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# NEWSPath

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## **ANATOMIC PATHOLOGY**

### **Metastatic Placental Site Trophoblastic Tumour in The Duodenum Presenting with a Gastrointestinal Bleed**

**Pai, Sanjay A; Kini, Dinesh; Deo, Ravi P**

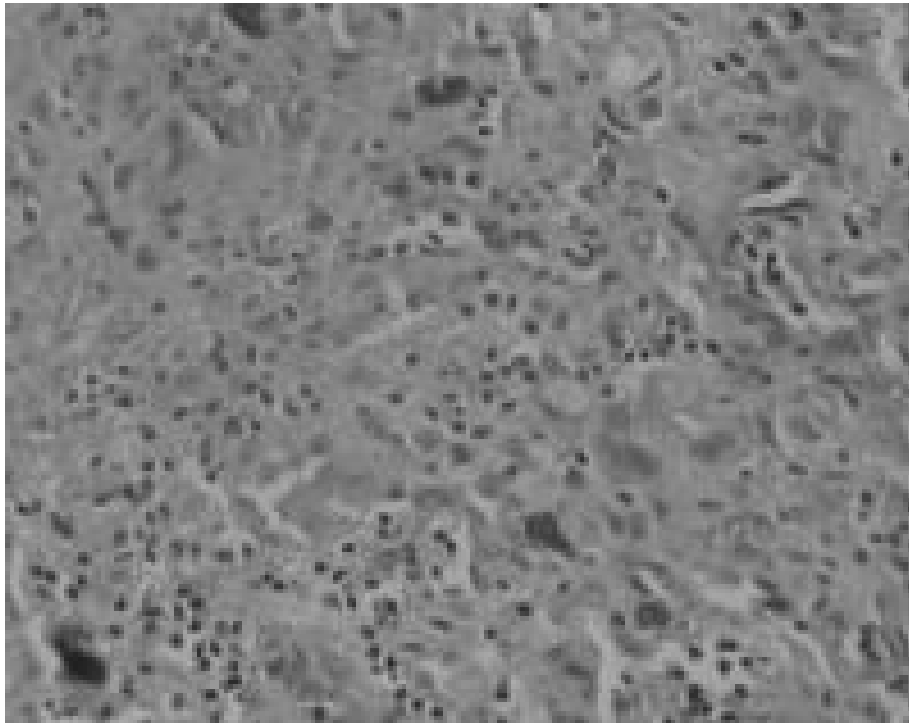
A 31-year-old woman presented with altered bowel habits, black stools and profound weakness. Abdominal ultrasonography performed elsewhere had shown hepatic metastases. Oesophago-gastroduodenoscopy had been reported as normal. A red blood cell labelled blood pool study had shown a slow bleed at the ileocaecal junction, caecum and/or ascending colon. Except for pallor, her general condition was good. There was no supraclavicular adenopathy or ascites. She underwent lower gastrointestinal endoscopy at our hospital and was detected to have a rectal polyp. Because the size and appearance of the polyp did not correlate with the symptoms, the possibility of a separate pathology was considered. Consequently, the patient underwent an upper gastrointestinal endoscopy, which showed friable ulcerated lesions in the third part of the duodenum. The endoscopic differential diagnosis included carcinoma, lymphoma and tuberculosis.

The rectal lesion was a juvenile polyp. The duodenal biopsy specimen showed ulceration with neutrophilic infiltrates. The lamina propria contained a neoplasm composed of confluent masses of polygonal to round cells with large, vesicular nuclei. Some of the cells possessed smudged nuclei; a few multinucleate giant cells were also present. There were moderate amounts of eosinophilic cytoplasm; some cells contained clear cytoplasm. Mitotic figures were scarce. There was no desmoplasia, haemorrhage, necrosis or calcification. The tumour cells expressed cytokeratin (1:50) strongly and diffusely and were negative for CD45 (1:50) and CD30 (1:20) (all antibodies from Dakocytomation, Carpinteria, CA, USA).

