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ANATOMIC PATHOLOGY

Challenges in Breast Pathology: New Twists on Old Problems

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Predicting the potential for recurrence and metastases of carcinomas at the time of their initial presentation and diagnosis remains a significant challenge in management of patients with breast cancer. This is particularly critical for early-stage carcinomas, for which adjuvant chemotherapy is unnecessary in nearly 75% of cases. Gene expression profiling assays of breast carcinoma in some specific groups of patients have been used to predict outcome and avert unnecessary adjuvant chemotherapy. These assays are being used to supplement traditional prognostic and predictive factors and may replace them in the future. It is crucial for pathologists to understand the nature of these assays and become familiar with the various assays available, the information provided by the assays, the differences between them, and their current limitations. Ein-Dor and Domany have noted that while various groups using different gene sets have shown good predictive performance, overlap between the gene lists is rather small. Furthermore, they suggest that thousands of tumors have to be studied in order to obtain a 50% overlap between 2 sets of predictive genes. We are clearly in a discovery phase and everyone involved with these assays, including the pathologists who select the block of tissue for assessment at the request of the oncologist, should ask critical questions in order to ascertain procurement of a robust gene list for future analysis. Recent microarray-based analyses of breast cancers have identified several clusters designated as luminal A (ER+, PR+, HER2/*neu*-), luminal B (ER+, PR-, HER2/*neu*+), HER2+ (ER-, PR-, HER2/*neu*+), basal-like (ER-, PR-, HER2/*neu*-; CK17+, CK14+, EGFR+, CK5/6+), and normal breastlike (ER-, PR-, HER2/*neu*-) subtypes, where ER indicates estrogen receptor, PR indicates progesterone receptor, EGFR indicates epithelial growth factor receptor, and CK indicates cytokeratin. The basal-like category has attracted particular attention because it was suggested that this group is associated with a more aggressive behavior. While hormonal therapy and Herceptin are clearly of no value in management of basal-like carcinomas, there are many cancers that would fit the immunophenotype of this group (ER;ms, PR;ms, HER2;ms, but EGFR+ and CK5, CK14, and/or CK17+) that are either amongst the least aggressive of breast cancers or show a range of aggressiveness; among these are adenoid cystic carcinomas, squamous carcinomas, malignant myoepithelial lesions, and a few others. Since a vast majority of carcinomas evaluated for their gene expression profile have been of epithelial origin and have been infiltrating duct carcinomas, very few tumors of myoepithelial derivation or differentiation have been evaluated to clearly define their spectrum. When several hundred basal-like tumors are evaluated, undoubtedly a spectrum with a range of clinical behavior and variable degrees of aggressiveness will emerge just as we now recognize a variety of epithelial-derived lesions. Will we continue to have basal-like carcinomas or rather a range of myoepithelial derived/basal cancers? While we should be applying novel technologies to the assessment and classification of tumors, it is important to exercise caution in making conclusions based on the relatively small number of cases studied. The definitiveness with which some of the microarray-based findings is promoted may be premature, though the findings may be confirmed in larger studies. The point

to keep in mind is that molecular-based classifications and gene expression-based predictive assays should not be treated as “The Emperor's New Clothes.”

In a more conventional arena, the introduction of novel approaches in sampling of tissues requires a constant and critical evaluation of our diagnostic criteria and adjustments to provide accurate diagnoses and recommendations for further therapy within the limitation of the samples available for analysis. Sentinel lymph node evaluation was introduced to prevent axillary node dissection and its consequences. While some form of assessment of one or more “sentinel” nodes has become a part of the routine practice for breast pathologists worldwide, not everyone would agree that the relatively common practice of assessing 3 to 4 nodes as “sentinel nodes” is a valid utilization of the concept of sentinel node examination, and there are substantial differences in how extensively these nodes are sampled. There is general agreement, however, that the significance of some findings in this setting is neither clear nor established and has not even been addressed by the TNM guidelines.

A lack of uniformity and consensus is anticipated and is not surprising in the application of novel approaches and technologies. It is surprising, however, that after more than a hundred years of examining the morphology of breast lesions through the microscope and despite lucid description of the earliest morphologically recognizable neoplastic alterations in the breast several decades ago the lesion now recognized as flat ductal intraepithelial neoplasia 1 or flat epithelial atypia was totally ignored until recently. This lesion became widely accepted as a significant lesion only after confirmation of its similarity to well-recognized invasive carcinomas at the molecular level in the year 2000. Although the presence of calcifications is a common feature of this lesion, which is frequently detected mammographically because of microcalcification, some mammographers had not even heard of it as recently as 6 years ago. What clinical action the diagnosis of this flat lesion in pure form should provoke remains to be determined. It is crucial that we accept new ideas and let go of those that have become obsolete.

Only a few decades ago, a diagnosis of papillary carcinoma was not always qualified as either intraepithelial (in situ) or invasive. Our understanding of papillary lesions has improved significantly. Nonetheless, papillary lesions have remained a source of diagnostic problems because of their wide morphologic spectrum; it is, therefore, important to share experiences and viewpoints on these lesions to enhance our diagnostic capability and provide useful recommendations for patient management.

In this issue of the *Archives of Pathology & Laboratory Medicine* several review articles will address some of these critical issues relevant to the routine practice of breast pathology.

Drs Chungyeul Kim, Yusuke Taniyama, and Soonmyung Paik will focus on gene expression-based prognostic and predictive assays and will specifically compare 2 currently available assays, OncotypeDX and MammaPrint. The authors clearly explain the reasons that these assays are needed, what information each provides, what type of tissue is required for each assay, and which aspects of the assay require further clarification. For example, in our practice, a high proportion of cases we send for OncotypeDX analysis come back with an intermediate

recurrence score. This intermediate category is currently under evaluation and further analysis in the hope of its eventual elimination and ultimate reduction of the recurrence scores to 2 categories, high-risk and low-risk, to facilitate the decision-making process in the use of chemotherapeutic agents.

Gene expression profiling has introduced a new vocabulary and novel clusters in the classifications of breast carcinomas. Drs Emad Rakha and Jorge S. Reis-Filho present an excellent review of basal-like breast carcinomas, a group of carcinomas characterized by ER, PR, and HER2 negativity (triple negativity) and expression of basal/ myoepithelial cell markers. The need for either further refinement of the category or restriction of the immunophenotypic criteria for inclusion among basal-like carcinomas is notable. We may witness a widening of the spectrum of “basal-like” carcinomas and a dilution of the uniformly aggressive behavior noted in the small number of cases evaluated until recently as more breast carcinomas are studied by gene expression profiling.

One of the major recent modifications in management of patients with breast carcinoma has been the assessment of the sentinel lymph node to avoid axillary lymphadenectomy whenever possible. Drs Aysegul A. Sahin, Merih Guray, and Kelly K. Hunt address some of the critical issues in the assessment of sentinel lymph nodes. With the thorough sampling of the small number of nodes obtained, we often encounter variably located small clusters of cells, the precise clinical significance of which is yet to be determined. How should we interpret a small cancer cell cluster within a vessel just outside of a nodal capsule—would it qualify as a positive node or not? How do we measure a metastatic tumor that displays a spray or dispersed distribution pattern in the lymph node as is the case in some lobular carcinomas? There are no guidelines offered by TNM for interpretation of these and some other scenarios that we encounter in assessment of the sentinel lymph nodes.

