisms, the possibility of NSG can be raised. However, conclusive diagnosis cannot be made on histologic features alone, and the final pathologic diagnosis of NSG should be made after thorough exclusion of the other possible diseases with similar features. Nodular sarcoidosis is excluded by the presence of necrotizing vasculitis and diffuse parenchymal necrosis.\(^3\) WG is excluded by the presence of sarcoid-like granulomas and granulomatous vasculitis distant from necrotic areas.\(^4\) Most importantly, granulomatous infections should be carefully excluded because they can also produce variable vasculitis, extensive necrosis, and sarcoid-like granulomas.\(^3,4\) Such distinction can be very challenging because most microbiological studies, including those that use the latest technologies such as polymerase chain reaction, can produce false-negative results.

In describing the characteristic pathologic findings of NSG, Katzenstein\(^2\) has mentioned that transmural vasculitis with fibrosis distant from necrotic area, as seen in the first case of this report, is an important defining finding of NSG. It is important to remember that these findings can be easily overlooked and considered as usual nodular sarcoidosis if pathologists do not pay enough attention to the lung biopsy samples of similar conditions. For this reason, Rosen\(^2\) suggests in his recent review that NSG is a specific form of sarcoidosis and should be referred to with the diagnostic term sarcoidosis with necrotizing sarcoideal granulomatosis pattern. However, since this specific form of sarcoidosis mimics granulomatous infections such as tuberculosis and results in inadequate anti-tuberculosis treatment, the distinction from usual pulmonary sarcoidosis should continue to be emphasized.

According to the guidelines on treatment of tuberculosis by the Centers for Disease Control and Prevention, a four-drug anti-tuberculosis regimen is recommended for the first two months of treatment for patients who are highly suspected as having an active tuberculosis infection based on clinicalradiographical findings, even without isolation of mycobacterium.\(^10\) The Korean Guidelines for Tuberculosis also recommend empirical chemo-prevention in sputum-negative patients while waiting for the results of culture studies.\(^9\) Thus, it is inevitable that patients with NSG in Korea will undergo the risks and inconvenience of anti-tuberculosis treatment considering the socioeconomic burden of tuberculosis. However, it should be noted that there may be more unrecognized patients with NSG in Korea, and that they might be overlooked in the endemic setting of tuberculosis, considering that NSG has been reported quite commonly in Japan, the most similar Asian country to Korea.\(^11\)

Collectively, for the accurate recognition of NSG, the following diagnostic steps should be performed. If pathologically suspicious features for NSG are found in the absence of identification of any causative microorganisms and the radiologic findings demonstrate multiple nodules with hilar lymphadenopathy, the possibility of NSG should be considered. If NSG is clinically suspected, the initial two to three weeks of anti-tuberculosis treatment can be used to monitor the disease response. If there is no change in symptoms or radiological findings, then systemic steroid treatment can be applied.

To our knowledge, this is the first case report of NSG in Korean patients, one of whom showed a dramatic response to corticosteroid therapy. A case of NSG was reported in 1997 by Kim et al.,\(^1,2\) but the evidence for NSG in their report is insufficient because polymerase chain reaction was not used to exclude tuberculosis, the patient was not appropriately treated, and there was no radiographic improvement.

In conclusion, the diagnosis of NSG should be made very carefully in suspected cases based on consistent radiologic and histologic findings and thorough exclusion of possible causative microorganisms.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**REFERENCES**

Salivary Gland Hyalinizing Clear Cell Carcinoma

Jung-Chia Lin1 · Jia-Bin Liao1,2 · Hsiao-Ting Fu1 · Ting-Shou Chang2,3,4 · Jyh-Seng Wang1,2,5

1Department of Pathology and Lab Medicine, Kaohsiung Veterans General Hospital, Kaohsiung; 2National Defense Medical Center, Taipei; 3Department of Otolaryngology, Kaohsiung Veterans General Hospital, Kaohsiung; 4Institute of Public Health, College of Medicine, National Cheng Kung University, Tainan; 5Department of Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan.

Hyalinizing clear cell carcinoma (HCCC) is a rare malignant salivary gland tumor that was characterized as a distinct entity by Milchgrub et al. in 1994.1 It has a slight female predominance with ages ranging from 25 to 87 years (mean, 59.4 years).2 Most cases occur in the oral cavity, mainly the palate and tongue.2 Histologically, HCCC is characterized by the predominance of clear cells embedded in a characteristic dual hyaline and fibrocellular stroma.3 In 2011, Antonescu et al.2 identified EWSR1 rearrangements in this tumor, which have been subsequently observed in 82% of cases. This genetic change allows the distinction of HCCC from other salivary gland neoplasms with a clear cell phenotype. Here, we present a typical case of HCCC, which we believe is the first case report from Taiwan.

