

June – July 2010



www.pathoindia.com

NEWSPATH

Dr. S.G. Deodhare

Former Professor of Pathology
Grant Medical College and Dean, J.J. Group of Hospitals, Mumbai

Table of Contents

- Immunohistochemical Classification of Amyloid Deposits in Surgical Pathology
- Diagnostic Accuracy and Limitations of Fine-Needle Aspiration Cytology of Bone and Soft Tissue Lesions: A Review of 1114 Cases with Cytological-Histological Correlation
- Recent Advances in Neuroblastoma
- Autopsy Findings in Eight Patients with Fatal H1n1 Influenza
- Uncommon and Recently Described Renal Carcinomas
- Enteric Fever: Emerging Trends

Immunohistochemical Classification of Amyloid Deposits in Surgical Pathology

BoGun Jang, Youngil Koh and Jeong-Wook Seo

Background and aim: Determining the specific type of amyloid is critical as prognosis and treatment of amyloidosis completely differs according to the type. As aggressive and radical treatments for systemic amyloidosis are newly emerging, accurate typing of amyloid has become more important.

Methods: Immunohistochemistry was performed with four antibodies to major amyloid proteins on tissue microarrays.

Results: Overall, of 49 cases from 39 patients, 32 specimens (65%) were successfully classified by immunohistochemistry (IHC) with clinical information. Immunoglobulin light chain related (AL) amyloid was the most common type, accounting for 49% of all. Among 19 patients with AL amyloid, 7 (37%) had multiple myeloma, while serum amyloid A related (AA) amyloid was found in 8 (16%) specimens, and all patients had chronic inflammatory diseases. In 18 specimens (35%), however, the type of amyloid could not be established due to lack of clinical clue and/or inconclusive immunostaining. Particularly, antibodies to lambda light chain and transthyretin frequently stained amyloid of other origin additionally, therefore complicated the typing of amyloid.

Immunohistochemistry could be applied as useful tools for classification of amyloidosis in most cases and should be carefully interpreted in the light of clinical information.

Amyloidosis encompasses a heterogeneous group of diseases of diverse cause characterized by deposition of extracellular fibrillar proteins in various tissues.¹ These fibrils are generated by a misfolding of normal proteins into insoluble, toxic protein aggregates having a predominantly anti-parallel sheet secondary structure.² The diagnosis of amyloidosis is largely dependent on the histologic demonstration of amyloid deposits in tissue specimens, which could be identified by their apple green birefringence under polarized light after Congo red staining and also by the presence of non-branching fibrils measuring 7.5 to 10 nm in width on electron microscopy.³ Once the diagnosis of amyloidosis is made, the distinct type of the amyloidosis, which is classified according to the amyloid fibril protein that forms the amyloid, should be determined because the prognosis and treatment plan vary greatly as the specific type of amyloidosis.⁴ Moreover, recent therapies of systemic amyloidosis include highly radical and aggressive approaches such as high-dose chemotherapy with autologous stem cell transplantation for immunoglobulin light chain related (AL) amyloidosis and liver transplantation for hereditary transthyretin-associated (ATTR) amyloidosis, which are often accompanied by treatment related mortality. Therefore, the need for definite and accurate amyloid typing has been growing during the last decade. Classification of amyloid is usually based on the immunohistochemistry with an aid of clinical information. To date, however, at least 21 different proteins are known to be able to form amyloid fibrils and additional amyloid

proteins are likely to be discovered in the future. Practically, it is unreasonable and impossible to always investigate all known amyloid fibril proteins for the typing of amyloid. In 2006, Kebbel et al. classified 169 biopsies from 121 patients diagnosed with amyloidosis immunohistochemically. They proposed that a panel of antibodies for immunohistochemical typing of amyloid should contain at least antibodies against immunoglobulin (Ig) light chain, transthyretin (TTR), and serum amyloid A protein (AA) since the majority of amyloid proteins fell into three amyloid types, that is, AL, AA, and ATTR amyloidosis. But also, it has long been recognized that there are some limitations and pitfalls concerning the immunohistochemistry for the typing of amyloid deposits. In particular, differential diagnosis of AL amyloidosis with rare hereditary amyloidosis is on occasion seriously challenging for surgical pathologists. In the present study, we reviewed all biopsy and resection specimens which were diagnosed as amyloidosis over the last 10 years in our hospital, and evaluated the applicability of immunohistochemistry for the classification of amyloid.