Dr Farid Moinfar provides a detailed review of the flat ductal intraepithelial neoplasia 1 (flat epithelial atypia), a lesion beautifully described by Azzopardi in 1979. Because of the emphasis placed on hyperplasias in the conventional classification of intraductal proliferative lesions, the role of this lesion in mammary carcinogenesis was totally ignored by many pathologists in the United States until its neoplastic nature and similarity to low-grade invasive and intraepithelial carcinomas at the molecular level was demonstrated in our laboratory in 2000. Since then, a substantial number of publications have focused on this lesion, and a variety of terms have been used for its designation. Flat ductal intraepithelial neoplasia 1 (flat epithelial atypia) is now recognized as the milieu in which low-grade ductal intraepithelial neoplasia (low-grade ductal carcinoma in situ) develops. Furthermore, not infrequently, it is the only intraepithelial lesion associated with tubular carcinoma. Commonly associated with microcalcifications, it is now recognized that these flat lesions account for a significant proportion of biopsies currently performed for microcalcifications. Optimal management of patients with this lesion remains to be determined, but its recognition is the first step toward its understanding. Moinfar provides a thorough historical review, an excellent enumeration of the diagnostic features of flat ductal intraepithelial neoplasia 1, and a summary of our current understanding of the clinical significance of the lesion when found in core or excisional biopsies.

Papillary lesions of the breast continue to present diagnostic problems despite the many studies and reviews of the topic. This is in great part due to the tremendous variations that are encountered in any subtype of this architectural phenotype. Drs Shir-Hwa Ueng, Thomas Mezzetti, and I address some of the more common variants and problems encountered among papillary lesions and present an approach in dealing with these issues. Only a few decades ago, a clear-cut distinction was not made between in situ (intracystic) and invasive papillary carcinomas; currently, even with the use of immunostains for myoepithelial cells, it is extremely difficult to assign some lesions to a purely noninvasive category or to one that is partially or completely invasive. The frequent use of needle aspiration and core biopsies has added another confounding factor; papillary lesions are highly friable, and generous fragments can be easily dislodged and implanted into the surrounding stroma, mimicking an invasive process.

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Histological Patterns In Drug-Induced Liver Disease

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The diagnosis of drug-induced liver injury (DILI) is a challenging problem, often confounded by incomplete clinical information and the difficulty of eliciting exposure to herbal products, over-the-counter agents and toxins. The task is further rendered difficult on biopsy, as drugs can mimic all the patterns found in primary liver disease. Acute hepatitis, with or without cholestasis, is the most common histological pattern of DILI, and drugs such as acetaminophen are the leading causes of acute liver failure. Most cases of DILI resolve on discontinuation of the drug, but recovery can take months or rarely the disease can progress despite drug withdrawal. Drugs such as methotrexate can lead to chronic hepatitis and cirrhosis, while others such as minocycline, nitrofurantoin and methyldopa are implicated in autoimmune hepatitis. Prolonged cholestasis and ductopenia resembling primary chronic biliary disease can occur. Drug-induced steatohepatitis is also an uncommon pattern, but is well described with drugs such as amiodarone and irinotecan. In the presence of risk factors such as obesity and diabetes, some drugs such as tamoxifen, oestrogens and nifedipine can precipitate or exacerbate steatohepatitis. Other observed patterns include granulomatous hepatitis, vascular injury (eg, sinusoidal obstruction syndrome), Ito cell lipidosis and neoplasms (eg, adenomas).

Evaluation of liver biopsy for adverse drug reaction is one of the most challenging problems in liver pathology. Drug-related injury can mimic all the patterns observed in primary liver disease, and an unequivocal histological diagnosis is not possible in the majority of cases. Inadequate clinical history and multiple drugs being taken simultaneously often compound the problem. It can be difficult to elicit information about herbal agents, over-the-counter medications, and exposure to household or industrial toxins. The list of drugs associated with hepatotoxicity is long, although the association of many drugs with liver injury remains tenuous and can be found only in case reports.

MECHANISMS OF INJURY

It is widely recognised that drug-induced liver injury (DILI) is mediated by two chief mechanisms: intrinsic and idiosyncratic hepatotoxicity. Intrinsic hepatotoxins cause hepatocellular damage in a predictable dose-dependent manner directly by the drug or indirectly by its metabolite. Some drugs, such as acetaminophen, cause intrinsic hepatotoxicity, but the majority of agents in this category are industrial, household or environmental toxins such as carbon tetrachloride and alkaloids in mushrooms. The majority of drugs lead to idiosyncratic liver injury and can be classified into metabolic and immunological categories. In the former, the drug is metabolised into a toxic metabolite in predisposed individuals, while the latter is akin to “drug allergy” or hypersensitivity following sensitisation to the drug. In general, intrinsic hepatotoxicity manifests with hepatocellular necrosis with little inflammation, while idiosyncratic drug reactions often show inflammation-dominant hepatic injury.

ESTABLISHING DRUG AS THE CAUSATIVE AGENT

The temporal profile is crucial to establish the diagnosis of DILI, as the onset of liver disease follows drug ingestion. However, the manifestation of liver toxicity may occur weeks or months after drug ingestion and even after the drug has been stopped. Liver enzyme elevations can persist for up to several months after the drug has been discontinued. In some instances, measurement of serum levels of the drug or its metabolite can be helpful in diagnosis, such as in acetaminophen toxicity. Since the list of drugs capable of causing liver injury is long, a systematic literature search for each drug that the patient has been taking is necessary. The case for DILI is strengthened if the reported pattern of injury in the literature is in keeping with the observed clinical and histological picture. Rechallenge with the drug can help establish the drug aetiology, but it is often not done due to the inherent risk involved. Since diverse histological patterns of DILI can mimic virtually any primary liver disease, appropriate imaging and laboratory tests are necessary to exclude other aetiologies before the diagnosis of DILI can be accepted.

Liver injury can be classified as hepatocellular, cholestatic or mixed, based on criteria established by the Council for International Organizations of Medical Sciences (CIOMS)

The CIOMS system also is used for causality assessment of DILI by scoring parameters such as time to onset of symptoms, laboratory data, additional drug regimen, known toxicity of suspected drug, non-drug causes, and response to rechallenge. The total score is categorised into ranges of causality: highly probable, probable, possible, unlikely and excluded. The remaining discussion is devoted to the patterns observed in DILI with emphasis on morphological features, common drugs and differential diagnosis for each pattern.

ACUTE HEPATITIS

DILI accounts for ~10% of acute hepatitis and is perhaps the most common cause of cholestatic hepatitis. A wide variety of drugs can cause acute hepatocellular injury.

Herbal and botanical drugs are an important but often overlooked cause of hepatotoxicity.

These are not regulated by the Food and Drug Administration and hence are not subject to rigorous testing. More than 20 000 herbal products are marketed in forms including powders, essential oils and teas, and more than \$5 billion are spent on these annually. Nearly 20% of American adults have used herbal remedies, and usage is higher in selected groups including Chinese, South African and Native American cultures. Definitive identification of an herbal product can require chemical analysis, as mistranslation or misidentification can be an issue. Eliciting a detailed herbal history is imperative. Certain commonly consumed herbal agents now being investigated for their hepatoprotective effects, such as turmeric (*Curcuma longa*) and mate tea (*Ilex paraguariensis*), are listed as potentially hepatotoxic in various patient literature. Finally, contaminants of herbal supplements should be considered, including heavy metals such as arsenic, cadmium, lead or mercury.

The following morphological patterns can be observed in acute hepatocellular injury.

