1649 Patch Tissue Microarray (TMA): A Novel Technique for Tma Construction Using Pre-Existing Slides as a Source of Tissue When Paraffin Blocks Are Unavailable

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Background: TMA technology achieves miniaturization of samples permitting entire studies to be performed on single slides. This is increasingly becoming the preferred platform for tissue based research studies. TMA construction however has required access to the paraffin block to enable needle core extraction from the tissue. Institutions that treat referral patients receive consultation slides from many different hospitals and laboratories, but as they do not have possession of the original paraffin block are precluded from including these cases in research studies. We describe a technique for construction of TMA slides based on transfer of tissue from pre-existing routine slides instead of from the original paraffin blocks.

Design: As proof of this technique, we constructed a 20 core prostate cancer TMA from radical prostatectomy slides instead of from paraffin blocks. Briefly, this technique entails removal of the coverslip on each slide and reinforcement of the tissue by covering with Mount-Quick liquid mounting medium. Once the medium has hardened, the coverslip with attached tissue is punched using a tissue microarray needle. The removed biopsy disks are arrayed on an adhesive tape and then transferred and adhered to a recipient slide. For this technique to be useful, we wished to demonstrate that morphology was not compromised and that the tissue was sufficiently adherent to the slide to enable immunohistochemical staining. We therefore subjected a patch TMA with a traditional TMA on the same slide to immunohistochemical staining (34BE12) with antigen retrieval, and compared for expression of antigen and loss of cores.

Results: After immunohistochemical staining, morphology was equally preserved on both types of TMA with only slightly greater tissue loss on the patch TMA (19 of 20 cores intact on the traditional TMA compared with 16 of 20 cores intact on patch TMA). Expression of the immunohistochemical marker (34BE12) was equally specific on both TMAs.

Conclusions: Patch TMA represents a viable solution for TMA construction when paraffin blocks are unavailable. This technique, although laborious and with a limited output, is still valuable in allowing use of material for studies which otherwise would not be possible in the absence of paraffin embedded tissue. This may prove a useful technique at referral institutions where slides sent for review are the only tissue available.

Ultrastructural

1650 Electron Microscopic Findings in Skin Biopsies from Patients with Infantile Osteopetrosis and Neuronal Storage Disease

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Background: Infantile osteopetrosis with neuronal storage is a rare lysosomal disease with early clinical manifestations that resemble some other lysosomal diseases. To date 51 different lysosomal diseases have been identified. Electron microscopic examination of skin biopsy is a cost effective screening tool for diagnosis of lysosomal storage diseases (J Child Neuro 11:301,1996). Skin biopsies from patients with infantile osteopetrosis with neuronal storage have not previously been described.

Design: Skin biopsies obtained from 1 month affected boy and his twin sister their family has a history of severe infantile autosomal recessive osteopetrosis. A biopsy was also obtained from 3 and a half months old boy. Molecular biology studies revealed unique mutations of *OSTM1* gene in each of the boys. The biopsies were fixed in Trumps fixative, post-fixed with osmium tetroxide, embedded in Epon, cut and stained with uranyl acetate and led citrate, and examined with Phillips EM 201.

Results: The skin biopsies of the normal and affected children contains epidermis, vascular endothelium, pericytes, fibroblasts, eccrine gland ducts, mast cells, macrophage, smooth muscle cells, nerves with myelinated and unmyelinated axons, Schwanns cell and perineural cells. The skin morphology of the twin sister appears to be normal. The biopsies of both boys revealed secondary lysosomes containing lipofuscin in Schwanns cells and endothelial cells, swollen axons, irregular thin myelinated sheaths and unmyelinated axons that contains spheroid inclusions.

Conclusions: The morphological changes seen in skin biopsies of infants with infantile osteopetrosis with neuronal storage due to mutation in *OSTM1* gene are unique and are different fromthose previously reported in skin biopsies of patients with other lysosomal storage disease.

1651 Intraneural Perineurioma: Meta-Analysis with Illustrative Cases

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Background: Intraneural perineurioma (INP) may be confused with other "onion-bulb" Schwann cell entities (localized hypertrophic neuropathy-LHN, reactive/demyelinating processes, or inherited polyneuropathies of Charcot-Marie-Tooth/Dejerine Sottas) due to similar clinical, radiologic, and histologic features. The nerve is expanded by concentric whorls of spindle-shaped perineurial or Schwann cells, which can only be differentiated by ultrastructure (US) and immunohistochemsitry (IHC).

Design: Meta-analysis was performed on definitive INPs obtained via Medline. Inclusion criteria were a) nerve expansion by concentric whorls of spindle-shaped perineurial cells with thin elongated eosinophilic, cytoplasmic processes; b) epithelial membrane antigen (EMA) positive, S-100 protein negative; and/or c) US confirmed perineurial lineage (thin cytoplasmic processes, incomplete basal lamina, poorly formed tight junctions, pinocytotic vesicles). Baylor College of Medicine-affiliated hospitals databases yielded illustrative INP cases (n=2).