MATERIALS AND METHODS

Patients

This study included 44 patients diagnosed as amyloidosis between July 1999 and June 2008 at the Department of Pathology of the Seoul National University of Hospital. A total of 54 amyloid deposits in 47 biopsies, two excisions and five resection specimens were examined. Specimens were obtained from 44 patients, 17 women and 27 men (women: men ratio, 1:1.6). The mean patient age was 58 years (range, 35–77 years).

Construction of tissue microarrays

In each case, all specimens were formalin fixed and paraffin embedded. Amyloid deposits were demonstrated by apple green birefringence under polarized light after Congo red staining. A slide with representative amyloid deposits was selected from each case, and coupled to the corresponding formalin-fixed paraffin-embedded block. In 49 out of 54 cases, duplicate 2-mm cores could be taken from the specimen (donor blocks) and inserted into new recipient paraffin blocks (tissue array blocks) using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). Two tissue array blocks were constructed and they contained 54 and 49 cores respectively.

Immunohistochemistry

Immunohistochemical staining was performed with a panel of antibodies against four major amyloid fibril proteins, which included serum AA protein, kappa light chain, lambda light chain and transthyretin (TTR). And, antibody to amyloid P (AP) component was added to the panel of antibodies (Table 1). All antibodies were purchased from Dako (Glostrup, Denmark). For antigen retrieval of AA, AP component and TTR, the specimens were pretreated with microwave antigen retrieval procedure in 10 mM ethylenediaminetetraacetic acid (EDTA) buffer for 15 min. For kappa light chain, 10 mM citrate buffer (pH 6.0) was used instead, but antibody to lambda light chain did not

require antigen retrieval. After incubation in 0.3% hydrogen peroxide for 20 min, immunostaining was carried out by an automated immunostainer, the TechMate 500 plus (Dako) with antibodies to kappa light chain (1:50), lambda light chain (1:20.000), AA (1:100), TTR (1:400) and AP component (1:300) respectively. To visualize the immunostaining, avidin biotin complex method applying a cap-plus detection kit (Invitrogen, Carlsbad, CA, USA) was used, and diaminobenzidine (DAB) served as a chromogene. Finally, they were counterstained with Meyer's hematoxylin.

Table 1 Antibodies used for classification of amyloid

Antibody	Species	Monoclonal/polyclonal	Dilution	Pretreatment
kappa	mouse	monoclonal	1:50	EDTA
lambda	rabbit	polyclonal	1:20.000	None
amyloid A	mouse	monoclonal	1:100	EDTA
transthyretin	rabbit	polyclonal	1:400	EDTA
amyloid P component	rabbit	polyclonal	1:300	EDTA

EDTA, ethylenediaminetetraacetic acid

The diffuse and strong immunoreactivity only was considered to be positive. Weak or focal staining was regarded as nonspecific reaction. The decision of staining intensity was based on the intensity of the plasma present in the luminal space of small vessels. Because all of the amyloid fibril proteins which we aimed to detect in this study were derived from normal plasma proteins, it is natural to find strong stain in the luminal space of small vessels in the tissue. Also, in the case of antibodies to kappa and lambda light chains, the plasma cells present in interstitium could be an internal control.

Determination of AL, AA and ATTR amyloid

When amyloid of a patient with monoclonal gammopathy which was proven by serum immunoglobulin electrophoresis (IEP) was positive for corresponding light chain by IHC, the amyloid was classified as AL amyloid irrespective of immunoreactivity for other amyloid proteins. Likewise, amyloid of a patient with chronic infection or inflammatory disease was categorized into AA amyloid if the amyloid was positive for antibody to amyloid A regardless of immunostain for other amyloid proteins. When amyloid of a patient with family history of amyloidosis was immunoreactive for transthyretin only, it was diagnosed as ATTR amyloid.

RESULTS

Forty nine specimens were obtained from 17 different organs or tissues of 39 patients. Thirty patients had single specimen, eight patients had two specimens, and three specimens were available from one patient. Finally, amyloid deposits in 32 (65%) of 49 specimens (24 of 39 patients) were conclusively classified by immunohistochemical

staining with an informative clinical data. Most common amyloid type was the AL amyloid, which accounted for 49% (24 of 49 specimens) of all specimens examined, followed by AA amyloid (16%, 8 of 49). Of the total 49 obtained tissues, eight (16%) were derived from stomach and rectum, followed by heart (14%), colon (10%), and duodenum (8%). Overall, a half of tissues (53%) examined in this study were originated from the gastrointestinal (GI) tract and among extra-GI tract organ, most common biopsy site was heart (Table 2).