* Acute hepatitis. The hallmarks of acute hepatocellular injury are portal and parenchymal inflammation, hepatocellular injury, and/or necrosis). By definition, fibrosis is absent. Regenerative features such as binucleate hepatocytes and thick cell plates are common. Prominent Kupffer cells often are present in the sinusoids. The term “cholestatic hepatitis” is used when these changes are accompanied by cholestasis (see Acute cholestatic injury).

* Necrosis. Acute hepatocellular injury can result in necrosis affecting single (spotty necrosis) or groups of hepatocytes (confluent necrosis). In some cases, confluent necrosis can be zonal and may be helpful in diagnosis. Centrizonal (zone 3) necrosis is characteristic of acetaminophen and halothane, and toxins such as carbon tetrachloride. Isolated necrosis affecting zones 1 and 2 is rare; toxins such as cocaine and ferrous sulfate typically affect zone 1, while beryllium has been implicated in zone 2 necrosis. When extensive, confluent necrosis can lead to acute hepatic failure.

* Resolving hepatitis. If biopsy is performed later in the disease course, hepatocellular injury and inflammation may be minimal). The presence of numerous macrophages in the sinusoids is a helpful clue for the diagnosis of resolving hepatitis. The stain periodic acid–Schiff with diastase can be used to highlight the macrophages.

Differential diagnosis

The histological features can be indistinguishable from other causes of acute hepatitis such as acute viral hepatitis, initial presentation of autoimmune hepatitis and Wilson disease. The presence of bile duct injury, prominent eosinophilic infiltrate, granulomas, sharply defined perivenular necrosis, or cholestasis out of proportion to hepatocellular injury, favours adverse drug reaction, but none of these features is specific.

ACUTE LIVER FAILURE (FULMINANT HEPATITIS)

Acute liver failure (ALF) is defined as the onset of hepatic encephalopathy within 8 weeks of onset of symptoms. Drugs are the most common cause of ALF in the USA, accounting for 25–50% of cases

Based on morphological features, ALF can be subdivided into three categories.

* Extensive microvesicular steatosis. This pattern is rare and has been observed with tetracycline and nucleoside analogues such as zidovudine (see Steatosis and steatohepatitis).

* Necrosis with marked inflammatory activity. This is the most common pattern seen in idiosyncratic adverse drug reactions. It is similar to the acute hepatitis pattern discussed above except that the confluent necrosis involves most of the liver parenchyma (massive/submassive hepatic necrosis). The most commonly implicated drugs are isoniazid, other antimicrobial agents (sulfonamides, cotrimoxazole, ketoconazole), monoamine oxidase inhibitors, and anticonvulsants (phenytoin, valproate). Any drug that causes acute hepatitis can potentially cause ALF.

* Necrosis with little or no inflammation. This pattern is seen with acetaminophen, recreational drugs such as cocaine and 3,4-methylenedioxymethylamphetamine (MDMA; ecstasy), industrial organic compounds such as carbon tetrachloride, and some herbal preparations. Necrosis can be accompanied by steatosis.

Acetaminophen

Acetaminophen toxicity is the leading drug-related cause, implicated in nearly 40% of ALF, the remaining being attributed to idiosyncratic drug reactions. Acetaminophen is a very safe drug within its therapeutic window (3–4 g/day), but can cause dose-dependent toxicity with overdose whether accidental (1/3 of cases) or with suicidal intent (2/3 of cases). At low doses, the drug is conjugated to water-soluble metabolites in the liver and is excreted in the urine. At higher doses, glutathione depletion leads to saturation of the conjugation mechanism, leaving the parent compound to be metabolised to toxic intermediates. The minimum toxic dose in adults is 7.5–10 g, but severe liver damage occurs with ingestion of 15–25 g. Acetaminophen blood levels taken 4–16 h after ingestion are the best predictor of outcome. Chronic alcohol consumption, obesity, and drugs that induce the P-450 cytochrome system, such as isoniazid, phenytoin, carbamazepine or cimetidine, can lower the toxic threshold of acetaminophen.

Patients typically experience gastrointestinal symptoms for the first 12–24 h and a latent phase at 24–48 h. The onset of acute hepatitis/acute liver failure occurs 72–96 h after drug ingestion. Hepatotoxicity can be prevented with early presentation and institution of acetyl-cysteine therapy within 12 h. The highest mortality is encountered in late presenters.

CHRONIC HEPATITIS

Chronic liver disease typically refers to persistent biochemical abnormalities beyond 6 months. In some series, the cut-off of 3 months has been used for hepatocellular injury and 6 months for cholestatic or mixed injury. Progression to chronicity has been reported in 5–10% of adverse drug reactions and is higher for the cholestatic/mixed injury pattern. Histologically proven drug-induced chronic hepatitis with fibrosis is a rare phenomenon. Some specific patterns and clinicopathological situations are discussed below.

Chronic hepatitis with negative autoimmune markers

The histological features are indistinguishable from chronic viral hepatitis, and progression to fibrosis and even cirrhosis can occur. The features of acute hepatitis may be seen to a variable degree. Drugs associated with this pattern include lisinopril (antihypertensive), sulfonamide (antibiotic), trazodone (antidepressant), and chemotherapeutic agents such as uracil, 5-fluorouracil prodrug tegafur and tamoxifen. Isolated case reports implicate numerous other drugs including phenytoin and the Chinese herb Jin bu huan. Progression to fibrosing cholestatic hepatitis has been reported in a hepatitis C patient after administration of cyclophosphamide and corticosteroids for glomerulonephritis. Discontinuation of the drug may lead to a favourable outcome, but if the fibrosis is advanced the resolution may be slow or the disease may progress.

Autoimmune hepatitis

Several drugs can cause chronic hepatitis that is serologically and morphologically indistinguishable from de novo autoimmune hepatitis (AIH). The hepatic disease may be accompanied by features of hypersensitivity such as rash, arthralgia and peripheral eosinophilia.

Minocycline

Long-term use of minocycline, a synthetic tetracycline for treatment of acne, can lead to hepatitis that can mimic lupus-related hepatitis, AIH or overlap syndrome. Autoimmune disease can develop within days of starting the drug or may be delayed for many years. High titres of antinuclear antibodies (ANAs) are common, but smooth muscle (SMA) and other autoantibodies often are negative. Autoimmune markers may be elevated in chronic hepatitis due to drugs (drug-induced autoimmune hepatitis). Inflammatory activity can be minimal to mild, and eosinophils are typically inconspicuous. Marked fibrosis and cirrhosis are rare, and patients often improve after drug withdrawal. Microvesicular and macrovesicular steatosis in response to minocycline have been reported, but these followed high-dose intravenous therapy rather than oral administration.

Nitrofurantoin

Nitrofurantoin is used to treat urinary tract infections. The hepatic injury can manifest as self-limited acute hepatitis, chronic hepatitis, and rarely as hepatic failure. Chronic hepatitis can be indistinguishable from de novo AIH and is often associated with ANA and SMA. Discontinuation of the drug generally leads to clinical and biochemical improvement. In some cases, the disease

may progress despite drug withdrawal. In contrast to minocycline, significant fibrosis and cirrhosis can occur.

Others

Other drugs implicated in AIH include methyldopa (antihypertensive) and clometacin non-steroidal anti-inflammatory drug (NSAID). Antibodies to liver-kidney-microsomal antibodies, akin to type 2 AIH, have been described in hepatitis related to hydralazine (antihypertensive) and tienilic acid (ticrynafen, a diuretic withdrawn from the American market), but this association is not clearly defined.