Initially, a diagnosis of a high grade undifferentiated carcinoma involving the duodenum was considered. However, the unusual clinical features of a massive gastrointestinal bleed occurring in an otherwise healthy woman and the presence of hepatic metastases without abdominal adenopathy on ultrasonography were contradictory to a histological diagnosis of a high grade epithelial neoplasm. Moreover, there were some unusual morphological features: the bulk of the neoplasm was deep in the lamina propria and there was no dysplasia in the overlying epithelium. Further, the smudged cells raised the possibility of a trophoblastic neoplasm (Figs. 1 and 2). Further investigation showed a serum [beta]-human chorionic gonadotropin ([beta]-HCG) level of 11 000 mIU/ml. The slides were re-evaluated and a diagnosis of placental site trophoblastic tumour (PSTT) was made. The tumour cells expressed [beta]-HCG focally and human placental lactogen diffusely. The patient was re-interviewed and we learnt that she had had an abortion recently. However, she refused further investigation or treatment and died a month later.



**Figure 1. Duodenal neoplasm composed of sheets of polygonal to round cells with vesicular/smudged nuclei.**



**Figure 2. High power view of the tumour cells.**

Primary duodenal choriocarcinoma as seen at autopsy and PSTT involving the duodenum and retroperitoneum in a known case of PSTT have been reported earlier. However, we are unaware of any reports of metastatic PSTT presenting primarily as a duodenal lesion. Choriocarcinoma usually shows haemorrhagic areas, with focal expression of human placental lactogen and strong expression of [beta]-HCG in the tumour cells. PSTTs possess sheets of tumour cells with diffuse human placental lactogen and focal [beta]-HCG expression. The moderate rise in serum [beta]-HCG levels in patients with PSTT, as in our case, correlates with the focal expression of [beta]-HCG in the tumour cells. The cells of epithelioid trophoblastic tumour are smaller than those of a PSTT and form cords and nests but no sheets; moreover, epithelioid trophoblastic tumour cells express human placental lactogen only focally. Differentiating between choriocarcinoma and PSTT is of importance because the former are exquisitely chemosensitive. PSTTs are relatively resistant to chemotherapy, though a few long term remissions to etoposide “methotrexate “actinomycin-D or cisplatin” treatment have been reported.

There are many lessons to be learnt from this unfortunate patient. Pathologists must always consider, especially in an unusual clinical setting, the possibility of a trophoblastic neoplasm, a potentially curable disease, before labeling a tumour as a high grade carcinoma or an incurable disease. For physicians, it underscores the fact that sound clinical judgement followed by good communication with the pathologist is the key to a correct diagnosis. Finally, it is a reminder that it is mandatory to take an obstetric history, even in apparently non-obstetric cases.

*Journal of Clinical Pathology, Volume 60(11), November 2007, pp 1295-1296*

## **Pathology of the Appendix in Children: An Institutional Experience and Review of the Literature**

**Rabah R.**

**BACKGROUND:** The appendix can be affected by a variety of congenital and acquired diseases, but acute appendicitis is the most common pathology found in the pediatric population.

**OBJECTIVE:** This is a retrospective review of all appendectomies performed during a 2-year period at a major children's hospital with a review of the literature regarding the most common pathologic findings.

**MATERIALS AND METHODS:** The pathology database was reviewed for appendectomy specimens, and patient medical records were evaluated to determine the age, gender, race and operative diagnosis. All slides were reviewed and the histologic findings were recorded.

**RESULTS:** A total of 392 appendectomies were performed, including 68 incidental appendectomies and 324 performed for clinical suspicion of acute appendicitis. In 247 of the latter, acute appendicitis was confirmed histologically, and of the remainder 14 were interval appendectomies, 2 had findings suspicious for Crohn disease, 1 confirmed diverticulitis and 60 were histologically negative for appendicitis.

CONCLUSION: Acute appendicitis is the most common pathologic cause of appendectomy, but various other pathologic entities are found in children. Examination of the appendix is warranted even when it appears normal on exploration.