Table 2 Biopsy sites and amyloid type

	N (%)	AL (n)	AA (n)	pAL (n)	ATTR (n)	Undetermined (n)
Gastrointestinal tract	26 (53)	16	8	1	0	1
Stomach	8	5	3	0	0	0
Duodenum	4	2	1	0	0	1
Small intestine	1	1	0	0	0	0
Colon	5	4	1	0	0	0
Rectum	8	4	3	1	0	0
Heart	7 (14)	3	0	2	0	2
Respiratory tract	5	0	0	2	0	3
Lung	2	0	0	1	0	1
Larynx	2	0	0	0	0	2
Liver	1	0	0	1	0	0
Soft tissue [§]	2	2	0	0	0	0
Oral cavity [¶]	2	1	0	0	0	1
Muscle	2	1	0	0	0	1
Urinary bladder	1	0	0	0	0	1
Eyelid	1	0	0	0	0	1
Cornea	1	0	0	0	0	1
Conjunctiva	1	0	0	0	0	1
Lymph node	1	1	0	0	0	0
Total	49 (100%)	24	8	5	0	12

AA, amyloid A; AL, immunoglobulin light chain related amyloid; ATTR, transthyretin-associated amyloid; pAL, probably AL. §The location of soft tissue included shoulder and submandibular area. ¶Oral cavity included gingiva and lip.

AL amyloidosis and probably AL amyloidosis

AL amyloidosis was diagnosed in 24 (49%) of 49 specimens (19 of 39 patients). The mean age of the 19 patients at the time of diagnosis was 60 years (range, 35–77 years). All patients had clinical history or laboratory data related with the monoclonal Ig overproduction: A total of seven (37%) out of 19 patients had overt multiple myeloma and two patients had monoclonal plasma cell disorder associated with POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy and Skin change) syndrome (Fig. 1). Another two patients had excess IgM production which was caused by Waldenstrom's macroglobulinemia and Castleman's lymphadenopathy of plasma cell type respectively. Although remaining eight patients had no evident clinical diseases which belong to plasma cell dyscrasia with broad spectrum, monoclonal light chain was detected in serum or urine by immunoglobulin electrophoresis (IEP). The multiorgan involvement was common and observed in 13 (65%) patients. The most commonly affected organ was the heart (42%), followed by stomach (32%), and kidney (32%).

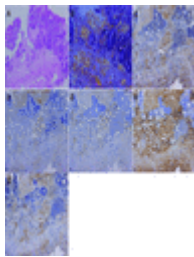


Figure 1 Immunoglobulin light chain related (AL) amyloidosis (lambda type) in the duodenum of the patient with POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy and Skin change) syndrome: Amorphous material deposited in the mucosa and submucosa (A) showing apple green birefringence after Congo red staining (B) and positive immunostain for amyloid P component (C).

Immunohistochemistry revealed weak and focal stain for amyloid A (D), kappa (E), and transthyretin (F), whereas diffuse and strong stain for lambda (G).

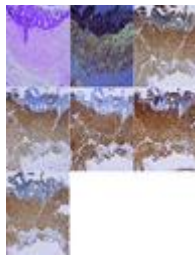


Figure 2 Multiple stain of immunoglobulin light chain related (AL) amyloid (kappa type) of a patient with multiple myeloma: Hematoxylin and eosin stain (A), Congo red stain (B) and immunostain for amyloid P component (C). Although the amyloid was definitely positive for kappa light chain (E), it also exhibited unequivocal stain for all the other amyloid proteins such as amyloid A (D), lambda (F), and transthyretin (G).

Amyloid P (AP) component

All amyloid deposits were stained with the antibody against AP component regardless of its amyloid fibril protein type and location of deposition. Compared to Congo red stain, immunostain for AP component tended to be more diffuse and homogenous, which made small amyloid deposit easily identifiable and overall extent of amyloid in the specimen evaluable.