Methotrexate

Methotrexate is a folate antagonist that is used for long-term treatment of rheumatoid arthritis, psoriasis and inflammatory bowel disease. The canals of Hering may be the target of methotrexate-related scarring. The risk of liver toxicity is exacerbated with heavy alcohol use, pre-existing liver disease, daily dosing and high cumulative dose. Minor elevation in liver enzymes occurs in 20–50% of patients but does not necessarily imply significant toxicity.

The histological features of methotrexate-related toxicity range from minor fatty change, hepatocyte anisonucleosis, mild portal-based inflammation, and focal necrosis to more severe hepatocellular necrosis, fibrosis and cirrhosis. Methotrexate may exacerbate or precipitate steatohepatitis in patients with risk factors such as obesity and diabetes. Some patients with high cumulative dose can have steatohepatitis-like histology without other risk factors.

Patients on long-term methotrexate need close monitoring. Liver biopsy is necessary in patients who develop deranged liver function following methotrexate therapy. A grading scheme has been proposed to assess toxicity (Roeningk classification).

ACUTE CHOLESTATIC INJURY

Drug-induced cholestatic injury can manifest clinically with jaundice, pruritus, dark urine and pale stools. Liver enzyme studies typically reveal elevation of alkaline phosphatase and [gamma]-glutamyl transferase. Transaminases can be variably elevated. A Danish study of 1100 cases of drug-associated injury reported 16% with the acute cholestatic pattern.

The histological patterns of injury can be divided into two forms. (1) Pure (bland) cholestasis in which bile plugs are seen in hepatocytes or canaliculi and are most prominent in zone 3. Inflammation and hepatocellular injury are not observed. This pattern is typically observed with anabolic steroids and oral contraceptives. Other drugs that have been incriminated include prochlorperazine, thiabendazole and warfarin. (2) Cholestatic hepatitis in which the cholestasis is accompanied by inflammation and hepatocellular injury. Bile ductular reaction may be present. This pattern also has been referred to as cholangiolitic or hypersensitivity cholestasis. This pattern manifests as mixed-type injury on liver biochemical tests. Cholestatic hepatitis can

result from a wide variety of drugs; it is the classic pattern seen with toxicity due to macrolide antibiotics such as erythromycin and the antipsychotic agent chlorpromazine.

Differential diagnosis

Drug-induced cholestatic injury can be histologically indistinguishable from obstructive biliary disease. While the latter typically results in portal tract oedema and ductular reaction with inflammation, cholestasis may be the only significant feature in early stages. Drug-induced cholestatic hepatitis also needs to be distinguished from autoimmune hepatitis and acute viral hepatitis.

Bland cholestasis can occur in several systemic disorders such as sepsis, cardiac failure and shock, and hence clinical information is necessary to establish the aetiology. In the appropriate clinical setting, benign recurrent intrahepatic cholestasis, postoperative cholestasis and intrahepatic cholestasis of pregnancy have to be considered. Benign recurrent intrahepatic cholestasis is a mild, non-progressive variant of bile transporter disorder characterised by intermittent episodes of cholestasis. Intrahepatic cholestasis of pregnancy also is due to bile transporter gene variation, although it additionally appears affected by hormonal status, as twin pregnancies and patients on oral contraceptives are reported to be more susceptible to intrahepatic cholestasis of pregnancy

Chronic biliary diseases such as primary biliary cirrhosis and primary sclerosing cholangitis do not show cholestasis on biopsy early in the course of the disease; serological tests such as antimitochondrial antibodies and cholangiography, respectively, can more definitely rule out these diagnoses.

CHRONIC CHOLESTASIS AND DUCTOPENIA

Cholestatic symptoms and biochemical findings usually resolve with cessation of the offending drug but may persist in some instances. Drugs causing prolonged cholestasis (defined as greater than 3 months in duration) and ductopenia include antibiotics such as amoxicillin–clavulanic acid and flucloxacillin, antifungals such as terbinafine and, rarely, oral contraceptives. Amiodarone can also cause prolonged disease.

Vanishing bile duct syndrome

Cholestasis with variable degree of inflammation, bile duct injury and hepatocellular damage is seen early in the course of the disease. If the disease persists for a few months or beyond, loss of bile ducts and overt ductopenia may be observed, termed “vanishing bile duct syndrome”. Persistent inflammation and bile ductular reaction also may be present. Rare cases can progress to cirrhosis. Vanishing bile duct syndrome can be triggered by anticonvulsants such as carbamazepine and zonisamide, antipsychotics such as chlorpromazine and sulpiride NSAIDs such as ibuprofen and tenoxicam, and antibiotics such as amoxicillin, flucloxacillin, clindamycin and trimethoprim-sulfamethoxazole. The histological picture can mimic primary

biliary cirrhosis or obstructive biliary disease. Absence of antimitochondrial antibodies and normal imaging of the biliary tree help in establishing drug-related aetiology.

Other patterns

Biliary sclerosis can result from intra-arterial infusion of 5-fluorodeoxyuridine for treatment of hepatic metastasis of colorectal carcinoma. Ischaemic injury to the large intrahepatic and extrahepatic bile ducts can lead to strictures that resemble primary sclerosing cholangitis radiologically and histologically. Similar injury can occur with other agents such as formaldehyde and sodium chloride injected into hydatid cysts.

GRANULOMATOUS HEPATITIS

The most common causes of granulomas in the liver are infections, sarcoidosis, primary biliary cirrhosis and drugs.

Granulomas are uncommon in hepatitis C but can occur in patients treated with interferon. Talc granulomas can occur in intravenous drug users and can be detected by viewing under polarised light. Other systemic granulomatous disease such as chronic metal toxicity (such as beryllium or copper) can also involve the liver. Finally, a study of granulomatous hepatitis cases over a 13-year period identified 11% as idiopathic. These cases can present with fever of unknown origin and generally respond favourably to steroids.

The granulomas can be present in the portal tracts or the parenchyma and lack necrosis. Unlike in primary biliary cirrhosis, the granulomas are not centred on the bile ducts. Granulomas also can occur with other patterns of liver injury such as acute hepatitis, cholestasis or steatosis.

The term fibrin-ring granuloma has been used for small granulomas that consist of a ring of fibrin arranged around a central fat vacuole. Epithelioid histiocytes are present around the ring of fibrin. In atypical cases, the fibrin is intermixed with the histiocytes and does not form a well-defined ring. More typical granulomas without the fibrin ring generally are present in other areas of the biopsy. Fibrin-ring granulomas have been described with allopurinol, BCG vaccination and intravesical therapy for carcinoma. These granulomas were first described in the rickettsial disease Q fever (*Coxiella burnetii*) but also occur in boutonneuse fever (*Rickettsia conorii*), leishmaniasis, toxoplasmosis, cytomegalovirus infection and Hodgkin lymphoma.

STEATOSIS AND STEATOHEPATITIS

Macrovesicular steatosis

Macrovesicular steatosis includes large and small droplet fat. The term “large droplet fat” is used when at least half the hepatocyte cytoplasm is occupied by a single lipid vacuole, while multiple lipid vacuoles are seen in small droplet fat. The latter often is confused with true microvesicular steatosis which, unlike small droplet fat, affects the liver in a diffuse fashion. Macrovesicular steatosis can be seen in association with steroids, nitrofurantoin, gold, methotrexate, NSAIDs

such as ibuprofen, indomethacin and sulindac, and antihypertensives such as metoprolol, chlorinated hydrocarbons such as carbon tetrachloride and chloroform, or chemotherapeutic agents such as 5-fluorouracil, cisplatin and tamoxifen.