CASE REPORT

A 37-year-old female patient visited our clinic complaining of painless swelling on the ventral tongue that had been present for months. Intraoral examination revealed a 1 × 1 cm nodule on the left ventral tongue and an excisional biopsy was done. Light microscopy showed an infiltrative tumor composed mostly of clear cells with spindle cell stroma. The tumor cells were immunoreactive for cytokeratin AE1/3 (CKAE1/3) and p63, and the spindle cell stroma for smooth muscle actin (SMA). The original pathology report was myoepithelial carcinoma (MC) due to misinterpretation of the p63 immunoreactivity as myoepithelial differentiation. The diagnosis was soon revised to HCCC after recognizing this new entity.

The tumor comprised of cords and nests of tumor cells with skeletal muscle infiltration and perineural invasion (Fig. 1A). The tumor cells had clear and pale eosinophilic cytoplasm (Fig. 1B), fine nuclear chromatin with occasional small nucleoli, and no mitotic figure. The tumor cells were embedded in a characteristic dual hyaline and fibrocellular stroma (Fig. 1C). No ductal formation was seen. On immunohistochemical stains, the tumor cells were positive for CKAE1/3 and p63 (Fig. 1D), but negative for myoepithelial cell markers, such as SMA, CD10, S100, myosin, calponin, glial fibrillary acidic protein (GFAP), muscle specific actin (MSA), and desmin. The spindle cells in the fibrocellular stroma were positive for SMA and CD10 (Fig. 1E) but negative for CKAE1/3, p63, myosin, calponin, GFAP, MSA, S100, and desmin. The clear cells contained abundant glycogen highlighted by a diastase sensitive periodic acid-Schiff positive reaction (Fig. 1F, G). The result of the mucicarmine stain for mucin was negative. Fluorescence in situ hybridization for EWSR1 showed a break-apart signal pattern in 25% of the tumor cells (Fig. 1H), confirming the presence of EWSR1 gene rearrangement. Since the margin was focally involved by the tumor, the patient received further excision with safe resection margin, which showed no residual tumor.

DISCUSSION

HCCC was first described by Milchgrub et al. in 19941 as a rare salivary gland carcinoma made up of clear cells forming cords and nests in a hyalinized stroma. This tumor was often
confused with other clear cell mimics, such as epithelial-myoepithelial carcinoma (EMC), mucin-depleted mucoepidermoid carcinoma (MEC) and MC. It was separated from these entities because of its lack of apparent squamous, mucinous, and myoepithelial differentiation. HCCC is currently classified by the World Health Organization (WHO) as a diagnosis of exclusion: “clear cell carcinoma, not otherwise specified.” Many small case series and case reports have been described, but none added new insights into HCCC until Antonescu et al. recently identified a consistent EWSR1-ATF1 fusion in HCCC.

In the differential diagnosis, EMC has a prominent ductal structure of tumor cells and does not show characteristic dual hyalinizing and fibrocellular stroma of HCCC. HCCC can be distinguished from MC by lack of myoepithelial cell markers such as S100, calponin, and SMA. Neither EMC nor MC has the EWSR1 gene rearrangement as seen in HCCC. Mucous cells may be absent in mucin-depleted MEC and is very difficult to differentiate from HCCC. However, mucin-depleted MEC lacks the characteristic dual stroma and EWSR1 rearrangement of HCCC. It should be noted that clear cell odontogenic carcinoma also has EWSR1 rearrangement as well as dual hyalinizing and fibrocellular stroma, and cannot be separated reliably from HCCC by the morphology or molecular marker, except by its location in the jaw bone.

Treatment usually involves primary resection with negative margins. Cases of HCCC with recurrence or metastasis have been reported and proven by molecular analysis; however, the vast majority of HCCC have had good outcomes.

In summary, we present a typical case of HCCC with characteristic dual stroma, lack of myoepithelial differentiation and

Fig. 1. (A) Hyalinizing clear cell carcinoma (HCCC) showing cords and nests of tumor cells with perineural invasion. The tumor cells have a clear cytoplasm (B) and are embedded in a characteristic dual hyaline and fibrocellular stroma (C). (D) Almost all clear cells are positive for p63. (E) The tumor cells are negative for CD10 while the myofibroblastic spindle cells in the stroma are positive for CD10. (F, G) Tumor cells with clear cytoplasm contain abundant glycogen which is positive for periodic acid–Schiff and sensitive to diastase digestion. (H) Fluorescence in situ hybridization shows a break-apart signal pattern in 25% of the tumor cells confirming the presence of EWSR1 rearrangement.
presence of EWSR1 rearrangement. This is the first case report from Taiwan. Molecular genetic methods have become an integral part of modern oral pathology and play an important role in helping to categorize cases that defy traditional morphologic analysis. In the molecular era, “NOS” is no longer fitting for HCCC. The term “NOS” is at odds with the presence of the specific EWSR1-ATF1 fusion that clearly sets HCCC apart from other salivary neoplasia. Adding “hyalinizing” is also recommended, even though this feature is not present in all cases. The benefit of its inclusion is the mental association with a salivary clear cell malignancy.¹

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

REFERENCES