DISCUSSION

Immunohistochemistry (IHC) with a panel of antibodies against various amyloid fibril proteins has been used most widely as a method for typing of amyloid. Numerous reports have validated the applicability of IHC for the purpose, and they reported a wide range of success rate in immunohistochemical typing of amyloid, ranging from 38 to 100%. Generally, the overwhelming majority of amyloid deposits are divided into three most common types, which include AL, AA, and ATTR amyloid, and they should be managed by distinct therapeutic strategies respectively. Therefore, we used a panel of antibodies which aimed to detect the three amyloid types. Antibody against amyloid P component was additionally included in a panel to localize the amyloid deposit in the specimen. In this study, we could classify the amyloid deposits as AL or AA amyloidosis in 32 (65%) out of 49 specimens based on the immunohistochemical findings and clinical and laboratory information. However, 35% of all cases (17 of 49 cases) remained inconclusive state demanding further studies for correct classification of amyloid.

Of the cases successfully classified immunohistochemically, AL amyloidosis was the most common type, accounting for 49% of all cases, and AA amyloidosis was diagnosed in 16%. These figures are consistent with the prevalence of amyloidosis in the developed world. Compared to other published reports, it was surprising to find that none of ATTR amyloidosis was identified in our cases. ATTR amyloidosis was initially thought to be limited to some geographic areas such as Portugal, Sweden, and Japan, but it is now believed to occur throughout the world. Considering a fact that ATTR amyloidosis caused by mutant transthyretin is not only the most common hereditary amyloidosis but also the second most common type of amyloidosis in heart and peripheral nerve biopsies, the absence of ATTR amyloid is hard to explain even though no family history was noted in our patients. In the study by Kebbel et al., ATTR amyloid accounted for even 12% of the 169 biopsies of German people diagnosed as amyloidosis. Thus, it seems likely that ATTR amyloidosis was unrecognized than being really absent in our cases.

As expected, the diagnosis of AA amyloidosis was straightforward in the cases where the patient had long standing chronic inflammatory disease and corresponding stains for amyloid A were definitely positive. On the other hand, the diagnosis of AL amyloidosis was sometimes very challenging especially due to non-restrictive staining patterns of amyloid with antibodies to light chains. In particular, when those patients had no evidence of monoclonal gammopathy with one light chain type, it was very difficult to determine the type of amyloid. Therefore, those cases were mostly referred to as probably AL amyloidosis or undetermined type in this study. Some true AL amyloids might be

included among them because it has been documented that monoclonal Ig or light chains cannot be detected by means of immunofixation electrophoresis in 10% of patients with AL amyloidosis and some of those patients present plasma cell dyscrasia during follow-up. However, without clinical data supporting the monoclonal gammopathy, the diagnosis of AL amyloidosis is actually impossible solely depending on the immunohistochemical finding in the equivocal cases, and this problem largely stems from the fact that amyloid of nonimmunoglobulin origin often can be stained with antibodies to light chains. In 2007, Anjali et al. designated such a nonspecific binding activity of amyloid to immunoglobulin components in the serum as "sticky" property of amyloid.

It has been also demonstrated that there was no specific immunostaining of amyloid deposits with antibodies to kappa or lambda light chains in almost 195 (62%) of 316 patients with AL amyloidosis. Such a nonspecificity of antibodies to light chains has long been an obstacle in determining the type of amyloid. And, generally, it has been explained by the contamination of amyloid by serum proteins during amyloid for although it should be further elucidated whether the contamination results from truly nonspecific affinity of amyloid to immunoglobulins or a selective interaction. In our study, antibody to lambda light chain was found to stain nine of 16 amyloids of non-lambda light chain origin (44% of specificity), and such a low specificity seriously complicated the immunohistochemical typing of AL amyloid as well as other types of amyloids. And, this problem was not confined to the antibodies to lambda light chain only because antibodies to transthyretin and amyloid A also showed 53% and 67% of specificity respectively in this study. Overall, we observed that amyloid deposits showing multiple staining patterns were reaching up to 51% (25 of 49 specimens) of all amyloids. To escape this pitfall of IHC, it has been suggested that immunofluorescence should be the method of choice for amyloid typing, as the background contamination with serum proteins is much less in frozen sections. However, antibody to kappa light chain showed highly specific immunostain, whose specificity was amazingly 96%. This finding could not be explained by the nonselective contamination of amyloid by serum proteins containing kappa as well as lambda light chains. Therefore, it is more likely that somewhat specific interaction between amyloid fibril proteins and distinct serum proteins occurs during amyloid formation even though the exact mechanism remains unknown. Anyway, in the cases of amyloid with multiple staining, IHC could not help to recognize which protein is the real fibril protein to form amyloid and which is the second or contaminating protein. Until now, clinical and laboratory information is the most important factor to decide the type of amyloid in those cases. However, in rare cases where patients have multiple diseases that can cause systemic amyloidosis of different origin or informative clinical data are not available, more advanced tools such as proteomic technology may be necessary to unravel the exact chemical nature of amyloid and reach the definite diagnosis.