Microvesicular steatosis

Exclusive or predominant microvesicular steatosis diffusely affecting the liver is a result of mitochondrial injury and often occurs as an adverse effect of drugs/toxins such as cocaine, tetracycline, valproic acid and zidovudine. Acute exposure to alcohol (alcohol foamy liver degeneration) and paediatric Reye syndrome also show diffuse microvesicular steatosis. Other non-drug related aetiologies include acute fatty liver of pregnancy and genetic diseases such as carnitine deficiency.

Steatohepatitis

By definition, steatohepatitis is characterised by steatosis, lobular inflammation and hepatocellular injury in the form of hepatocellular ballooning (with or without acidophil bodies or Mallory hyaline) or pericellular fibrosis. A few drugs (notably amiodarone and irinotecan) play a direct aetiological role in steatohepatitis. Most other drugs exacerbate or precipitate steatohepatitis in the presence of other risk factors such as obesity and diabetes.

Amiodarone

Amiodarone, a potent antiarrhythmic agent, causes elevated liver enzymes in up to 30% of patients and steatohepatitis in 1–2% of patients. The majority of cases display liver enzyme abnormalities within 24 h of intravenous infusion. Even low oral dosing (200 mg daily) may trigger steatohepatitis with cumulative use. Occasionally, jaundice is the major clinical presentation. These cases often show hepatocellular necrosis and fibrosis, and have a poor prognosis.

Amiodarone steatohepatitis is characterised by prominent Mallory hyaline (occasionally in zone 1) and neutrophilic satellitosis, while steatosis is less conspicuous. The findings can be similar to alcoholic steatohepatitis. Reversal of liver injury often occurs with discontinuation of the drug but may be delayed by weeks or months. In addition, amiodarone is also associated with a different type of lipid accumulation called “phospholipidosis” characterised by accumulation of drug in the lysosomes. This leads to “foamy” appearance of hepatocytes and Kupffer cells. The foamy areas show lamellar lysosomal inclusion bodies on electron microscopy. Phospholipidosis is not always seen in amiodarone toxicity and is independent of steatohepatitis.

Perhexiline maleate (Pexid), an antianginal drug, and diethylaminoethoxyhexestrol (Coralgil), a vasodilator, have been used extensively in Europe and Japan, respectively. Both drugs can cause steatohepatitis and phospholipidosis similar to amiodarone.

Chemotherapy-induced steatohepatitis

Steatosis and steatohepatitis have been reported with chemotherapeutic agents. The latter especially is associated with irinotecan, a drug often used preoperatively in colorectal cancer with hepatic metastases. This has been referred to as chemotherapy-associated steatohepatitis in the oncology literature. Other chemotherapeutic agents such as oxaliplatin have been variably implicated

Others

Drugs such as tamoxifen, steroids, oestrogen and diethylstilbestrol often lead to hepatic steatosis, but steatohepatitis is rare. These drugs may exacerbate or precipitate steatohepatitis in patients with risk factors for steatohepatitis rather than play an aetiological role. The evidence linking steatohepatitis and calcium channel blockers such as nifedipine also is anecdotal. Risk factors for steatohepatitis were present in many reported cases, creating uncertainty about the association of these drugs with steatohepatic injury

VASCULAR ABNORMALITIES

Several vascular patterns of injury are recognised, each with distinctive morphological features and drug associations.

Sinusoidal obstruction syndrome

Sinusoidal obstruction syndrome (SOS; veno-occlusive disease) is due to endothelial cell injury to small hepatic venules that manifests histologically as endothelial swelling and thrombosis). The resultant venous outflow obstruction leads to sinusoidal dilatation, congestion, hepatocellular necrosis, and can result in centrilobular fibrosis.

Cytotoxic/chemotherapeutic drugs such as oxaliplatin (used in colorectal cancer) can cause injury to sinusoidal endothelial cells and hepatic stellate cells. SOS can also occur due to myeloablation before stem cell transplantation, chemotherapy for acute lymphocytic leukaemia, bone marrow transplantation and pyrrolizidine alkaloids. Genetic polymorphisms in methylenetetrahydrofolate reductase have been implicated in SOS in post-transplant patients. Recently, defibrotide has been used with success to resolve cases of SOS, although in some cases, transplantation can be required.

Peliosis hepatis

Peliosis is characterised by blood-filled cavities without an endothelial lining in the hepatic parenchyma. This phenomenon most commonly is associated with androgens or contraceptive steroids. Thiopurine-derived chemotherapeutic drugs also have been implicated. Peliosis also occurred with the intravenous contrast agent thorium dioxide (Thorotrast), which has been discontinued due to the high risk of angiosarcoma. Sinusoidal dilatation may accompany peliosis or may occur independently, particularly with androgenic or oestrogenic steroid use.

Hepatic vein thrombosis

Hepatic vein thrombosis is a rare complication of some drugs, including oral contraceptives and dacarbazine, and presents clinically as Budd-Chiari syndrome.

OTHER PATTERNS

Stellate cell lipidosis

Hepatic stellate cells (Ito cells) are modified fibroblasts that store lipids and vitamin A in the normal liver. They are located in the space of Disse between the sinusoidal endothelium and the hepatocytes but generally are not easily visible. In certain conditions, especially hypervitaminosis A, excessive lipid gets stored in the stellate cells (stellate cell lipidosis). The nuclei of stellate cells are crescent shaped, dark staining, and indented by the lipid droplets. Thin strands of cytoplasm separate the lipid droplets. These lipid-laden cells easily can be mistaken for hepatocytes with steatosis. Their characteristic morphology and location along the sinusoids between the hepatic plates distinguishes them from steatotic hepatocytes.

Hypervitaminosis A results from excess dietary/supplementary vitamin A intake or use of oral/topical retinoids (such as etretinate for acne). Stellate cell lipidosis also has been reported with methotrexate, valproate and steroids, as well as in other clinical settings such as cholestasis, alcoholic liver disease and hepatitis C.

It is important to recognise this condition, as activation of stellate cells can lead to fibrosis, non-cirrhotic portal hypertension and, rarely, cirrhosis. One case of liver transplantation for subacute vitamin A toxicity has been reported. Early recognition can prompt reduced intake of vitamin A to avert progression and fibrosis. The contribution of stellate cell lipidosis to disease progression when present with other disease processes such as alcoholic liver disease and chronic hepatitis C is unknown.

Cytoplasmic inclusions

Ground glass change in the cytoplasm occurs in a minority of patients with hepatitis B and is characterised by pale eosinophilic cytoplasmic inclusions in hepatocytes. Similar changes (often termed “pseudo ground glass change”, can be seen with drugs such as cyanamide, a drug used in alcohol treatment programs. This phenomenon has also been described with other drugs such as barbiturates and diazepam, diabetic patients on insulin, and transplant patients on multiple immunosuppressive drugs such as steroids, tacrolimus and mycophenolate mofetil. Similar to hepatitis B, this change reflects hypertrophy of smooth endoplasmic reticulum with use of drugs such as barbiturates, while most other drug-induced cases are due to accumulation of abnormal glycogen. Rare metabolic disorders such as type IV glycogenosis, hypofibrinogenaemia, and Lafora disease can lead to the same morphological findings.

Pigments

Some drugs/toxins such as gold, titanium and thorium dioxide (Thorotrast) can be deposited as pigments in the liver. Drugs that cause prolonged cholestasis can lead to copper accumulation in periportal hepatocytes. Lipofuscin, a lysosomal pigment often seen in centrilobular hepatocytes, can be increased with exposure to anticonvulsant drugs such as phenothiazine and phenacetin.