*Pediatr Radiol. 2007 Jan; 37(1):15-20*

## **Typing of Amyloidosis in Renal Biopsies Diagnostic Pitfalls**

**Anjali A. Satoskar; Kelly Burdge; Daniel J. Cowden; Gyongyi M. Nadasdy;  
Lee A. Hebert; Tibor Nadasdy**

*Context.*—Amyloidosis represents a group of diseases with extracellular deposition of congophilic fibrils of similar morphology but differing chemical composition. The types commonly involving the kidney are AL (light chain amyloid) and AA (serum amyloid A). Familial amyloidosis can also affect the kidney, but we have not encountered such a case during the study period. Distinguishing between the AL and AA forms of amyloid is clinically important because of the different treatments and outcomes. The classification of amyloidosis is made by immunostaining with antibodies to  $\kappa$  and  $\lambda$  immunoglobulin light chains and for serum amyloid A protein.

*Objective.*—To draw attention to the nonspecific immunofluorescence staining patterns in renal biopsies with amyloidosis, causing potential diagnostic pitfalls.

*Design.*—Renal biopsies from 15 patients, including 13 cases of AL and 2 cases of AA amyloidosis, were studied. Immunofluorescence staining with routine antibody panel and immunoperoxidase staining for amyloid A were performed.

*Conclusions.*—Immunofluorescence staining for immunoglobulin light chains on renal biopsy, as the first step to differentiate between AL and AA amyloidosis, may sometimes be inconclusive or even misleading. Applying amyloid A immunostain on a routine basis and detailed clinical history are essential to avoid misclassification.

### COMMENT

Although amyloidosis is a relatively uncommon cause of renal disease and proteinuria, the renal pathologist is commonly involved in the diagnosis of amyloidosis. Familial/ hereditary forms of amyloidosis are rare but occasionally may involve the kidney. As indicated in the introduction, clinically relevant renal amyloidosis is overwhelmingly caused by AL or AA amyloidosis. In this study, we focused on AL and AA amyloidosis. More recently, treatment with high-dose melphalan and autologous stem cell transplantation has led to improved outcomes in cases with AL amyloidosis. Treatment of AA amyloidosis is targeted at controlling the underlying medical condition as well as at inhibiting fibril formation (eg, colchicine). Because of the divergent treatment approaches, the differentiation of AL and AA amyloid in a renal biopsy is very important. This is usually not a problem if there is monoclonal spike in serum and urine, monoclonal plasmacytosis in a bone marrow biopsy, and bright immunostaining for the corresponding light chain on renal biopsy tissue. However, sometimes there may be no history of monoclonal spike, and the immunostaining on biopsy may be

inconclusive. The main pitfalls that we have encountered are (1) we do not always see a striking predominance of one light chain over the other in AL am.

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## **The Burden of “Sticky” Amyloid Typing Challenges**

### **Editorial**

Although rare, co-deposition of more than 1 amyloid protein has been reported, either in different compartments of an organ or at the same site. A possible improvement in the diagnosis of AL amyloidosis is the use of antibodies to different subgroups of the immunoglobulin light chains. Similar considerations also pertain to AH, that is, amyloid derived from immunoglobulin heavy chain. Heavychain immunoreactivity in the absence of light-chain staining is considered diagnostic of AH. However, because (similar to the light chains) heavy chains can also be truncated, they may not be detected by commercial antibodies, and in all probability, we miss most of these cases today. Interestingly, other antibody-based techniques, such as ultrastructural immunogold labeling and Western blot, have been reported to yield better results, being both more sensitive and more specific. In the case of the former technique, nonspecific staining and lack of labeling are minimized, with the outcome that more specific and cleaner results are obtained at the ultrastructural level. Many cases that were challenging and confusing at the light microscopic level have been accurately typed using ultrastructural immunogold labeling. Direct typing of the amyloid protein extracted from formalin-fixed, paraffin-embedded specimens has been tried for several years by a handful of laboratories. A more recent approach is the application of proteomics technologies to amyloid typing. Of these methods, tandem mass spectrometry of tissue deposits is the most developed at present, although still essentially restricted to research laboratories. These methods permit precise and full characterization, based on protein sequencing, of the proteins associated with amyloid deposits. Amyloid typing is not a trivial matter, and misinterpretation may have profound consequences. A knowledge of the limitations of immunohistochemistry, and its potential pitfalls, is important. It is essential that the criteria for amyloid-type diagnoses be stringent. To diagnose AL or AH, light- or heavy-chain restriction must be demonstrated by antibody-based or molecular methods. In cases in which this is not evident, a pathologic diagnosis of AL is unwarranted. AL should not be diagnosed “by default” in cases that are inconclusive or negative for light chains because this would result in an inappropriate overdiagnosis of AL amyloidosis, miss the correct amyloid type, and lead to incorrect patient management and therapy. Inconclusive or negative cases should be diagnosed as such and considered for evaluation by reference laboratories that have experience in amyloid typing using a wider antibody panel as well as the capability to apply methods that are more sophisticated.