To make matters more complicated, it has been reported that misdiagnosis of hereditary amyloidosis as AL amyloidosis occurred in 34 (10%) of 350 patients of presumptive AL amyloidosis. Conversely, in 8 of those 34 patients with a genetic mutation in an amyloidogenic protein, monoclonal immunoglobulins were detected in the serum. Monoclonal gammopathy of undetermined significance (MGUS) is known to be found in

about 3% of elderly people aged 50 years and older, which means that evidence of monoclonal gammopathy does not guarantee the diagnosis of AL amyloid especially in old age. These findings indicate that the differential diagnosis of AL amyloidosis with hereditary systemic amyloidosis on occasion may be very difficult unless ancillary studies such as DNA sequencing or proteomics techniques are undergone. Therefore, it is mandatory to consider another type of amyloidosis and carry out further molecular studies when definite diagnosis of AL amyloidosis is suspected due to ambiguous immunostain results. Even in that case, Immunohistochemical analysis would play a role to guide the direction of further diagnostic approach. For example, if amyloid deposits with nonrestrictive immunostaining for light chains is also strongly stained for TTR, genetic analysis targeting transthyretin should be applied even though the patient has a clear-cut evidence of monoclonal gammopathy.

It was interesting to find that the location of amyloid deposit of undetermined type tended to be unusual, such as lip, larynx, eyelid, cornea, conjunctiva, and urinary bladder (Table 2). The amyloidosis in these sites is known to usually occur as localized form, which is associated with the lack of systemic disease. Moreover, amyloid deposits in those unusual sites have been reported to be formed by very rare amyloid proteins. For example, ocular amyloidosis could be caused by gelsolin, lactoferrin, and keratoepithelin. Therefore, if we cover antibodies against those amyloid proteins, more classification of amyloid might be possible.

Amyloid P component, the normal plasma glycoprotein, is universally present in amyloid deposits, which binds to amyloid fibrils and protects them from degradation by phagocytic cells and proteolytic enzymes. In addition, AP component in amyloid deposits persists for a long time with completely unmodified state. Therefore, diagnostic and therapeutic use of AP component has been extensively investigated. Consistent with the previous studies, we also observed immunostain for AP component in all amyloids in our study. Immunohistochemical staining for AP component revealed not only very tiny foci of amyloid deposit but also even unrecognized deposit, which made the analysis of immunostain much more convenient. This advantage in sensitivity of immunohistochemistry could be very useful in the diagnostic process and maybe replace the Congo red staining and electron microscopy. Already, some reports have shown that non-amyloid deposits in light chain deposition disease, which is distinguishable from amyloidosis only by Congo red staining or electron microscopy, contains no AP component contrast to amyloid deposit. However, further studies that include a large number of cases are required for its diagnostic validation.

In conclusion, we could successfully classify amyloids in 64% of cases diagnosed as amyloidosis by immunohistochemistry with a panel of antibodies against major amyloid fibril proteins including AA, AL and TTR. Most common type was the AL amyloid, accounting for 48% of all cases. Although a substantial number of amyloids remained ambiguous or unknown type, immunohistochemical analysis can give definite or important information about the amyloid deposits in most cases. Therefore, as a component of the multidisciplinary approach for the correct typing of amyloid,

immunohistochemical staining should be carefully interpreted, and in the cases suspected as hereditary amyloidosis gene sequencing should be performed.

*Basic and Applied Pathology, Volume 2, Issue 1, Pages 1-8,
Published Online: 29 Mar 2009*

Diagnostic Accuracy and Limitations of Fine-Needle Aspiration Cytology of Bone and Soft Tissue Lesions: A Review of 1114 Cases with Cytological-Histological Correlation

Khalbuss WE, Teot LA, Monaco SE.