Hepar lobatum

This term generally refers to liver abnormalities in tertiary syphilis. In some instances, chemotherapy for metastatic liver cancer (especially from the breast) can lead to similar changes. The liver shows a lobulated contour with capsular indentations from which fibrous septa extend deep into the parenchyma. The fibrous septa can surround the degenerated centre of tumour nodules and may contain macrophages and residual tumour. Typical features of cirrhosis such as regenerative nodules are not observed. These features probably result from tissue collapse due to chemotherapy-related tumour regression that is followed by an organising phase of healing and scar contraction.

Drug-related neoplasms

The association of oral contraceptives and hepatic adenomas is well recognised. Association with focal nodular hyperplasia and hepatocellular carcinoma also has been reported, but the link is less convincing. Other agents such as anabolic steroids used by sportsmen, clomiphene, danazol and carbamazepine have also been associated with hepatic adenoma.

Exposure to vinyl chloride (an industrial chemical) and thorium dioxide (a discontinued radiographic contrast agent) can lead to angiosarcoma, sometimes after long latent periods exceeding 20 years. Hepatocellular carcinoma and cholangiocarcinoma also have been reported with thorium dioxide.

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Histopathology of Gestational Trophoblastic Disease. An Update

Horn LC, Einkenkel J, Vogel M.

The differential diagnosis of villous forms of gestational trophoblastic disease (GTD) includes hydropic abortion, complete and partial hydatidiform mole and placental mesenchymal dysplasia. In addition to histologic criteria, p57(KIP2) immunohistochemistry might be helpful. Choriocarcinoma represents the most immature form of GTD. This and downregulation of HSP-27 might contribute to the high chemosensitivity, compared to placental site (PSTT) and epithelioid trophoblastic tumor (ETT). Within the differential diagnosis of the non-villous forms of GTD an algorithmic approach of immunohistochemistry is very helpful. With an incidence of 1.6% of all abortions within the first trimester the exaggerated placental site reaction (EPS) is

rare. There is no molecular indication that the EPS represents a precursor lesion of PSTT. The morphologic prediction of the behaviour of PSTT is not well established. Factors which might be associated with adverse outcome are age >35 years, interval since last pregnancy >2 years, growth outside the uterus, deep myometrial invasion, destructive growth, extensive coagulative necrosis, presence of cells with clear cytoplasm, high mitotic rate and a Ki-67 labeling index >50%. Recent molecular data suggest a neoplastic transformation of (cyto-) trophoblastic stem cells, within the pathogenesis of (non-villous) GTD. The detection of target molecules for a targeted therapy is currently irrelevant.

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CYTOPATHOLOGY

Fine-Needle Aspiration Cytology of Thyroid Nodule: Does the Needle Matter?

Cappelli, Carlo; Pirola, Ilenia; Gandossi, Elena

Our data showed that the adoption of a stylet needle significantly improves diagnostic performance, reducing by about 14% the expenditure per diagnostic procedure. Therefore, we suggest that stylet needles should be widely adopted to perform US-FNAC of thyroid procedures.

Key Points

- * Ultrasound-guided fine needle aspiration cytology (US-FNAC) is the most effective test available to distinguish between benign and malignant thyroid nodules.
- * The major limiting factor of US-FNAC is the rate of inadequate specimens.
- * The adoption of a stylet needle to perform thyroid cytology significantly improves its diagnostic performance by increasing the number of diagnostic specimens.
- * The use of stylet needle reduces the expenditure per diagnostic procedure by about 14%.

Fine needle cytology (FNC) is currently the most accurate, safe and cost-effective method for identifying malignant thyroid nodules. First used in Sweden in the 1950s, it did not become widely used until the 1980s. Although there are a number of methodologies, the most efficient includes ultrasound guidance, needles of 21–27 gauge and specimen aspiration by a syringe or capillary action. False negative and false positive diagnosis may occur, but the main limitation of this procedure remains the number of nondiagnostic specimens, which are reported in up to 23% of specimens (mean 17%).

Different strategies have been proposed to improve the diagnostic performance of ultrasound-guided fine needle aspiration cytology (FNAC). In 2005, we focused our attention on the needle, hypothesizing that the presence of a stylet could prevent the entrance of blood during the insertion of the needle. We obtained a significant improvement in adequate FNAC by using spinal needles into nodules at high risk of nondiagnostic sampling, such as those with intranodular vascularity on

Doppler evaluation. In a subsequent study, we obtained the same results in complex thyroid nodules, demonstrating that the presence of the stylet could limit the contamination of the sample, reducing the presence of colloid. Recently, we have extended our investigation to solid nodules, confirming that the use of stylet needles is associated with a significant increase in adequate specimens. The aim of the present study was to evaluate whether the adoption of stylet needles may result in a significant improvement of diagnostic performance in a large, unselected series of all types of thyroid nodules.

Materials and Methods

Seven hundred and seventy-nine patients with nodules (solid and mixed) were recruited between January 2001 and July 2007 among those submitted to the thyroid outpatient clinic of our institution for neck ultrasound investigation. A single endocrinologist using an ultrasonography scanner performed fine needle aspiration cytology. The vascular pattern was assessed as type 0—absence of flow signals or vascular images only in peripheral position, and type 1—intranodular flow with multiple vascular images. Complex thyroid nodules were defined as cystic nodules with a significant solid component, ~40% or more of all thyroid nodules. One thousand and four hundred sixty-eight nodular lesions were detected and submitted to ultrasound-guided aspiration cytology (US-FNAC) by two different 25-gauge needles: a traditional hollow needle (Neolus, Chemil Wenzhou, China) or a stylet needle (Yale Spinal, Becton Dickinson, Spain) (Fig.1). All nodules of the patients of each US-FNAC session were submitted to cytology using the same type of needle (Neolus or Yale); we started by using the traditional one (Neolus). Two passes for each nodule were performed and the material was obtained by capillary action. After the introduction of the needle into the nodule(s), and also after removing the stylet in the case of the Yale, the needle was moved back and forth several times within the lesion with a rapid, gentle, stabbing motion. An air-filled syringe with its plunger already retracted was immediately attached to the needle after it was withdrawn, and the needle contents were expelled onto clean glass slides.

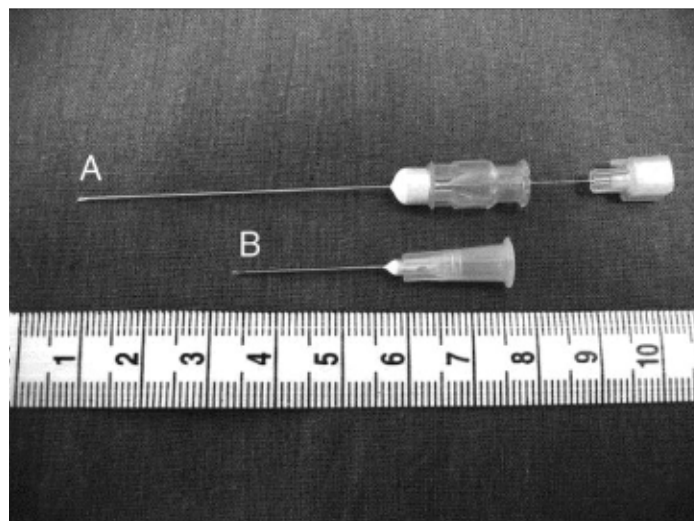


Fig. 1 Two type of needles: (A) Yale spinal and (B) Neolus.