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## **CYTOPATHOLOGY**

### **Fine-Needle Aspiration Biopsy of High-Grade Sarcoma**

#### **A report of 107 cases**

**Ranleigh Fleshman, Joel Mayerson, Paul E. Wakely Jr**

**BACKGROUND:** To the authors' knowledge, few studies exist demonstrating the reliability of fine-needle aspiration (FNA) biopsy for high-grade sarcoma (HGS).

**METHODS:** In the current study, the authors reviewed their cytopathology database (March 2001 through January 2007) and identified all FNA cases diagnosed as HGS. They also searched their tissue database for all HGS cases that had prior FNA biopsy findings.

**RESULTS :** A total of 107 FNA samples from 98 patients (age range, 13-90 years, with a male:female ratio of 1:1) had an FNA diagnosis of HGS, or had HGS and a prior FNA diagnosis of another entity. Ten cases were nondiagnostic. Of the 97 remaining samples, 6 were diagnosed as something other than HGS (sensitivity of 94%). The positive predictive value of an FNA diagnosis of HGS was 97% (88 of 91 cases). Fifty-four cases were diagnosed as HGS, not otherwise specified, 8 as myxofibrosarcoma, 8 as osteosarcoma, 5 as malignant peripheral nerve sheath tumor, 5 as leiomyosarcoma, 4 as Ewing sarcoma, 4 as liposarcoma, 2 as epithelioid sarcoma, and 1 as angiosarcoma. Approximately 71% of patients presented with a primary tumor, 23% with disease recurrence, and 7% with metastasis. Sites of disease included the lower extremity (59%), upper extremity (19%), trunk (15%), groin (4%), and head and neck (4%). FNA diagnosis was confirmed histologically in 88% of cases, clinically in 7% of cases, and cytogenetically in 1% of cases; 3% of cases had false-positive results and 1 patient was lost to follow-up. Sixteen of 19 patients received neoadjuvant chemotherapy based on the FNA diagnosis alone.

**CONCLUSIONS:** A cytopathologic diagnosis of HGS was found to be accurate in 88 of 97 cases (91%) with follow-up. A FNA biopsy diagnosis of HGS appears to be clinically reliable in a high percentage of cases when used in close conjunction with the orthopedic team.

*Cancer Cytopathology, Volume 111, Issue 6, Pages 491 - 498*

# **MOLECULAR PATHOLOGY**

## **Molecular Pathology in Anatomic Pathology Practice: A Review of Basic Principles**

**Jennifer L. Hunt**

Molecular testing in pathology emerged shortly after polymerase chain reaction became a standard molecular biology assay. Testing efforts began in the clinical laboratories primarily with assays for genetically inherited diseases and assays for clonality in hematologic malignancies. Today, the field has evolved into “molecular diagnostics,” which encompasses testing in almost every area of anatomic pathology. Molecular testing is now even making its way definitively into both surgical pathology and cytopathology, although molecular anatomic pathology is still young with few standard tissue-based molecular assays. As more clinically valuable information is gained from molecular pathology testing of tissues, unique challenges are also becoming apparent at the intersection between tissue diagnosis and DNA diagnosis. This review focuses on basic molecular pathology concepts, with particular emphasis on the challenge of tissue-based testing in anatomic pathology.