BACKGROUND: Fine-needle aspiration (FNA) cytology is increasingly being used as a diagnostic modality for soft tissue and bone lesions. These diagnoses can be challenging because of a variety of factors, including interpretation and sampling issues. This study investigates the diagnostic utility of FNA biopsy, in addition to the diagnostic pitfalls, in soft tissue and bone cytopathology.

METHODS: We retrospectively reviewed the soft tissue and bone FNAs over a 4-year period (2004-2008), along with available ancillary studies, pathological follow-up, and clinical data. The cases with a cytologic-histologic discrepancy were then reviewed.

RESULTS: A total of 1114 soft tissue and bone FNAs were identified. Of the 1114 aspirates, 525 (47%) were positive for malignant cells, 505 (45.5%) were benign aspirates (including 189 benign lesions/neoplasms), 37 (3.5%) were inadequate, 34 (3%) had atypical cells, and 13 (1%) were suspicious for malignancy. Of the 586 cases (53%) with follow-up, including 445 cases with histological follow-up and 141 with ancillary studies, the overall sensitivity was 96%, the specificity was 98%, the positive predictive value was 99%, and the negative predictive value was 92%. A total of 15 false negatives and 3 false positives were identified with errors because of sampling (9 cases), interpretation (7 cases), and screening (2 cases).

CONCLUSIONS: This large series demonstrates that there can be a high sensitivity and specificity in diagnosing bone and soft tissue lesions by FNA. Our data supports prior studies in the literature in showing that FNA cytology can be a valuable method for diagnosing these lesions.

Cancer Cytopathol. 2010 Jan 20. [Epub ahead of print]

Recent Advances in Neuroblastoma

Maris, John M.

Neuroblastoma is an embryonal tumor of the autonomic nervous system, meaning that the cell of origin is thought to be a developing and incompletely committed precursor cell derived from neural-crest tissues. As may be expected with a disease of developing tissues, neuroblastomas generally occur in very young children; the median age at diagnosis is 17 months. The tumors arise in tissues of the sympathetic nervous system, typically in the adrenal medulla or paraspinal ganglia, and thus can present as mass lesions in the neck, chest, abdomen, or pelvis. The clinical presentation is highly variable, ranging from a mass that causes no symptoms to a primary tumor that causes critical illness as a result of local invasion, widely disseminated disease, or both. The incidence of neuroblastoma is 10.2 cases per million children under 15 years of age; it is the most common cancer diagnosed during the first year of life.

For over a century, researchers have noted that neuroblastomas exhibit diverse and often dramatic clinical behaviors. On the one hand, neuroblastoma accounts for disproportionate morbidity and mortality among the cancers of childhood; on the other hand, it is associated with one of the highest proportions of spontaneous and complete regression of all human cancers. Outcomes in patients with neuroblastoma have improved, with 5-year survival rates increasing from 52% during the period from 1975 through 1977 to 74% during the period from 1999 through 2005, according to the Surveillance. This improvement, however, is attributable mainly to increased cure rates among patients with the more benign form of the disease; the rates among children with high-risk neuroblastoma have shown only modest improvement, despite dramatic escalations in the intensity of therapy provided.

Neuroblastoma may be considered a malignant manifestation of aberrant sympathetic nervous system development. Until recently, however, little was known about the genetic basis of this disease. As has been shown for many human cancers, a subgroup of cases display autosomal dominant inheritance. Mossé and colleagues recently reported that activating mutations in the tyrosine kinase domain of the anaplastic lymphoma kinase (ALK) oncogene account for most cases of hereditary neuroblastoma. These germ-line mutations encode for single-base substitutions in key regions of the kinase domain and result in constitutive activation of the kinase and a premalignant state. Mutations resulting in oncogene activation are also somatically acquired in 5 to 15% of neuroblastomas. Children with either sporadic or familial neuroblastoma in conjunction with congenital central hypoventilation syndrome, Hirschsprung's disease, or both usually have loss-of-function mutations in the homeobox gene PHOX2B. Thus, genetic testing for mutations in ALK and PHOX2B should be considered whenever a patient has a family history of neuroblastoma or has other clinical conditions that are strongly suggestive of a highly penetrant transmissible mutation, such as bilateral primary tumors of the adrenal glands. Such testing is currently available to practitioners though ALK and PHOX2B mutations account for the majority of familial cases of neuroblastoma, additional familial genes may still be discovered.