Cytological specimens were evaluated in blind conditions by the same cytopathologist and, according to the guidelines of the Papanicolaou Society of Cytopathology, the smear was

considered adequate if there were at least five groups of well-visualized follicular cells, with each group containing 10 or more cells. All the nodules with inadequate samples were submitted to a second procedure at least one month later. Written informed consent was obtained from all subjects.

Statistics

Analysis of variance or $[\chi]^2$ test were used for between-group comparisons of continuous or categorical variables, respectively. Logistic analysis was performed to examine the influence of the main experimental factor (needle type) and of potential confounders related to patient (age, gender) or nodule (size, multiplicity, cystic component, vascularity) on cytological sampling adequacy.

All data were analysed using SPSS version 11.5 (SPSS, Inc., Chicago IL). Data were reported as mean \pm standard deviation. Statistical significance was considered at $P < 0.05$.

Results

A total of 672 and 796 nodules were submitted for US-FNAC by Neolus (Ns) and Yale spinal (Ys) needles, respectively. No difference of age, gender, nodule size and multiplicity, presence/absence of a cystic component or echo color Doppler vascularity pattern were observed between the two groups (Ns vs. Ys) (Table 1). Inadequate specimens were observed in 93/672 (13.8%) and 29/796 (3.6%) nodules investigated by Ns and Ys, respectively ($P < 0.001$). A second US-FNAC was performed with the same type of needle in each nodule with nondiagnostic cytology at the first sampling, thus obtaining an adequate specimen in 32/93 (34.4%) and 7/29 (24.1%) of previously unsatisfactory sampling by Ns and Ys respectively ($P = 0.296$). Overall, US-FNAC performed with the stylet needle was associated with a significant reduction of nondiagnostic specimens (9.1% vs. 2.8% by Ns and Ys, respectively, $P < 0.001$). Cytological results obtained by each needle are summarized in Table 2; a diagnosis of carcinoma was histologically determined in 72 of the 124 nodules that were submitted to surgery based on suspicious or neoplastic findings at cytological evaluation (see Table 2). Logistic regression analysis, taking into account sex, age, needle type, nodule size, multiplicity and structure (presence of a cystic component and vascularity type), showed that needle type was the only significant predictor of successful sampling [odds ratio 3.6 (95.0% C.I 2.0–6.4), $P < 0.001$] (Table 3). In this series of US-FNAC procedures, there were no relevant complications (subcutaneous hematoma, infection or others) with either type of needle. Local pain or discomfort, though not formally assessed, appeared generally limited and similar.

Table 1. Characteristics of two groups^a

	Neolus	Yale	<i>P</i>
Age (mean \pm SD)	53.6 \pm 13.1	53.2 \pm 12.5	0.602
Female/male	293/102	279/105	0.690
Nodular dimension (mean \pm SD)	16.8 \pm 5.6	16.7 \pm 6.3	0.724
Single/multiple	136/536	163/633	0.961
Mixed/solid nodules	242/430	278/518	0.705
Vascularity type 0/1	351/321	389/407	0.199

^aSD, standard deviation.

Table 2. The final cytological results obtained by each needle

	Neolus (%)	Yale spinal (%)
Cytological diagnosis		
Benign	557 (91.1)	704 (90.9)
Suspicious	21 (3.5)	32 (4.1)
Cancer	33 (5.4)	38 (5.0)
Total	611 (100)	774 (100)
Histological diagnosis		
Benign	7 (12.9)	8 (11.4)
Follicular adenoma	16 (29.7)	21 (30)
Cancer	31 (57.4)	41 (58.6)
Total	54 (100)	70 (100)

Table 3. Logistic regression analysis of successful sampling predictors^a

Predictor	Odds ratio (95% CI)	P
Yale spinal needle	3.6 (2.0–6.4)	<0.001
Gender (male)	0.8 (0.4–1.4)	NS
Age	1.0 (0.9–1.1)	NS

Discussion

Since its introduction 75 years ago, thyroid biopsy has had modifications and refinements to improve its diagnostic performance. Initially, an 18-gauge needle was used. After a few changes, including cutting needles for biopsies such as Silverman or Tru-Cut that required histological fixation, most operators adopted fine needles, 21 to 27 gauge, with the aim to reduce complications such as large blood effusions or malignant implants in the needle track, and also to simplify the procedure itself.

Fine needle cytology of thyroid nodules was first used in Sweden in the 1950s, but did not become widely used in North America until the 1980s. Ultrasound imaging guidance permitted introduction of the needle into the core of the lesion, improving diagnostic accuracy and sensitivity. The most common procedures now are ultrasound-guided fine needle aspiration cytology and fine needle capillary cytology.

In the classic approach of fine needle cytology described by Löwhagen et al, the biological specimen is obtained by repetitively moving a needle (most frequently a 25-gauge attached to a 10–20 mL syringe for constant or intermittent suction) through the nodule. A more recent variant collects the specimen by moving through and twirling within the nodule a small needle, not attached to a syringe, in order to aspirate the material by capillary action. Both methods have advantages and drawbacks: the first one may yield more material due to the suction, but may aspirate more blood; aspiration by capillary action causes less blood contamination but often collects less material. The choice of the aspiration technique is largely dependent on subjective preference. Some reports suggest that the percentage of diagnostic specimens may be increased by capillary aspiration. The major limitation remains the number of nondiagnostic specimens, which are reported in up to 23% of patients (mean 17%). Considering that blood contamination is one major cause of nondiagnostic sampling, we hypothesized that a stylet needle would limit the amount of blood flowing into the needle and improve the diagnostic yield. In 2005, we demonstrated that this procedure was effective in a specific subtype of nodules which are presumably more exposed to the risk of blood contamination, i.e., those presenting with an

intranodular vascular pattern at US evaluation.¹ Shortly thereafter, we were able to show that the diagnostic performance of US-FNAC in complex nodules may profit by the use of a stylet needle, possibly because less dilution by colloid material occurs, and more recently also in solid thyroid nodules. In the present study, we confirm that the advantage of a stylet needle is independent of the US vascular pattern and the presence of a cystic component.

Given the large number of cytological procedures performed worldwide, the issue of cost is not irrelevant, as recently reported also by Borget et al. A stylet needle is certainly more expensive than a “regular” hollow needle, but we have been able to demonstrate also in this unselected series of consecutive US-FNACs, that the reduction of the number of repeated procedures overcompensates the cost of the stylet needle. Although we did not perform a subanalysis of indirect costs related to the repetition of the procedure, such as absence from work, travelling to the clinic, etc, it is reasonable to hypothesize a reduction of total costs.

The adoption of a stylet needle to perform thyroid cytology significantly improves its diagnostic performance by increasing the number of diagnostic specimens. The proposed approach is safe and cost effective; it reduces by about 14% the expenditure per diagnostic procedure. We suggest that stylet needles should be widely adopted to perform US-FNAC of thyroid nodules.