### **LABORATORY SETUP**

Setting up a molecular anatomic pathology laboratory requires investments in space, equipment, and personnel. As with any laboratory for clinical testing, the space requirements will depend on the anticipated volume of assays, the specific equipment that will be used, and the number of technicians/technologists that will be performing the assays. There is one general important principle that relates to space that is specific to molecular pathology testing. To reduce the risk of contamination, it is crucial to have a physical separation of the “clean” and “dirty” spaces whenever amplification procedures are used. There are many different configurations for laboratory space, but in the molecular anatomic pathology laboratory, separate space for microdissection should also be included. Clean space refers to all pre-PCR procedures, including microdissection, DNA or RNA extraction, and PCR setup. Dirty space is where post-PCR products (amplicon) are handled. Personnel will need to be trained to keep all reagents, equipment, and protective gear separate for these 2 spaces and not to transport anything from the dirty space back into the clean space. This physical separation of space greatly reduces the risk of contamination.

Equipment needed for molecular anatomic pathology varies based on the type of assays that will be performed. Laboratories may want to invest in a microdissecting platform: either a manual microscope or a laser microdissection system. All standard equipment for PCR and RT-PCR are required, along with some specialized product analysis equipment, depending on assays being performed. Common platforms may include machines for quantitative PCR, a capillary electrophoresis machine for fragment analysis, and a unit for direct sequencing. When budgeting for these items, it is also important to budget for service contracts for maintenance and repair, which can be relatively costly.

Trained personnel for a molecular laboratory are in high demand. Very few specific training programs for technologists in molecular techniques exist. Even in low-volume molecular laboratories, a minimum number of qualified technologists are required to provide adequate turnaround time and

coverage for vacation. Cross training of technologists from other areas has been used successfully in some laboratories. Many institutions do not have a separate laboratory for molecular testing in anatomic pathology, but these assays are part of a larger molecular diagnostics laboratory. It is still important to have dedicated technicians trained to manage tissue samples. The embedded tissues will be somewhat different from other standard operating procedures.

Molecular testing will almost certainly expand dramatically in anatomic pathology during the next decade. More and more clinical laboratories will begin to perform testing using the principles and techniques described in this review. As this occurs, practicing pathologists will need more familiarity with the underlying principles and will also need to be comfortable with the testing applications.

*Archives of Pathology and Laboratory Medicine: Vol. 132, No. 2, pp. 248–260.*

## **CLINICAL PATHOLOGY**

### **Prostate-Specific Antigen-Positive Extramammary Paget's Disease Association With Prostate Cancer**

**Hammer A, Hager H, Steiniche T**

Extramammary Paget's disease (EMPD) is a rare intraepidermal adenocarcinoma that primarily affects the anogenital region. Cases of EMPD reacting with PSA (prostate-specific antigen) have previously been associated with underlying prostate cancer. However, a recent case of EMPD in our department has led us to question the value of PSA as an indicator of underlying prostate cancer. Clinical and pathological data were obtained for 16 cases of EMPD. Formalin-fixed, paraffin-embedded tissue blocks from the primary skin lesions were investigated using PSA and other immunohistochemical markers. 5 of the 16 cases of EMPD stained positive for PSA (2 women and 3 men). However, no reactivity was seen for the prostatic marker P501S. Three of the five patients had been diagnosed with internal malignant disease—two with prostate cancer, stage 1. Immunohistochemical investigations of the tumour specimens from the prostate revealed an immunoprofile which was very different from that of the primary skin lesion. In our study, no cases of EMPD with PSA positivity seem to represent an extension of an underlying prostatic adenocarcinoma. PSA positivity can be seen in cases of EMPD without associated adenocarcinoma of the prostate.

*APMIS Volume 116 Issue 1 Page 81-88, January 2008*

## **MICROBIOLOGY**

### **Real-Time PCR Detects *Pneumocystis Jirovecii* DNA in HIV-Infected Patients**

NEW YORK (Reuters Health) Feb 12 - Rapid molecular identification of *Pneumocystis jirovecii* infection of the lower respiratory tract, using a real-time polymerase chain reaction (PCR) assay, is useful for the rapid diagnosis of *Pneumocystis pneumonia* (PCP) in HIV-positive patients.