In sporadic neuroblastoma cases, malignant transformation probably arises from the interaction of common DNA variants in which each individual variation has a relatively modest effect on susceptibility. A genomewide association study of neuroblastoma is currently under way, under the auspices of the Children's Oncology Group (COG). To date, the study has shown that alleles with common single-nucleotide-polymorphism variations within the putative genes FLJ22536 at chromosome band 6p22.3 and BARD1 (BRCA1-associated RING domain 1) at 2q35 are significantly enriched among patients in whom neuroblastoma has developed as compared with controls. In addition, the study has also shown that a relatively common copy-number variation at 1q21 is associated with the development of neuroblastoma. Taken together, these observations suggest that this developmental childhood cancer is influenced by common DNA variations, facilitating the development of a putative genetic model for this disease

The New England Journal of Medicine, Volume 362(23), 10 June 2010, p 2202–2211

Autopsy Findings in Eight Patients with Fatal H1N1 Influenza

Schmidt LA, Smith LB, et al

A novel H1N1 influenza A virus emerged in April 2009, and rapidly reached pandemic proportions. We report a retrospective observational case study of pathologic findings in 8 patients with fatal novel H1N1 infection at the University of Michigan Health Systems (Ann Arbor) compared with 8 age-, sex-, body mass index-, and treatment-matched control subjects. Diffuse alveolar damage (DAD) in acute and organizing phases affected all patients with influenza and was accompanied by acute bronchopneumonia in 6 patients. Organizing DAD with established fibrosis was present in 1 patient with preexisting granulomatous lung disease. Only 50% of control subjects had DAD. Peripheral pulmonary vascular thrombosis occurred in 5 of 8 patients with influenza and 3 of 8 control subjects. Cytophagocytosis was seen in all influenza-related cases. The autopsy findings in our patients with novel H1N1 influenza resemble other influenza virus infections with the exception of prominent thrombosis and hemophagocytosis. The possibility of hemophagocytic syndrome should be investigated in severely ill patients with H1N1 infection.

Am J Clin Pathol. 2010 Jul;134(1):27-35

Uncommon and Recently Described Renal Carcinomas

Srigley JR, Delahunt B.

Major consensus conferences held over a decade ago laid the foundations for the current (2004) WHO classification of renal carcinoma. Clear cell, papillary and chromophobe carcinomas account for 85-90% carcinomas seen in routine practice. The remaining 10-15% of carcinomas consist of rare sporadic and hereditary tumors, some of which had

been long recognized, but many of which only emerged as distinct entities in the decade leading up to the WHO publication. Collecting-duct carcinoma is a rare, often lethal form of carcinoma. Medullary carcinoma associated with sickle cell trait, has emerged as a distinctive tumor showing some overlapping features with upper tract urothelial carcinoma. Mucinous tubular and spindle-cell carcinoma and tubulocystic carcinoma were earlier considered as patterns of low-grade collecting-duct carcinoma, but are now recognized as separate tumor entities. Carcinomas associated with somatic translocations of TFE3 and TFEB comprise a significant proportion of pediatric renal carcinomas. Oncocytoid renal carcinomas in neuroblastoma survivors was recognized as a unique tumor category in the WHO classification. Renal carcinoma associated with end-stage renal disease is now recognized as having distinct morphological patterns and behavior. In addition there is a group of rare recently described carcinomas, including clear cell papillary carcinoma, oncocytic papillary renal cell carcinoma, follicular renal carcinoma and leiomyomatous renal cell carcinoma. It behooves the surgical pathologist to not only be capable of diagnosing the common forms of renal cancer, but also to be aware of the rare types of renal carcinoma, many of which have emerged in recent years.

Mod Pathol. 2009 Jun;22 Suppl 2:S2-S23.