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MICROBIOLOGY

Recent Discoveries in the Pathogenesis and Immune Response Toward *Entamoeba Histolytica*

Manigandan Lejeune; Joanna M. Rybicka; Kris Chadee

Entamoeba histolytica is an enteric dwelling human protozoan parasite that causes the disease amoebiasis, which is endemic in the developing world. Over the past four decades, considerable effort has been made to understand the parasite and the disease. Improved diagnostics can now differentiate pathogenic *E. histolytica* from that of the related but nonpathogenic *Entamoeba dispar*, thus minimizing screening errors. Classically, the triad of Gal-lectin, cysteine proteinases and amoebapores of the parasite were thought to be the major proteins involved in the pathogenesis of amoebiasis. However, other amoebic molecules such as lipophosphopeptidoglycan, perioxiredoxin, arginase, and lysine and glutamic acid-rich proteins are also implicated. Recently, the genome of *E. histolytica* has been sequenced, which has widened our scope to study additional virulence factors. *E. histolytica* genome-based approaches have now confirmed the presence of Golgi apparatus-like vesicles and the machinery for glycosylation, thus improving the chances of identifying potential drug targets for chemotherapeutic intervention. Apart from Gal-lectin-based vaccines, promising vaccine targets

such as serine-rich *E. histolytica* protein have yielded encouraging results. Considerable efforts have also been made to skew vaccination responses towards appropriate T-helper cell immunity that could augment the efficacy of vaccine candidates under study. Thus, ongoing efforts mining the information made available with the sequencing of the *E. histolytica* genome will no doubt identify and characterize other important potential vaccine/drug targets and lead to effective immunologic strategies for the control of amoebiasis.

Medscape Pathology and LabMedicine, 05/07/2009

H1N1 Flu Has Shorter Incubation Time, Strikes Younger Adults

Emma Hitt

May 5, 2009. The incubation period of the influenza A (H1N1) strain, now estimated at about 1 to 5 days, is shorter and more like seasonal influenza than originally thought, according to the World Health Organization (WHO).

The WHO continues to see a global increase in H1N1 cases. As of 8:00 am EDT, there were 1490 laboratory-confirmed cases and 30 deaths reported, with all but 1 death occurring in Mexico. The numbers represents an increase in 405 cases and 4 deaths since yesterday and are likely to be updated later today.

Keiji Fukuda, MD, MPH, assistant director-general ad. interim for health security and environment at the WHO spoke today at daily media briefing.

"There have been a significant number of countries in Europe reporting cases," with the United Kingdom and Spain reporting the most, Dr. Fukuda said. However, he added that they are not seeing community transmission in Europe "in the same way that we are seeing community transmission in the United States or Mexico." In Europe, the cases still seem to be mostly travel related, he said.

Currently, the pandemic alert level for H1N1 influenza is remaining at phase 5 and is not presently moving to the highest level of phase 6. The move to phase 6 will take place when there is community transmission in more than 1 WHO region.

At the time of the media briefing, a virtual meeting of international infectious disease experts was convening to focus on clinical presentation of H1N1 influenza and was still under way. According to Dr. Fukuda, the current consensus from that meeting was that people who are infected continue to be relatively younger people, mostly younger than 60 years. "The average age seems to be people in their mid-20s," he said.

The reason for the increased incidence in younger people remains unclear. According to Dr. Fukuda, it could be that younger people simply travel more and have greater exposure to the virus, or it could be that older people have developed a greater resistance than younger people. "With influenza, oftentimes we see the infections go to younger people first and then go to older people later," he said.

According to Dr. Fukuda, men and women are being infected at the same rate, which is similar to seasonal influenza. Severe respiratory illness, such as severe pneumonia requiring ventilation, seems to be the cause for most hospitalizations and deaths. "But the big question is...does this occur relatively infrequently or does this occur frequently," he said.

"In general we have seen a number of healthcare workers infected, but it's not certain that they got infected in the healthcare setting," Dr. Fukuda said. "And it has not been transmitted to a lot of healthcare workers."

The viral specimens in various regions continue to look very similar. The WHO maintains that the H1N1 influenza is susceptible to oseltamivir and zanamivir, the neuraminidase inhibitors, but the virus shows resistance to the class of agents that includes rimantadine and amantadine, he said.

Medscape Medical News

BOTTOM LINE

Autopsy Pathology: A Manual and Atlas

Edited by Walter E. Finkbeiner, Philip C. Ursell, and Richard L. Davis
2nd ed, 366 pp, \$179
Philadelphia, PA, Saunders/Elsevier Health Sciences, 2009
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There are those to whom the contributions of an efficient autopsy service to the overall standard of health care are unquestionable. To many, the postmortem observation lends itself to scientific discovery, stimulates clinical research, maintains public as well as family health, and contributes to jurisprudence, medical education, and pathology training. To these sanguine souls, the relentless and seemingly inexorable decline in autopsy rates is inexcusable. Various excuses (some more valid than others) have been proffered ad nauseam for declining autopsy rates. Among clinicians, the waning of interest in autopsies has been attributed to a disinclination to face failures, medicolegal misgivings, lack of rapport with the relatives of the deceased, and dissatisfaction with autopsy reports—typically with their timeliness. The apparent disinterest among families of the deceased can be attributed to cultural conviction, religious restraint, fear of defacement, desire to “spare further suffering,” possible delays in interment, and, most often,

sheer ignorance about autopsy, including its availability, benefits, and costs. Even pathologists are now considered increasingly reluctant purveyors of postmortem examinations because of other more immediate demands on their time and attention, the perceived underappreciation of their efforts, various remuneration-related issues, and their lack of proficiency in the performance of autopsies, owing mainly to decreasing emphasis on autopsies during training. Although each of the aforementioned issues would need to be addressed if there is to be a revival of the autopsy, all such efforts are destined to fail unless all involved medical professionals, including pathologists, reaffirm their commitment to this crucial procedure and reiterate its central role in medicine—not only in words but also in deeds.

Autopsy Pathology: A Manual and Atlas may not directly help with increasing the autopsy rate, but it could prove helpful as a primer for neophyte practitioners of autopsy. Needless to say, mastering the intricacies and complexities of the art and science of the hospital-based autopsy entails gaining experience and appreciating nuances that simply cannot be realized from a compact textbook. As the title implies, this book is primarily divided into 2 parts, a manual and an atlas. The manual portion consists of 14 chapters that address myriad mundane matters, including dissection technique, microscopy, and the principles of quality control—topics generally covered in the autopsy manuals of most pathology departments. The text is succinct and (perhaps appropriately) rather grave. The coverage of routine procedures is adequate but by no means encyclopedic (eg, evaluation of coronary artery bypass grafts is detailed, but examination of coronary stents is not mentioned). Specialized procedures are explained but only rather summarily (eg, 4 procedures to obtain cerebrospinal fluid are briefly described, but none are illustrated). A few suggested methods would be considered desirable but not particularly feasible (eg, use of an “isolation” autopsy room for biosafety purposes). Some of the baffling legal matters (eg, those relating to consent), bewildering social issues, and enigmatic ethical concerns (eg, organ and tissue donations) are delineated—but it must be realized that the vagaries of local and regional regulations preclude generalizations in these matters. Also outlined, albeit perfunctorily, are the performance, interpretation, and reporting of autopsies on fetuses, infants, and pediatric patients. The atlas portion passably illustrates common findings and a few rather uncommon diseases (eg, testicular infarction in vasculitis). The obligatory congenital abnormalities (eg, conjoined twins) and some of the more common findings in forensic autopsies (including a variety of gunshot wounds) are presented, some in a more resplendent manner than others.

Perhaps the most practical information may be found in the 2 appendices. One appendix offers various templates for the reporting of gross findings, while the other includes tables of expected measures and dimensions and for the assessment of growth and development. The book is not hefty, and portability is further enhanced via access to its entire content online (included in the purchase price).

In a valiant attempt to inspire the harassed autopsy pathologist, each chapter of *Autopsy Pathology* begins with a motivational-type quote.

Syed A. Hoda,
JAMA. 2009;301(22):2391