Results: Based upon IHC and/or US features, 53 INPs were identified (23M, 29F). Mean age was 23 years (range 2 to 64); 87% from patients ≤39 years. Affected nerves/sites (decreasing frequency) were ulnar, median, peroneal, posterior interosseous, sciatic, radial, brachial plexus, femoral, tongue, and tibial. Mean tumor size was 5.4 cm (range 0.5 to 18). No trauma preceding tumor diagnosis was noted in 37 cases; 16 cases did not comment on trauma history. Mean symptom duration was 53 months (range 2 to 300). Motor abnormalities were present in 43 cases, and absent or not reported in 5 cases each. Mean follow-up was 20 months (range 1 to 72). Surgical resection with nerve grafting or anastomosis resulted in motor improvement in most cases. Molecular features repeatedly showed monosomy of 22q and deletions of 22q11. Recurrences or metastasis were not reported. Only one patient had neurofibromatosis (NF).

Conclusions: INP is a neoplastic proliferation of perineurial cells with unique IHC (EMA positive, S100 negative) and ultrastructural (perineurial cell lineage) features. INP is distinct from other "onion-bulb" Schwann cell-derived entities (S100 positive, EMA negative). Despite unique molecular characteristics, INP has not been associated with NF. In conclusion, INP is a benign peripheral nerve sheath tumor, which does not recur or metastasize.

1652 Pathogenesis of Fibrillary Glomerulopathy (FG) with Emphasis on Ultrastructural Findings

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Background: The pathogenesis of FG remains controversial. Although the hypothesis that it represents an immune complex-mediated process is favored, this is not universally accepted. Characteristics of this renal-limited disorder include Congo red negativity, randomly disposed 15 to 25 nm in diameter fibrils containing amyloid-P component in various glomerular locations and rarely, along tubular basement membranes.

Design: Twenty FG cases were analyzed taking into account clinical histories, light microscopy (LM), immunofluorescence (IF) with routine battery of stains and EM findings to identify clues that would help in defining the pathogenesis of this disorder. The clinical presentation was primarily proteinuria. Two of the cases had documented diagnoses of systemic lupus erythematosus (SLE), 1 had antiphospholipid antibody syndrome, 2 diabetes mellitus and 2 plasma cell dyscrasias. One of these patients with SLE and FG, developed renal failure, was transplanted and in the allograft a classical lupus nephritis appeared.

Results: The LM appearance resembled a variety of glomerulopathies. Fourteen of 19 cases showed staining for both kappa and lambda light chains (74%) and in 17 ribbon-like IgG and C3 fluorescence was identified (89%). Four cases revealed "full-house" staining (21%) and 5 kappa restriction (26%). Ten of 20 cases revealed fibrils in mesangial areas and along peripheral capillary walls (50%) and 10 showed a "membranous" pattern with epi/intramembranous fibrillary deposits (50%), coexisting with immune complex type of deposits. Immunogold labeling for amyloid- P component in 2 cases showed colocalization with the fibrils.

Conclusions: The presence of both kappa and lambda light chains in association with the fibrillary deposits in the great majority of the cases, the coexistence of typical immune complexes with fibrils in cases with a membranous glomerulopathy pattern, and the identification of FG in the clinical setting of active SLE with IF and distribution of deposits most consistent with lupus nephritis, strongly supports that the fibrils represent polymerized immune complexes. The finding of kappa restriction in a few FG cases indicates that monoclonal light chains can also polymerize into fibrils. The specific mechanism/s responsible for fibrillogenesis may be related to the milieu and/or the presence of amyloid-P component in the deposits.

1653 A Rapid Method for Negative Staining of Fecal Samples for Diagnosis of Viral-Induced Gastroenteritis by Transmission Electron Microscopy

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Background: Common etiologic agents for viral gastroenteritis are rotavirus, adenovirus, and "small round structure viruses" (enterovirus, Norwalk virus, calicivirus, astrovirus). Rapid viral infection diagnosis can avoid unnecessary antibiotic therapy, extensive costly medical workups and reduce hospital stays. Pseudo-replication for identifying viruses concentrates viral particles, but also concentrates fecal debris, making the screening process difficult and time consuming.

Design: A rapid negative staining method for viral particle identification in fecal samples is presented (modified Cubitt), that reduces debris and improves viral particle staining, while concentrating viral particles. This method requires <1ml of stool and may be prepared in <3m. Method is as follows: 1) Place small portion (0.5cm) of stool on glass slide/dish; 2) Add few drops of 2% phosphotungstic acid (pH 7.3) and mix thoroughly with stool; 3) Place drop of mixture on carbon-coated grid for 1m; 4) Remove as much fluid from grid as possible by touching torn edge of filter paper; 5) Before grid dries, place fresh drop of PTA on grid for 15-20s; 6) Remove excess fluid by gently touching grid to torn edge of filter paper. Allow grid to air-dry; 7) Examine grid for viral particles.

Results: To compare the modified Cubitt method of negative staining for stool samples to the pseudo-replica technique, 25 known samples of rotavirus, adenovirus, picornaviruses and negative controls were prepared by both methods. Each preparation was screened for 15m. Viral particles were counted per grid square and then rated as 1+, 2+ or 3+. Of the 14 positive samples, the modified Cubitt method was more sensitive than pseudo-replica with 10 of 14 samples and equally sensitive in 2 of 14 samples.

Conclusions: The rapid negative staining method for fecal sample preparation is preferable to the pseudo-replica technique for several reasons. This modified Cubitt method provides improved viral particle detection due to decreased background fecal debris and enhanced viral particle staining. The etiologic agent for viral gastroenteritis