Enteric Fever: Emerging Trends

Brig AK Nagpal, Lt Col A Jairam

Enteric fever is still regarded as a major public health problem globally. Worldwide there were an estimated 22 million cases of enteric fever, with 2,00,000 deaths in 2002. The incidence is highest (>100 cases per 1,00,000 population per year) in South Central and South East Asia. It is estimated that there is one case of paratyphoid fever for every four cases of typhoid fever, but the incidence of infection associated with *S paratyphi A* appears to be increasing, especially in India. Gupta et al, from Bangalore report that 20% of their 81 cases of culture positive Enteric fever were due to *S paratyphi*. Another study of 119 culture positive cases from Mumbai shows that 39% of their cases were of *S paratyphi* infection. Traditionally, it has been believed that enteric fever has a lower incidence and a benign course in preschool children. However, recent population based studies in South Asia show that the incidence is highest in children less than five years of age with higher rates of complications and hospitalization. Enteric fever is a potentially lethal disease but its course can be altered favourably if appropriate antibiotics are exhibited early in the illness. However, given the non specific nature of presentation there are no discerning clinical clues which aid the physician. One can only resort to a high index of suspicion. In the tropics, the presentation is indistinguishable from malaria, viral hepatitis, bacterial enteritis, dengue, rickettsial fever, leptospirosis and amoebic liver abscess. Given the name Enteric Fever, one would expect an illness dominated by gastro intestinal manifestations. However, abdominal pain (30-40%), vomiting (18%), diarrhea (22-28%) and constipation (13-16%) do not constitute the hall mark features of the disease. Constipation was not encountered at all in the Mumbai series of 119 cases. The oft quoted relative bradycardia is an inconsistent finding. However, two thirds of the 81

cases reported in this issue by Gupta et al., had relative bradycardia. The classic mode of presentation with as low and “step ladder” rise in fever is seldom seen and “Rose Red” spots are elusive in Asians. The incidence of splenomegaly is variably quoted. The Kathmandu group found splenomegaly in 5-6% of their 669 cases while the Mumbai study encountered splenomegaly alone or as part of hepatosplenomegaly in 20% of the cases. Gupta et al, however report a much higher occurrence of splenomegaly to the tune of almost 60%. It can well be concluded that there is nothing typical about the presentation of enteric fever and the threshold for considering the diagnosis has to be low. To make matters worse, a “hepatic” presentation maybe encountered in 4.8 to 8.6% of the cases. How far does the laboratory come to the rescue? Leucopenia or a normal leucocyte count is the norm in enteric fever but leucocytosis is common in children and in those with complications. However, the presence of leucocytosis casts a doubt on the diagnosis of typhoid fever. Leucocytosis was seen only in 4 out of 119 culture positive cases of the Mumbai study. The same study found absolute eosinopenia (AEC=0) in 77% of the cases and the authors propose this to be a useful marker of enteric fever. The Widal test lacks sensitivity and specificity and at best is a rough screening tool. The “Gold Standard” for diagnosis is culture positivity. The Mumbai study, found cultures positive in 52.6% of their cases in whom “Enteric Fever” was the diagnosis at discharge. It was heartening to observe that a substantial (46.2%) number of cases which were culture positive had received prior antibiotics. Therefore, antecedent antibiotic therapy should not dissuade a physician from sending cultures. Bone marrow cultures are said to retain a high sensitivity (90%) despite antibiotic therapy but the obvious constraints are that the test is invasive and more applicable to the hospital setting. Culture of intestinal secretions (best obtained by a noninvasive duodenal string test) can be positive despite a negative bone marrow culture. It would be interesting to extrapolate this to endoscopically obtained duodenal secretions at least in the centers where endoscopy is available and see if a relatively non invasive method for culture becomes available. Newer serological diagnostic techniques such as Typhidot, Typhidot M and Tubex detect the IgM response and are stated to have good sensitivity and specificity but have not proved to be robust enough in the community setting. A high incidence of nalidixic acid resistant *Salmonellatyphi* (NARST) isolates is reported in the study by Gupta et al and the study by Jog et al, viz 86% and 79% respectively. In therapeutic terms, this portends fluoroquinolone resistance. Fortunately, resistance to third generation cephalosporins is not an issue at present, though a substantial relapse rate with ceftriaxone is an area of concern. Oral azithromycin is an exciting prospect that gives cure rates which are better than quinolones and comparable to ceftriaxone, with the added advantage of lower relapse rates. Salmonella infection has challenged physicians since the times of Aristotle and still continues to do so today. Diagnosis can be elusive since the disease can be protean in its manifestations. The third world physician would do well to consider enteric fever in all the short term febrile illnesses that he encounters. Multi drug resistance is an area of concern but thankfully the pharmacological armamentarium has kept pace. Above all, it is the early exhibition of an appropriate antibiotic which will salvage many a patient.

MJAFI 2009; 65: 298-299