A British team, led by Dr. J. F. Huggett of University College London, studied 132 adults with HIV infection who were undergoing diagnostic bronchoscopy. Sixty-one consecutive subjects had 62 PCP episodes, defined by identification of *P. jirovecii* in bronchoalveolar fluid and confirmed by cytochemical staining. The patients all had clinical presentations typical of PCP. A control group consisted of 71 consecutive HIV-infected patients with alternative confirmed diagnoses and no evidence of Pneumocystis infection.

The investigators used PCR to quantify *P. jirovecii* DNA in bronchoalveolar lavage fluid. They employed primers designed to hybridize with the *P. jirovecii* heat shock protein 70 (HSP70) gene.

Dr. Huggett's team compared this assay with conventional PCR targeting the *P. jirovecii* mitochondrial large subunit rRNA gene sequence.

Quantifiable HSP70 DNA was detected in 61 of 62 PCP episodes. It was detectable but below the limit of quantification in the remaining case, the researchers report in the February issue of *Thorax*.

The 71 control patients had 74 episodes of respiratory illness with alternative diagnoses. Quantifiable HSP70 DNA was detectable in 6 of the 74 (8%) episodes. HSP70 was detectable but below the limit of quantification in 34 of 74 cases (46%).

Analysis showed a clinical sensitivity of 98% and specificity of 96% for diagnosis of PCP with the real-time assay for HSP70 DNA. In contrast, there was a clinical sensitivity of 97% and a specificity of 68% using the mitochondrial large subunit rRNA gene sequence.

Dr. Huggett and colleagues conclude, that "the HSP70 real-time PCR assay detects *P. jirovecii* DNA in bronchoalveolar fluid and may have a diagnostic application. Quantification of *P. jirovecii* DNA by real-time PCR may also discriminate between colonization with *P. jirovecii* and infection."

*Thorax 2008; 63:154-159*

## **BOTTOM LINE**

### **Patient Safety and Error Reduction in Surgical Pathology**

**Raouf E. Nakhleh**

*Objective.*—To review issues relevant to patient safety and error reduction in surgical pathology in the context of continuous quality improvement.

*Data Sources.*—The literature is reviewed.

*Conclusions.*—Patient safety goals can and should be addressed within the context of a quality improvement plan. Multiple factors that contribute to errors in surgical pathology are discussed. The current literature defines the extent of these problems within specific segments of the test cycle (preanalytic, analytic, and postanalytic). Potential solutions are presented that may reduce or avoid

errors. In addition, general principles are outlined that enhance the laboratory's ability to successfully and continuously address patient safety and error reduction.

### **DEFINITION**

Patient safety is defined as freedom from accidental injury in the delivery of health care. However, the definition that is currently used further imposes that health care institutions ensure that patient safety involves the establishment of operational systems and processes that minimize the likelihood of errors and maximize the likelihood of intercepting them when they occur. This operational definition highlights how patient safety is an essential and inseparable part of a QA plan and how patient safety and QA need to be an integral part of a laboratory's operations.

### **GENERAL FACTORS THAT LEAD TO ERRORS**

To eliminate errors, there must be a good understanding of how and why errors occur. Spath summarizes the literature and describes how errors occur in medicine. Several of these factors apply directly to surgical pathology, including the following: (1) Variable input; a consistent quality product is dependent on consistent inputs. In surgical pathology, the most important input includes specimen identification and the accompanying clinical information. Both inputs are discussed later as contributors to diagnostic error. (2) Complexity; with every step in a process the risk of error increases. It has been estimated that if there is a 1% error rate at each step, with 25 steps the risk of error goes up to 22%. Surgical pathology has numerous steps in receiving, processing, and reporting a specimen. (3) Inconsistency; errors may occur when there is inconsistency in the level of training, in individual performance, in how procedures are used, in the extent of communication by different individuals, and in the use of language or diagnostic taxonomy. (4) Human intervention; humans do poorly at routine repetitive tasks. They are susceptible to boredom or distraction. Machines on the other hand are best for routine repetitive tasks but tend to have problems with unanticipated situations. (5) Time constraints; batch work and deadlines may force individuals to cut corners or at least work in a hurried mode.

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